Genetic analysis and response to selection for resistance to two stem borers, *Busseola fusca* and *Chilo partellus,* in tropical maize germplasm

By

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Thesis Abstract

Maize is the principal staple food in sub-Saharan Africa (SSA), but production lags behind population growth. The African stem borer, *Busseola fusca*, Fuller (Lepidoptera, Noctuidae), and the spotted stem borer, *Chilo partellus*, Swinhoe (Lepidoptera, Crambidae) are serious insect pests of maize in tropical environments. The damage can be managed by breeding stem borer resistant maize varieties but there is limited information that can be used to devise appropriate breeding programs. Therefore breeding investigations were conducted to appraise germplasm screening methods, and to determine combining ability, heterosis and response of maize populations to S1 progeny recurrent selection. The study was conducted in Kenya during 2010 to 2013.

The results showed that most of the test genotypes were susceptible to *B. fusca* and less so to *C. partellus,* indicating that breeding for *B. fusca* would be more challenging. Therefore more resources would be required to improve maize germplasm for resistance to *B. fusca* to broaden the base from which breeders will select suitable lines for breeding. There was a highly significant ($r=0.947$, $p \leq 0.01$) correlation between rank selection index in the greenhouse and laboratory. The detached leaf disk bioassay method was effective for screening maize genotypes for resistance to both stem borers. Therefore it will be recommended for use in screening maize genotypes in future studies. The line x tester studies indicated a preponderance of the additive gene effects for borer resistance traits. Specific combining ability effects were significant for resistance traits and grain yield indicating that non-additive effects were also influential. Findings from the breeding investigations will impact positively on both food security and plant breeding capacity. The completed study was successful in identifying new maize inbred lines with resistance to both stem borers. These lines have high utility to maize breeding programmes that emphasise stem borer resistance in tropical environments. For the hybrid-oriented programmes, combining ability and heterotic orientation data for the 66 maize inbred lines will be crucial. In this regard the study was very successful in classifying the lines into three heterotic groups according to single cross testers (CML395/CML444, and CML312/CML442) that are widely used at CIMMYT, and by public breeding programs throughout SSA. Importantly, this was done based on grain yield potential of hybrids under *B. fusca* and *C. partellus* infestations in three mega environments.

The study demonstrates that S1 progeny recurrent selection is effective for improving stem borer resistance, without compromising yield. There was significant reduction (69%) in maize plant damage by both pests, and yield gains of 25% to 70% were realised in two populations. This represents significant contribution to plant breeding capacity, especially to maize breeding programmes that emphasise stem borer resistance in hybrids.

Declaration

I **Murenga Geoffrey Mwimali** declare that:

1. The research work reported in this thesis, except where otherwise indicated, is my original investigation

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or information unless explicitly acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless explicitly acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a) their words have been re-written but the general information attributed to them has been referenced;

    b) where the exact words have been used, their writing has been placed inside quotation marks, and referenced.

5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the sources being detailed in the thesis and the reference section.

Signed

…… Date……

Murenga Geoffrey Mwimali (Candidate)

We agree to the submission of this thesis, as the candidate's supervisors

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…… .. Date……
Professor John Derera (Supervisor)

Signed
…… .. Date……
Professor Pangirayi Tongoona (Co-Supervisor)

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Dedication

This research work is dedicated to

My one Loving Wife

ANNE A. MWIMALI

For your relentless reminders that I am a father and a husband

OUR sons VICTOR MUTOLA and STANLEY WESONGA and OUR daughter JESSICA OSUNDWA

For the perseverance during my times away from home for work and data collection

May you excel and achieve better than I have!

Parents

My Father JOEL MURENGA MUTOLA and Mother ELIZABETH KANAIZA MURENGA

For your nurturing, strong faith in my abilities and the cold nights you spent away from home for our good

Sisters

Carolyn, Lillian, Jacklyn, Roselyn, and Christine

For your motivation and love for me

Brothers

Phanuel and Eric

For your motivation and love for me

Late grandparents

For your revelation for me

I have done it!

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1.0 Significance of maize in Kenya

Maize is the dominant staple crop grown by a vast majority of rural households in Kenya. It is both a staple food and a cash crop in terms of area under production, total consumption and income generation for small-scale farmers (Brooks et al., 2009; Government of Kenya, 2010). Despite its importance in Kenya, the area harvested, the production levels, the amount of seed maize produced and grain yield (t ha^{-1}) has been fluctuating in the last ten years (Figures 1 and 2).

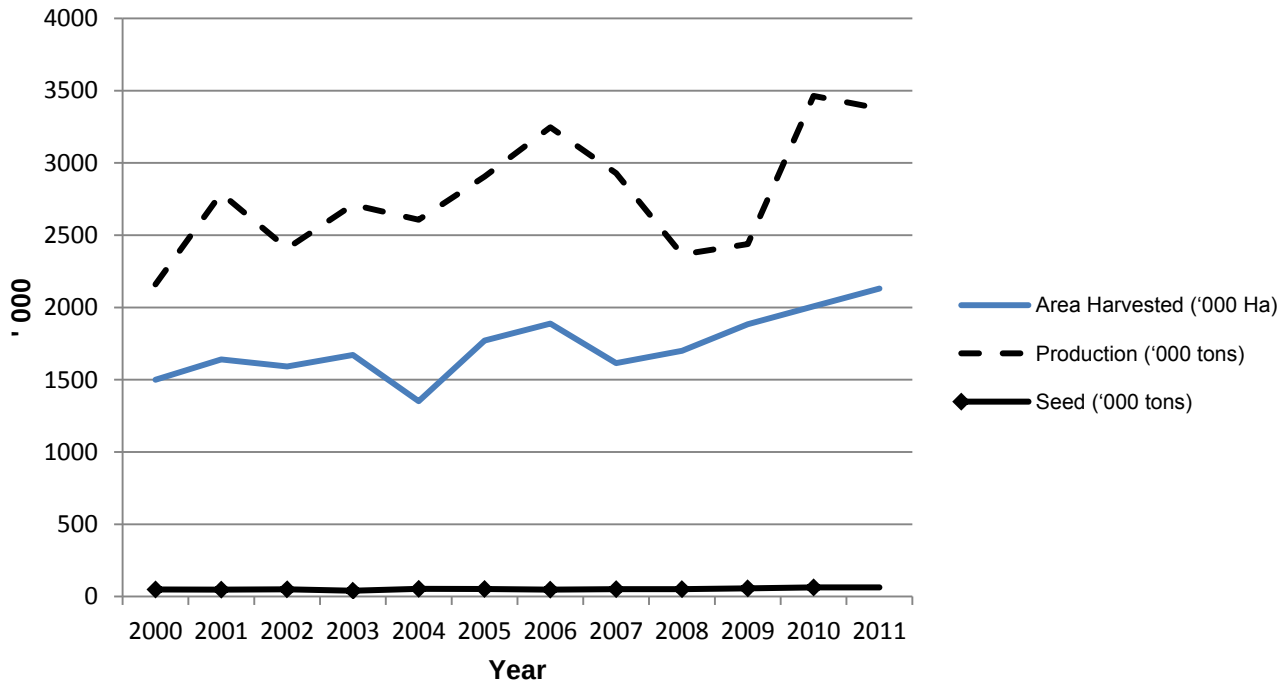

Figure 1. Maize area harvested, production and amount of seed in Kenya between 2000 and 2011

Source: (FAOSTAT, 2013)

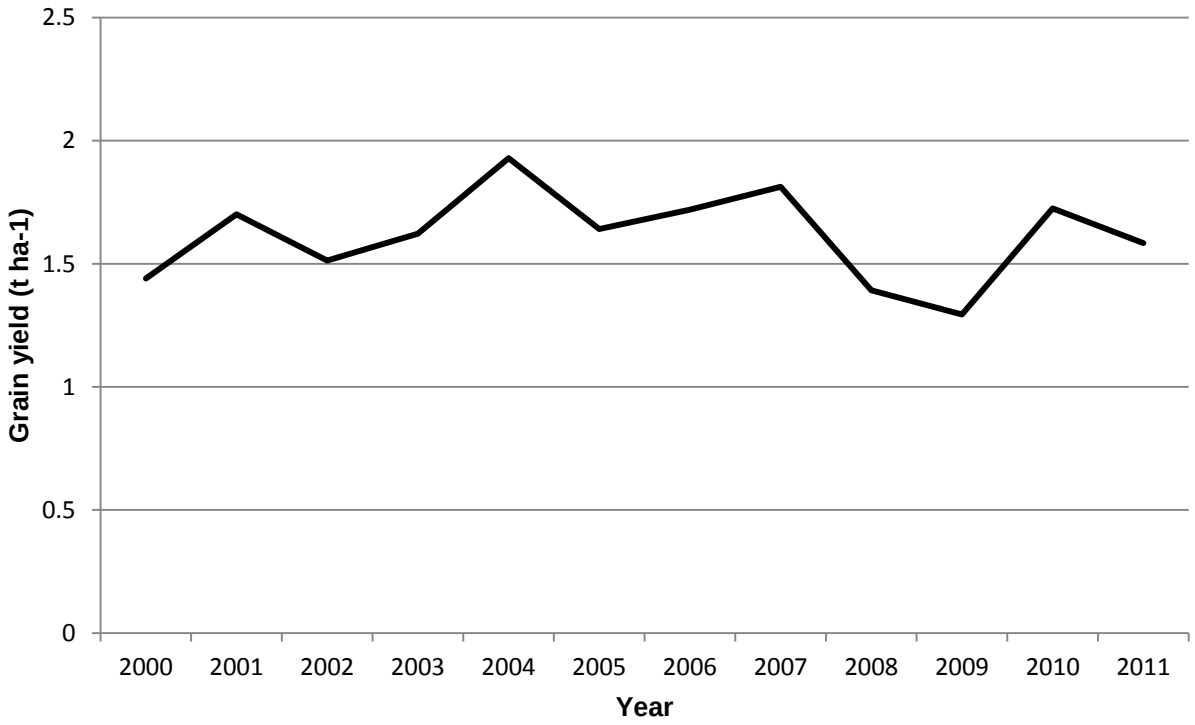

Figure 2. Maize yield (t ha^{-1}) in Kenya between 2000 and 2011

Source: (FAOSTAT, 2013)

Maize accounts for approximately 20% of the total agricultural production, and 25% of employment in the agricultural sector. It comprises about 3% of Kenya's gross domestic product (GDP), 12% of the agricultural GDP and 21% of the total value of primary agricultural commodities (Brooks et al., 2009; FAOSTAT, 2013). It contributes about 68% of daily per capita cereal consumption, 35% of total dietary energy consumption and 32% of total protein consumption, amounting to a per capita consumption of 98 kg yr^{-1} (Government of Kenya, 2010). It is grown both for subsistence and as a commercial crop by large-scale farmers (25%) and smallholders (75%) (Oscar, 2009). This translates to between 2.7 - 3.1 million metric tons annually (Government of Kenya, 2009; FAOSTAT, 2013). In Kenya, the various maize agroecologies have different characteristics (Table 1).

Table 1. Characteristics of maize agroecologies in Kenya

Characteristic	Highland	Mid-altitude Transitional	Mid-altitude moist	Mid-altitude dry	Lowland
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	1.0

Source: Government of Kenya (2009).

The national maize production average yield is approximately 1.6-1.8 t ha^{-1} and does not meet the annual national requirements of about 3.0 million MT. This shortfall may be filled by importations and cross-border trade (FAOSTAT, 2013). The Government of Kenya may have to continue extending duty waivers on maize imports to facilitate filling of the gap. Nevertheless, maize prices have remained well above average levels, by over 60% in most local markets nationally (Government of Kenya, 2010; Mutunga et al., 2010). Surveys in major maize ecologies in Kenya indicate that most farmers consistently rank poor and erratic rains, low soil fertility, *Striga* and stem borer infestation as their most important problems (De Groote et al., 2010; Mutunga et al., 2010). In Kenya, the farmers average maize yield is low when compared to world average of 4.3 t ha^{-1} (FAO, 2011; FAOSTAT, 2013). Yet an estimated five million farmers in Kenya grow maize at least once in a year on two out of every three farms (Government of Kenya, 2009; Oscar, 2009). Maize can be grown in almost every agro-ecological zone in Kenya (Figure 3) (Hassan, 1998). Three of the agro-ecological zones namely; lowland tropics, dry mid-altitudes, and the dry transitional zones are characterized by low yields (<1.5 tha^{-1}). Although these zones cover 29% of Kenya’s maize area, they only produce 11% of the maize. The highland tropics, moist transitional, and the mid-altitude agro-ecological zones achieve high yields (>2.5 t ha^{-1}) and produce 80% of Kenya’s maize (Government of Kenya, 2009; Oscar, 2009).

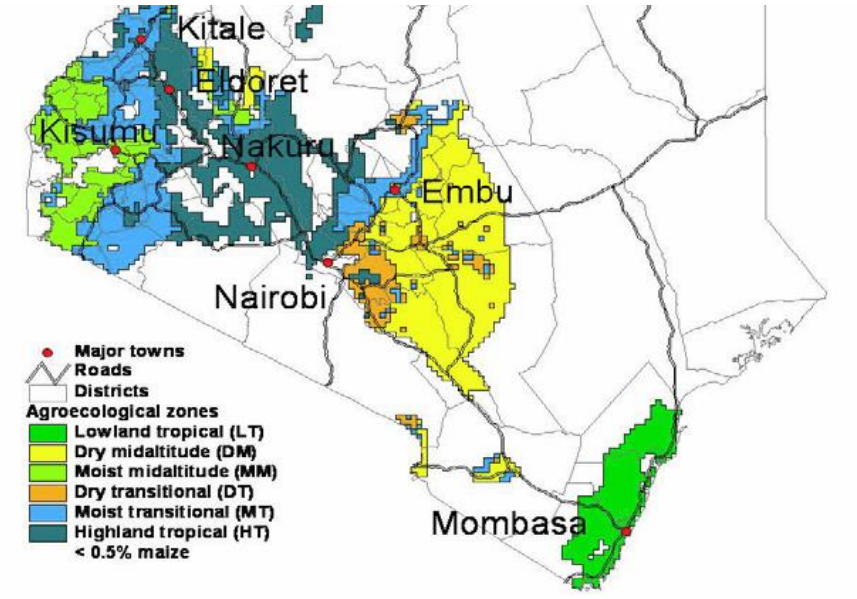

Figure 3: Maize agro-ecological zones in Kenya.
Source: Hassan, 1998

2.0 Maize production constraints in Kenya

Despite the importance of maize and its widespread production and consumption, recent reports indicate dramatic reductions of expected maize yield in many counties in the Eastern, Coast, and the Rift Valley regions of Kenya with respective decreases of 79%, 32%, and 14% (Government of Kenya, 2013). These regions are considered the food grain basket of Kenya. However, reduction in production of maize is approximated at about 250, 000 metric tons (Government of Kenya, 2009). At the national level, this is likely to impact negatively on livelihoods, market prices, and overall food security (Government of Kenya, 2009; Oscar, 2009; Government of Kenya, 2010).

The decline in grain yield can be attributed to various maize production constraints. These production constraints can be grouped into socioeconomic, technological, policy, abiotic and biotic constraints (Oscar, 2009). Socioeconomic, technological and policy limitations facing farmers include use of poor quality seeds, population pressure, land constraints, limitations to market access, poor state of infrastructure, and high costs of farm inputs (De Groote et al., 2004; Government of Kenya, 2010). Abiotic factors affecting maize production include declining soil fertility, low soil pH with associated nutrient deficiencies and toxicities, and low and unreliable rainfall leading to recurrent droughts. Biotic constraints affecting maize production are foliar diseases (turcicum leaf blight, grey leaf spot, maize streak virus and maize lethal necrosis), parasitic weeds (*Striga* spps and *Allectra vogelli*), and insect pests (stem borers, leaf hoppers, chafer grubs, cut worms, wireworms, maize weevils and the larger grain borer) (De Groote et al., 2004; Ajala et al., 2010; Wangai et al., 2012).

Previous studies have indicated a clear link between stem borer damage traits (leaf damage, number of exit holes, cumulative tunneling, number of dead-hearts etc.) and grain yield losses (De Groote et al., 2004; Morais et al., 2012). The values of these losses provide a basis for the setting research priorities to justify studies to understand the genetics of stem borer resistance, and selection for the borer resistance in tropical maize.

2.1 The maize stem borer problem

In sub Saharan Africa (SSA), stem borers are a major constraint to maize production because of their significant contribution to yield losses and grain quality degradation. The insect pests are more destructive in the tropical than temperate environments because of the favourable climatic conditions that are more conducive for accelerated insect development with numerous overlapping generations leading to high infestation levels and losses. Stem borers, are most damaging in the larval stages when they tunnel inside the maize stem after hatching and therefore very difficult to control. Successful infestation of these borers into plants, and their feeding may cause death of growing points, reduction in number of harvestable ears or may cause structural damage that increases the likelihood of lodging. In some cases these pests also attack maize ears making the cob and the kernels vulnerable to ear rots due to fungal attacks which produce harmful mycotoxins.

2.2 Economic importance of stem borers

Currently, about 50% of the maize area in 25 key maize growing countries in the tropics and subtropics has approximately 60% area under infestation with lepidopteran pests (CIMMYT, 2008; FAOSTAT, 2013). These lepidopteran pests include maize stem borers which are most serious in Asia and Africa (Morais et al., 2012). In Africa, they are mainly the African stalk borer (*Busseola fusca* Fuller*)*, the spotted stem borer (*Chilo partellus* Swinhoe*)*, the pink stem borer (*Sesamia calamistis* Hampson*)* and the sugar cane borer (*Eldana saccharina* Walker) (Mailafiya et al., 2009). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al., 2003; De Groote et al., 2005). This amount represents an average of 14% of the farmers' total annual harvest of maize.

3.0 Problem statement and justification

Maize (*Zea mays* L.) is a major staple food crop in Kenya and, because of its contribution to food security, the shortage of maize in Kenya always results in famine among the rural and urban poor communities. However, the high incidence and damage by stem borers (the spotted stem borer,

(*Chilo partellus Swinhoe*) and the African stem borer (*Busseola fusca* Fuller) greatly reduces maize grain yield with serious consequences on food security. Other stem borer species are less significant. In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al., 2003; De Groote et al., 2005). This amount represents an average of 14% of the farmers' total annual harvest of maize. In the highlands and dry areas grain yield losses due to stem borers range from 11 to 21% respectively. More than half of the losses occur in the moist transition agro-ecological zone. Incidentally, the moist transition agro-ecological zone has the highest adoption of improved maize varieties (95%) making this area a promising target for insect resistant varieties (De Groote et al., 2005). In maize growing zones of Kenya, the potential yield ranges from 2.7-6.7 t ha^{-1} compared to the farmers' yield which ranges from 0.5-2.0 t ha^{-1}. Therefore by reducing stem borer damage, the current farmer yield may be increased to above the average of 1.6-1.8 t ha^{-1} (De Groote et al., 2003; De Groote et al., 2005; Government of Kenya, 2009; Mutunga et al., 2010).

Numerous strategic options for managing maize stem borers have potential to either prevent or mitigate the damaging effects of these borers on crops but each option has its own limitations. For example, chemical control methods are most effective; however, they are expensive to most small scale farmers and pose risks to humans, livestock, and the environment. Biological control methods are efficient, cost-effective and environmentally safe; still, they may be insufficient in maintaining the pest populations below economic injury levels (Mailafiya et al., 2009). Cultural control methods are easy to use and may not involve costs *per se;* however*,* they have a limited mode of application, may not be applicable to large scale farms, and they have a difficulty in the timing. The use of genetically engineered *Bacillus thuringiensis* (Bt) crops is a very effective method in the control of stem borers and other lepidopteran pests because the proteins are highly specific in their mode of action, and they control a narrow range of target pests (Yuan et al., 2009). Nevertheless, there are biosafety concerns ranging from ethical and moral, intellectual property restrictions and the payment of royalties, environmental health considerations on biodiversity, food safety and human health, labeling and trade issues, traceability, and the need for monitoring of Bt-derived products (Tabashnik et al., 2009). Research on Bt-maize is in progress for the implementation in Kenyan farming systems under collaborative projects between the Kenya Agricultural Research Institute (KARI) its partners. However, even with the current biosafety law and the Agriculture, Fisheries and Food Authority Act in place it may take longer before farmers realize the products (Mugo et al., 2005; Government of Kenya, 2013). Host plant resistance using conventional methods is an acceptable method for protecting plants against insect pests; however, it is may be limited due to the polygenic nature of the insect resistance trait inheritance and the high costs of plant breeding (Hallauer et al., 2010). Nonetheless, host plant

resistance forms an important part of integrated pest management. It provides inherent control without environmental concerns and that it is mostly compatible with other pest management approaches (Morais et al., 2012). It is with this background that a large body of literature provides evidence that farmers would be probably continue to grow their accustomed varieties alongside the improved maize from conventional breeding.

The knowledge of the genetics of stem borer (*Busseola fusca* and *Chilo partellus*) resistance is restricted to a few crosses of maize inbred lines. These challenges may be attributed to the lack of resistant varieties, limited genetic information on stem borer resistance and limited information on response to selection for borer resistance. Therefore the need to study and increase the understanding of the genetics of plant damage traits by (*Busseola fusca* and *Chilo partellus*) and yield under artificial infestation. Both line x tester analysis, and divergent selection in populations were used in testing for gene action, combining abilities, and S1 progeny recurrent selection for predicting genetic gain for stem borer resistance and grain yield among cycles of selection.

4.0 Research objectives, hypotheses, and structure of thesis

To understand the genetics of stem borer resistance in maize the following specific objectives were addressed through studies to:

a) evaluate tropical maize inbred lines for resistance to two stem borers, *Busseola fusca* and *Chilo partellus*,
b) determine combining ability for resistance and heterotic orientation of maize inbred lines under *Busseola fusca* infestation,
c) determine combining ability and heterotic orientation of maize inbred lines under *Chilo partellus* infestation,
d) appraise a detached leaf disk bioassay method for screening for *Busseola fusca* and *Chilo partellus* resistance maize in the greenhouse and laboratory trials, and
e) separately improve resistance to two stem borers *Busseola fusca* and *Chilo partellus* in two tropical maize populations through S1 progeny recurrent selection.

5.0 Research hypotheses

In the understanding of the genetics of stem borer resistance in maize, the following assumptions have been made, namely:

a) There is resistance to *Busseola fusca* and *Chilo partellus* only and combined resistance to both stem borers maize inbred lines and populations included in this study.

b) Resistance to *Busseola fusca* and *Chilo partellus* stem borers is governed by minor genes with additive effects suggesting that resistance can be enhanced through selection approaches
c) There are adequate genetic variations; both additive and non-additive involved in resistance to stem borers, *Busseola fusca* and *Chilo partellus* in the maize lines and populations included in this study
d) It is possible to detect resistance to stem borers, *Busseola fusca* and *Chilo partellus* in maize in the greenhouse and laboratory trials using the detached leaf disk bioassay and whole plant bioassays method in maize inbred lines included in this study
e) Genetic gain for resistance to two stem borers *Busseola fusca* and *Chilo partellus* in two tropical maize populations through S1 progeny recurrent selection, and a significant increase in grain yield is achievable in populations included in this study

6.0 Structure of thesis

This thesis addresses the specific objectives in chapter form. Each chapter is independent and potentially a manuscript for journal publication. There may be repetition of content and references with other chapters. These chapters are divided as follows:

a) Introduction to Thesis
b) Chapter 1: Literature review
c) Chapter 2: Evaluation of tropical maize inbred lines for resistance to two stem borers, *Busseola fusca* and *Chilo partellus*
d) Chapter 3: Combining ability for stem borer resistance and heterotic orientation of maize inbred lines towards CIMMYT testers under *Busseola fusca* infestation
e) Chapter 4: Combining ability for stem borer resistance and heterotic orientation of maize inbred lines towards CIMMYT testers under *Chilo partellus* infestation
f) Chapter 5: Appraisal of leaf disk bioassay method for screening for resistance to stem borers, *Busseola fusca* and *Chilo partellus* in maize inbred lines in laboratory and greenhouse trials
g) Chapter 6: Response to two cycles of S1 progeny recurrent selection for resistance to two stem borers, *Busseola fusca* and *Chilo partellus* in two tropical maize populations
h) Chapter 7: Overview, general discussions, conclusions and recommendations

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Chapter 1

Literature Review

1.1 Introduction

This chapter provides a context for the research in maize improvement for resistance to stem borers (*Chilo partellus* and *Busseola fusca*) in tropical environments. The following aspects are reviewed a) major productions constraints in East Africa, b) the stem borer problem in maize, c) genetic studies on maize resistance to stem borers. The explanations of key technical issues on progress and challenges in breeding for stem borer resistance in maize, inheritance of stem borer resistance and combining ability in maize, maize heterotic patterns, determination of heterotic orientations, application of the line x tester mating design, screening methods, selection indices, genotype x environment interactions, and response to selection for resistance to stem borers are addressed. Therefore, this chapter forms a setting of reference for the study.

1.2 Maize in Kenya

Maize is the dominant staple crop grown by a vast majority of rural households in Kenya. It is both a staple food and a cash crop for small-scale farmers (Government of Kenya, 2009). The production statistics of maize in Kenya is depicted in Table 1.1. Maize accounts for approximately 20% of the total agricultural production, and 25% of employment in the agricultural sector. It constitutes about 3% of Kenya's gross domestic product (GDP), 12% of the agricultural GDP and 21% of the total value of primary agricultural commodities (FAOSTAT, 2013). It contributes about 68% of daily per capita cereal consumption, 35% of total dietary energy consumption and 32% of total protein consumption, amounting to a per capita consumption of 98 kg yr^{-1} (Government of Kenya, 2009). It is grown both for subsistence and as a commercial crop by smallholders (75%) and large-scale farmers (25%). This translates to between 2.7-3.1 million metric tons annually. In Kenya, the various maize agroecologies have different characteristics (Table 1.2).

Table 1.1: Maize area harvested, production, yield and amount of seed in Kenya between 2000 and 2011

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Area Harvested ('000 Ha)	1500	1640	1592	1671	1351	1771	1888	1615	1700	1884	2008	2131
Production ('000 tons)	2160	2790	2409	2711	2607	2906	3247	2929	2367	2439	3464	3376
Yield (t ha^{-1})	1.44	1.701	1.513	1.622	1.929	1.641	1.72	1.813	1.392	1.294	1.725	1.584
Seed ('000 tons)	49.20	47.77	50.13	40.54	53.13	53.00	48.46	51.00	51.00	57.00	63.96	63.96

Source: FAO Statistics Division 2013

Table 1.2. Characteristics of maize growing regions in Kenya

Characteristic	Highland	Mid-altitude Transitional	Mid-altitude moist	Mid-altitude dry	Lowland
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	1.0

Source: Government of Kenya (Government of Kenya)

In Kenya, '*when there is no maize, there is no food'* because of the strong link between food security and the amount of annual maize produced. Unfortunately the yield is very low ranging between 1.6 and 2.0 t ha^{-1} (see Tables 1.1 and 1.2). Thus the farmers average maize yield is low when compared to world average of 4.3 t ha^{-1} (FAOSTAT, 2013). Yet an estimated five million farmers in Kenya grow maize at least once in a year on two out of every three farms (Government of Kenya, 2010). A yield potential of up to 6 t ha^{-1} is achievable with the use of improved maize hybrids, irrigation, and the use of fertilizers accompanied with good management depending on the agro-ecological zone (see Table 1.2) (Government of Kenya, 2010). However the yield is still compromised by stress factors. Surveys in major maize ecologies in Kenya indicate that most farmers consistently rank poor and erratic rains, low soil fertility, *Striga,* and stem borer infestation as their most important constraints (Mutunga et al., 2010). The production constraints are discussed in detail in the next section.

Maize can be grown in almost every agro-ecological zone in Kenya (Figure 1.1) (Hassan, 1998). Three of the agro-ecological zones namely; lowland tropics, dry mid-altitudes, and the dry transitional zones

are characterized by low yields (<1.5 tha^{-1}). Although these zones cover 29% of Kenya's maize area, they only produce 11% of the maize. The highland tropics, moist transitional, and the mid-altitude agro-ecological zones achieve high yields (>2.5 t ha^{-1}) and produce 80% of Kenya's maize (Government of Kenya, 2010). However, even in this zone grain yield of maize has not reached its full potential due to many constraints.

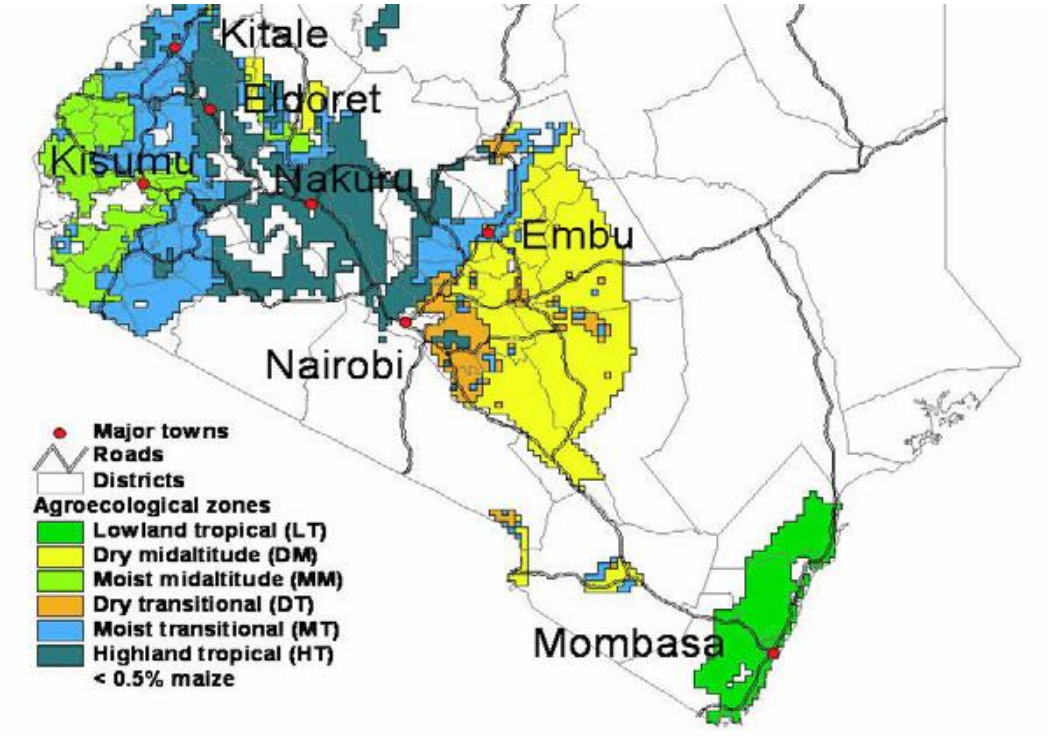

Figure 1.1. Maize agro-ecological zones in Kenya.

Source: Hassan, 1998

1.2.1 Maize production constraints in Kenya

Despite the importance of maize and its widespread production and consumption, recent reports indicate dramatic reductions of expected maize yield in counties in the Eastern, Coast, and the Rift Valley regions of Kenya with respective decreases of 79%, 32%, and 14% (Government of Kenya, 2010). These regions are considered the food and grain basket of Kenya. However, reduction in production of maize is about 250,000 metric tons (Government of Kenya, 2010). At the national level, this is likely to impact negatively on livelihood, market prices, and overall food security (Government of Kenya, 2009; Oscar, 2009).

The decline in grain yield can be attributed to various maize production constraints. These production constraints can be grouped into socioeconomic, technological, policy constraints, abiotic and biotic constraints (Oscar, 2009). Socioeconomic, technological and policy limitations facing farmers include use of poor quality seeds, population pressure, land constraints, limitations to market access, poor state of infrastructure, and high costs of farm inputs (De Groote et al., 2004; Government of Kenya, 2010). Abiotic factors affecting maize production include declining soil fertility, low soil pH with associated nutrient deficiencies and toxicities, and low and unreliable rainfall leading to recurrent droughts (Government of Kenya, 2010).

Biotic constraints that affect maize production are foliar diseases (maize lethal necrosis (MLN) disease, turcicum leaf blight, grey leaf spot, and maize streak virus), parasitic weeds (*Striga* and *Allectra vogelli* spps), and insect pests (stem borers, leaf hoppers, chafer grubs, cut worms, wireworms, maize weevils and the larger grain borer) (Ajala et al., 2010; Morais et al., 2012; Wangai et al., 2012). However the lepidopteran pests, mainly stem borers are one of the most devastating insect pests of maize in sub Saharan Africa (SSA) (Belay et al., 2010). The stem borers are major constraints to maize production because of their significant contribution to yield losses and grain quality degradation. The favourable climatic conditions are more conducive for the accelerated insect development with numerous overlapping generations leading to high infestation levels and losses. Stem borers, are most damaging in the larval stages when they tunnel inside the maize stem after hatching and therefore very difficult to control. Successful infestation of these borers into plants, and their feeding may cause death of growing points, reduction in number of harvestable ears or may cause structural damage that increases the likelihood of lodging (Morais et al., 2012). In some cases these pests also attack maize ears, cobs and the kernels, predisposing them to rots due to fungal attacks which produce mycotoxins.

Among these lepidopteran pests, primarily stem borers, the African stalk borer (*Busseola fusca* Fuller) and the spotted stem borer (*Chilo partellus* Swinhoe) are the most serious pests of maize in Kenya. Their biology of *Busseola fusca* and *Chilo partellus*, and distribution, and economic importance are discussed below.

1.4.1 Biology of *Busseola fusca*

The first concise information about the life cycle and the economics of *Busseola fusca* was carried out by Fuller in the 1900's (Kfir, 1997). *B. fusca* has two generations in one year; however it may have more than three generations in warm areas of sub Saharan Africa. Its importance increases at higher

altitudes. *B. fusca* forms tunnels in stems of host plants towards the end of the rainy season, and the larvae may diapause in areas that experience winter or dry seasons. The eggs are white at first, but later turn darker with time. These eggs are globular and about 1 mm in diameter. They are laid in a long column stretching up the stem, under the leaf sheath. They hatch after about 10 days and the young larvae are deep purple or black in colour. In the early stages, the caterpillars feed on leaves in the whorl of the host plant, resulting in characteristic lines of holes and 'windows'. The larval period takes about 35 days or more. The fully grown caterpillar is about 40 mm long with a pinkish white colour and small black spots along the sides of the body. A mature caterpillar cuts a hole in the stem before pupating within the tunnel and eventually uses this hole to emerge. The pupa is about 25 mm long. The pupal stage lasts about 14 days. Before the crop ripens there are usually two generations whose eggs may be laid on the cob. The caterpillars feed on the cob and later move into the stem. Before pupating they may diapause for long which may last till the next rains. They prepare a pupal chamber in the stem and pupate. The adult is a pale brown nocturnal moth with a wing span of 35-40 mm (Kfir, 1997). The detailed description of the biology of *B. fusca* is given by Mally (1920).

1.4.2 Biology of *Chilo partellus*

The first concise information about the life cycle and the economics of *Chilo partellus* was carried out by Swinhoe in the 1900's (Kfir, 1997). The eggs are laid on the underside of the host plant near the midrib in 3-5 rows and in groups of 50-100. These eggs are flattened, ovoid, and about 8 mm long. Hatching takes place after 7-10 days. The young caterpillars form characteristic holes on leaves and 'windowing' from their feeding. In early stages they may mine in the leaves causing streaks. After a few days the young caterpillars bore down through the whorl into the stem of the host plant. In general, *C. partellus* young caterpillars resemble those of *B. fusca* larvae. They are creamy pink with groups of dark spots along the back. The head capsule is brown. When mature they are about 25 mm long. These caterpillars can be distinguished from *B. fusca* and *Sesamia calamistis* larvae by the presence of circular hooks on their prolegs. In *B. fusca* and *S. calamistis* these hooks are arranged in a crescent manner. The larval period takes about 28-35 days. Pupation takes place in 7 to 10 days in a small chamber in the stem of a host plant. The adult moths have a wing span of 20-30 mm. The males are smaller and darker than females. The forewings of males are pale brown while those of females are paler with the hind wings almost white (Kfir, 1997). The detailed description of the biology of *C. partellus* is given by Päts (1992).

1.3 Geographical distribution of *Busseola fusca* and *Chilo partellus* stem borers

The biology, habits, distribution and control measures for these injurious insect pests to maize have been described in various literature sources (see Figure 1.2) (Belay et al., 2010; Chaudhary, 2013). *B. fusca* and *C. partellus* as pests of maize have generated a lot of interest for researchers since the last century. The geographical distribution of these two most damaging cereal stem borers of maize and sorghum are probably altitude-dependent (Kfir, 1997; De Groote et al., 2004). *Chilo partellus* reportedly occurs below 1500 m asl, whereas *B. fusca* is found at elevations greater than 600 m asl (Kfir et al., 2002). However, other studies have suggested that temperature, rainfall and humidity are key factors responsible for their distribution, with temperature being most important (Kfir et al., 2002; Ajala et al., 2010). Kfir et al. (2002) indicated that *B. fusca and C. partellus* are found in warmer and cooler regions respectively.

The distribution and occurrence of *B. fusca* and *C. partellus* stem borers is diverse in Africa (Figure 1.2; (Mailafiya et al., 2011). Several factors affect their population dynamics specifically; host availability, location and suitability, mate location, success of oviposition, larval survival and establishment, temperature and altitude (Mailafiya et al., 2011). The basic hosts are cultivated cereal crops mainly maize, sorghum, pearl millet, finger millet and sugarcane. The non-cultivated hosts are the wild grasses namely; wild Sudan grass (*Sorghum verticilliflorum*), elephant grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), Johnson grass (*Sorghum halepense*), *Hyparrhenia rufa* and *Rottboellia exaltata* (Kfir, 1997; Mailafiya et al., 2011).

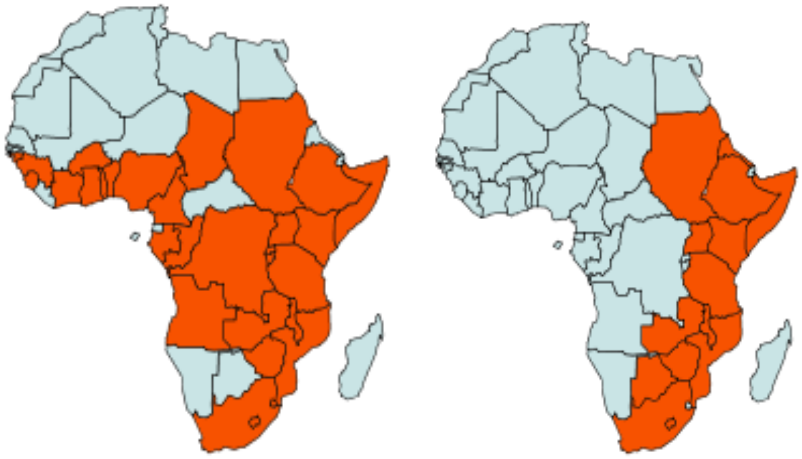

Figure 1.2. Geographical distribution of *Busseola fusca (left)* and *Chilo partellus (right)* in Africa
Source: http://www.infonet-biovision.org/default/ct/92/pests and http://www.infonet-biovision.org/default/ct/102/pests (accessed 27.11.2013)

1.4 Economic importance of *Busseola fusca* and *Chilo partellus*

Currently, about 50% of the maize area in 25 key maize growing countries in the tropics and subtropics has approximately 60% area under infestation with lepidopteran pests (Ong'amo et al., 2012; FAOSTAT, 2013). These lepidopteran pests include maize stem borers which are most serious in Asia and Africa. In Africa, they are mainly *Busseola fusca* and *Chilo partellus*, the pink stem borer (*Sesamia calamistis* Hampson) and the sugar cane borer (*Eldana saccharina* Walker) (Mailafiya et al., 2011). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al., 2005). This amount represents an average of 13.54% of the farmers' total annual harvest of maize.

1.5 Management of the stem borers

A number of strategic approaches for the management of stem borers have potential to either mitigate the damaging effects of these borers; however, each option has its own limitations. For illustration, chemical control methods are most effective; though, they are expensive to most small scale farmers and pose risks to humans, livestock, and the environment. Biological control methods are efficient, cost-effective and environmentally safe; still, they may not be sufficient to manage the pest populations at below economic injury levels (Mailafiya et al., 2009).

Cultural control methods are easy to use and may not involve costs *per se;* however*,* they have a limited mode of application, may not be applicable to large scale farms, and they have challenges in the timing. The use of genetically engineered *Bacillus thuringiensis* (Bt) crops is a very effective method in the control of stem borers and other lepidopteran pests because the proteins are highly specific in their mode of action, and they control a narrow range of target pests (Yuan et al., 2009). Nevertheless, there are biosafety concerns ranging from ethical and moral, intellectual property restrictions and the payment of royalties, environmental health considerations on biodiversity, food safety and human health, labeling and trade issues, traceability, and the need for monitoring of Bt-derived products (Tabashnik et al., 2009). Research on Bt-maize is in progress for implementation in Kenyan farming systems under collaborative projects between the Kenya Agricultural Research Institute (KARI) and its partners. However, even with the current biosafety law and the Agriculture, Fisheries, and Food Authority Act in place it may take longer before farmers realize the products (Mugo et al., 2005; Government of Kenya, 2013). Thus application of biotechnology that involves genetically engineered products is still an evolving option in sub-Saharan Africa.

Host plant resistance using conventional methods is an acceptable method for protecting plants against *B. fusca* and *C. partellus* because there are no biosafety concerns. However, its application is still limited due to the polygenic nature of the insect resistance trait, limited understanding of its inheritance and the high costs associated with plant breeding (Hallauer et al., 2010). Nonetheless, host plant resistance forms an important part of integrated pest management. It provides inherent control without environmental concerns. Host plant resistance is compatible with other pest management approaches (Morais et al., 2012). It is with this background that a large body of literature provides evidence that; farmers would probably continue to grow their accustomed varieties alongside the improved maize from conventional breeding. The genetics of *B. fusca* and *C. partellus* resistance is restricted to a few crosses of maize inbred lines. These challenges may be attributed to the lack of resistant varieties, limited genetic information on stem borer resistance, and limited information on response to selection for borer resistance. Therefore the need to study and improve the understanding of the genetics of *B. fusca* and *C. partellus* resistance in maize, and grain yield under artificial infestation. This will form the basis of a viable breeding strategy for deploying stem borer resistant maize hybrids.

1.6 Breeding for resistance to maize stem borers

Suitable maize germplasm should have resistance to both *B. fusca* and *C. partellus* species because the pests may occur together. Recent studies (Kfir, 1997) show that *C. partellus* is progressively displacing *B. fusca* from the high altitude areas in Kenya due to climate change. The problem is further exacerbated by farmers who exchange maize germplasm across agro-ecologies. Currently, there is lack of resistant varieties to both, limited genetic information on stem borer resistance, and limited information on response to selection for borer resistance. Therefore the need to identify resistance in tropical maize inbred lines to both *B. fusca* and *C. partellus* species key for the maize programme in Kenya. It is with this background that effective breeding methods for both pests could be designed by plant breeders using both improved and new sources of stem borer resistance.

1.5.1 Progress and challenges in breeding for resistance to maize stem borers

Various efforts have been undertaken by the International Maize and Wheat Improvement Center (CIMMYT) to include breeding for insect resistance in its breeding programs (CIMMYT, 2008; Tefera et al., 2010). The Insect Resistant Maize for Africa (IRMA) project was a collaborative initiative with the local partners to develop and deploy insect resistant maize for African farmers. Currently, the Water Efficient Maize for Africa (WEMA) project is testing the insect protected maize (Bt maize) at KARI,

Kiboko with the long term view of deployment to Kenyan farms. Additionally, the international collaboration continues to enable exchange of germplasm with insect resistance to various countries in Africa, Asia and others (CIMMYT, 2008). Maize varieties (open pollinated varieties and hybrids) have been identified and released by KARI; resistance levels are low to moderate (Ajala et al., 2010; Tefera et al., 2010) which has limited efficacy of the technology. Consequently, the identified sources of stem borer resistance have not been used extensively. Probably, this is due to linkage drag, pleiotropic effects, or low heritability which discourages breeders to emphasise insect resistance when there is huge pressure from donors to release new varieties in real time. Despite all these ominous efforts, there is a need to increase resistance levels through selection and other strategies, and to manipulate the basis of resistance through use of diverse resistance sources in cultivated germplasm. A lot of pre-breeding work needs to be done so that commercial breeders and their NARS counterparts to readily find stem borer resistance in the right genetic background, and from the right heterotic group to make maize hybrids.

1.6.1 Inheritance and combining ability for resistance to stem borers

Effective plant breeding programs for the development of stem borer resistant maize germplasm requires an elaborate understanding of the gene action involved in the inheritance of the traits. Breeders also want to know whether breeding for stem borer resistance can be achieved without affecting the grain yield potential of the hybrids. Gene action denotes how the expression of traits, separately or in combinations is affected through inheritance. The genetic components affecting quantitative or polygenic traits may be classified as additive, dominance, and epistasis variance (Falconer et al., 1996). Additive variance represents the proportion of a trait that can be transmitted from parents to the progeny, and it characterizes the degree of resemblance between offspring's and their parents (Falconer et al., 1996). It may be expressed by narrow sense heritability. Higher values of narrow sense heritability imply a higher probability of the transmission of the trait from the parent to the progeny. Non-additive gene action is not transmissible to the progeny, and represents all types of deviations that may not be explained by the additive model, and may include dominance and epistasis (Falconer et al., 1996). Given the foregoing, it is imperative to establish the mode of inheritance for stem borer resistance so that appropriate breeding strategies are devised.

Most studies on stem borer resistance in maize indicate both significant general and specific combining abilities, showing that additive and non-additive gene effects are important in governing the resistance (Udaykumar et al., 2013). Stalk resistance to stem borers is complicated because it is polygenic, and involves additive, dominance, and epistatic effects (Sandoya et al., 2010; Barros et al., 2011) which

partly explains why breeding for resistance in maize has been really difficult. Furthermore, both additive and dominance effects influence the expression of resistance to *B. fusca* and *C. partellus* (Andre et al., 2003; Kamala et al., 2012) which compromises heritability especially when the non-additive portion is preponderant. However other previous genetic studies have indicated that at least 10 genes are involved in borer resistance, and that gene action is primarily additive (Singh et al., 2012) indicating that higher heritability could be found in some populations . Other studies indicate that in sweet corn resistance to ear damage caused by *Helicoverpa zea*, is controlled by epistatic and, additive-dominance effects (Butrón et al., 2009; Singh et al., 2012). In addition, in different maize populations both GCA and SCA effects explain significant levels of variation for resistance to fall army worm, *Spodoptera frugiperda,* and the sugarcane borer, *Diatraea grandiosella* (Dyar) (Oloyede-Kamiyo et al., 2011). Given that stem borer resistance is a polygenic trait with low heritability (Falconer et al., 1996), recurrent selection approaches would be the most appropriate for the accumulation of favourable alleles for resistance.

1.7 Recurrent selection in maize

Recurrent selection is a method of that involves selection, recombination, and evaluation of the best test genotypes in successive cycles (Ana Paula et al., 2013) to accumulate high allele frequencies for traits of interest. Generally, the method improves the mean performance of the population, while at the same time maintains the genetic variation. The method is applied for the population improvement for polygenic traits hence it would be appropriate for improving stem borer resistance in maize.

Six different types of recurrent selection strategies have been identified namely; full sib, half sib, S1 progeny, S2 progeny, simple recurrent selection (SRS), and reciprocal recurrent selection (RRS) (Ana Paula et al., 2013). The traits under selection and the number of populations under consideration determine the method to be used in the selection. Both intra-population and inter-population recurrent selection approaches are used, but more commonly the former is applied for improvement of a single population (Sandoya et al., 2010). It is predominantly applied for improvement of resistance to insect pests and germplasm adaptation. The effectiveness of recurrent selection approaches depend on trait heritability, selection intensity, and the level of genetic variation in the base population (Acquaah, 2009).

The application of the S1 progeny recurrent selection exposes lethal recessive alleles and reduces the genetic load in the target population and at the same time, it emphasizes additive gene effects which

are more appropriate and effective in the improvement of most maize traits. Recent studies in quantitative genetics theory indicate that S1 progeny recurrent selection can be used in breeding for resistance to stem borers in maize populations (Sandoya et al., 2010). Through the S1 progeny recurrent selection, the expected genetic variation considering only the additive genetic effects is four times greater among half-sib families and two times among full-sib families (Sandoya et al., 2008; Hallauer et al., 2010). For these reasons, the S1 progeny recurrent selection was considered relevant for the current study. The strategy is most appropriate given the low heritability of the polygenic traits that constitute stem borer resistance (Hallauer et al., 2010). Given that there are limited studies on response of maize populations to selection for pest resistance, the present study serves as the reference for determining the value of S1 progeny recurrent selection for the improvement of *B. fusca and C. partellus* resistance in maize.

1.8 Line x tester mating design

Another strategy for breeding stem borer resistance would be exploitation of heterosis in hybrids. Therefore information regarding combining ability of insect resistant inbred lines would be required to expedite development of hybrids. The line x tester mating design developed by Kempthorne (1957) provides consistent information on the general and specific combining ability effects of parents and their hybrid combinations, respectively. The design has been applied in many previous quantitative genetic studies in maize (Sanghera et al., 2012). The design is mainly used to generate data on nature and magnitude of gene action, combining ability effects, heritability and nature and extent of heterosis for different traits. For example, Sprague et al. (1942) on studies in maize yield observed that general combining ability is mainly due to the additive gene effects while specific combining ability is attributed to dominance or epistatic gene effects. The line x tester mating design has been used in determining the pattern of gene action for stem borer (*B. fusca* and *C. partellus)* resistance in maize (Sanghera et al., 2012). The application of line x tester mating design is generally in the early generations of breeding mostly S2 or S3 generations to reduce the genetic load. Populations and inbred lines or single cross hybrids have been used as testers (Aguiar et al., 2008). This mating design continues to be applied in determination of the maize heterotic orientations using different testers (Hallauer et al., 2010; Fato et al., 2012). The design was therefore applied in the current study to evaluate the experimental inbred lines and hybrids in the target environments in Kenya.

1.9 Heterotic orientations in maize

For efficient development of hybrids knowledge of heterotic groups and patterns is essential. A heterotic group is defined as a group of related or unrelated genotypes from the same or different populations that indicate similar combining ability and heterotic response when crossed with genotypes from other genetically diverse germplasm groups. Furthermore, a heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and therefore high hybrid performance in their crosses (Hallauer et al., 1988).

In maize hybrid breeding, the concept of heterotic groups and patterns is basic (Hallauer et al., 1988; Flint-Garcia et al., 2009). Genetic diversity of the maize germplasm is a key consideration in the design of hybrid-oriented breeding program, where preference is given to the choice of heterotic groups and patterns from divergent populations. The more genetically diverse the parent lines selected for crossing for the formation of hybrids, the higher the hybrid vigour or expression of heterosis (Aguiar et al., 2008). Variations in the gene and allelic frequencies in the inbred lines is the basis for the diverse heterotic orientations.

The basis for selection of the best parents into different heterotic groups varies. Some breeders use trait performance (Estakhr et al., 2012), pedigree information and testcross evaluation (Barata et al., 2006), adaptability and grain yield stability (Badu-Apraku et al., 2011). Also various mating designs (Carena et al., 2010), biometrical approaches (Mather et al., 1982) have been used to determine heterotic groups. Both morphological and genetic markers (Wang et al., 2011) have been widely used to determine genetic groups for maize germplasm. Generally, an array of approaches has been applied to simplify separation of parent lines into heterotic groups (Hartings et al., 2008) that are manageable.

The number of heterotic groups depends on the objective of the programme, but it is generally simplified into two groups, namely A and B. Derera (2005), for example, reported at least nine heterotic groups of maize used in breeding programmes in Eastern and Southern Africa. Similarly, in Kenya, there are nine major heterotic maize groups classified according to the maize growing agro-ecological zones (Hassan, 1998). The mid altitude programme has six heterotic groups; Embu 11, Embu 12, Muguga A, Muguga B, Kakamega pool A, and Kakamega pool B (KARI, 1992; KARI, 2000), while the high altitude programme has three heterotic groups; Ecuador 573 and Kitale Synthetic I and II (Hassan, 1998). At CIMMYT heterotic groups have been simplified into three groups, namely A, B and AB, which can affect effective utilization of new inbred lines in the programmes. Good maize inbred lines may be

discarded when effective testers with high discrimination capacity are not used in hybrid oriented programmes. Among other objectives the current study aims to determine whether CIMMYT single cross testers would be effective for discriminating new stem borer resistant inbred lines according to grain yield under stem borer infestation.

1.9.1 Determination of heterotic orientations

Germplasm variation is of primary importance for hybrid breeding and population improvement programs. Characterization of the maize germplasm and its assignment into different heterotic orientations is useful in providing information about the genotypes (Hallauer et al., 2010). Numerous methods have been applied in the allocation of maize lines into different heterotic orientations (Schnable et al., 2013). Heterotic orientations among inbred lines and the best hybrid combinations can be identified using information from several approaches namely: quantitative genetic analysis; testcrosses to testers; pedigree information; morphological traits; and molecular markers (Fato et al., 2012; Sanghera et al., 2012; Liberatore et al., 2013). Quantitative genetic analysis based methods depend on gene frequency variations among the parental genotypes used in the crossing. Variations in the genetic structure determine the relationships among heterotic orientations of germplasm. Inbred lines are assigned to different groups' relative heterosis to the mean of the testers or based on the SCA estimates. Based on the heterosis data, lines that display significant heterosis in their crosses are allocated to the opposite groups.

Clustering of lines into heterotic groups depends on the direction of the specific combining ability such that lines exhibiting positive SCA with tester are allocated to the opposite heterotic group, and vice versa, whereas lines displaying positive SCA to both testers are designated as both groups (Hallauer et al., 2010; Fato et al., 2012; Sanghera et al., 2012). In the literature, the SCA effects based classification is considered to be more reliable because they have better predictive value for F1 grain yield than heterosis based classification (Aguiar et al., 2008; Hallauer et al., 2010; Fato et al., 2012; Sanghera et al., 2012). The heterosis based grouping is subject to environmental effects which might mask expression of heterosis or that heterosis changes from one site to another due to genotype x environment interactions.

Applications of molecular markers are a more powerful tool to discriminate heterotic orientations and to allocate inbred lines into current heterotic groups and for diversity analysis (Aguiar et al., 2008) because the markers are not affected by genotype x environment interactions. It is reported in the

literature that a combination of various approaches in the allocation of inbred lines into dissimilar heterotic orientations is more meaningful than a single method (Aguiar et al., 2008). In the current study, both SCA effects based classification and heterosis based grouping were applied in designation of genotypes to their different heterotic orientation.

1.10 Methods of screening maize germplasm for resistance to stem borers

Successful screening of maize materials for selection or evaluation requires normal vigorous plants. Plants exposed to different stress conditions (drought, salinity, heat, low soil fertility etc) may obscure the expression of resistance or plants may be 'escapes' which contributes to low heritability or low repeatability for insect resistance in maize. Heterosis or different maturity groups may also determine screening methodology for comparison. The use of local resistant and susceptible checks may help in determining the threshold of comparison of maize test genotypes.

1.10.1 Screening methods and rating

Artificial infestation is the most effective manner for screening maize germplasm. However, the larval colonies used especially the insectary-reared stem borer larvae and egg masses, should be vigorous and survive to cause feeding damage to the test genotypes under field conditions. Infestation should be carried out mid-morning or in the late afternoon to limit desiccation of larvae. Consistency for the number of insect larvae per plant used for infestation is most critical in discriminating the test genotypes. Factors, such as plant vigour, plant age, temperature and relative humidity may influence the observations on the test genotypes (Ajala et al., 2010; Tefera et al., 2010). In maize screening for resistance to stem borers, the level of plant damage on leaves is used in the rating. Mostly, the visual rating scale system is used (see Table 1.3).

Table 1.3. Scale for scoring stem borer damage from seedling to whorl-stage in maize

Numerical scores	Visual ratings of plant damage	Reaction to resistance
0	No damage	Probable escape
1	Few pin holes	Highly resistant
2	Few shot holes on a few leaves	Resistant
3	Several shot holes on leaves (<50%)	Resistant
4	Several shot holes on leaves (>50%) or small lesions (<2cm long)	Moderately resistant
5	Elongated lesions (>2cm long) on a few leaves	Moderately resistant
6	Elongated lesions on several leaves	Susceptible
7	Several leaves with long lesions with leaf tattering	Susceptible
8	Several leaves with long lesions with severe leaf tattering	Highly susceptible
9	Plant dying due to death of growing points (dead-hearts)	Extensively sensitive to damage

Source: Adapted and modified from CIMMYT (1989).

There are two methods of infestation with stem borers namely; natural and artificial. Natural infestation is the use of hotspot areas where the pest incidence is very high and mostly coincides with the critical stage of crop growth. Uniformity in the distribution of the infestation is challenging due to lack of stable pest populations over seasons, and the possibility of test genotypes being 'escapes' or be over infested. In contrast, artificial infestation is the most reliable and most effective method of screening maize germplasm. Through artificial infestation consistency is achieved since each test plant is infested with at least 20 first instar larvae or neonates or egg masses at the whorl stage 14 days after planting. Infestation may be carried out manually using camel hair brushes or through the use the bazooka applicator for large-scale testing (Tefera et al., 2010).

1.10.1.1 Leaf disk bioassays method

Breeding methods for resistance to borer damage requires reliable screening approaches. However, quick screening methods for maize genotypes for stem borer resistance are limited. Currently, screening involves splitting of stalks for measurement of cumulative tunneling, counting the number of exit holes and dead hearts, which are time consuming and labour intensive, therefore, the need to optimize a detached leaf bioassay screening method in the greenhouse and laboratory is essential. The use of isolated leaf bioassays for artificial screening of maize genotypes for stem borer resistance may

be a practical alternative method than the splitting of stalks for measurements and counts. Natural infestation may not be reliable due to lack of uniformity and seasonal variations that occur (Tefera et al., 2010). The use of artificial infestation in a controlled environment allows multiple screenings within a short time. Leaf screening bioassays have been used as rapid methods for screening materials in a wide range of horticultural and agronomic crops against pests and diseases including Bt maize trials (Mugo et al., 2001; Murenga et al., 2011; González et al., 2013). However this has not been tested for its efficacy in discriminating genotypes for stem borer resistance in maize breeding. Therefore the current study, aimed at appraising this approach against traditional screening methods with a view to lower costs and increase speed, and heritability in breeding maize for stem borer resistance.

1.10.3 Selection indices

Selection indices are multivariate techniques that combine information of different traits of agronomic interest with the genetic properties of a population. In the application of selection indices, numerical values are weighted and serve as an additional hypothetical trait resulting from a combination of various traits of interest (Mutinda et al., 2013). Selection for resistance to stem borers*, B. fusca* and *C. partellus* based on a single parameter is difficult since a resistant genotype has a certain aspect of damage that may be susceptible to another form or when pressure is increased. Trait interactions associated with a reduction in the amount of grain yield include: leaf feeding damage, dead hearts tunneling and exit holes. Appropriate indices, are useful in assisting breeders for concurrent selection for resistance *per se*, in addition to grain yield performance. Various examples in the applications of selection indices with improvements in stem borer resistance and grain yield in maize have been reported in the literature (Ajala et al., 2010).

1.10.4 Genotype x Environment Interactions

Genotypes x environment interactions are of considerable influence to response to selection and efficiency of resistance breeding programmes (Butrón et al., 2004). There are two types of genotype x environment interactions namely; cross over and non-cross over interactions which affect genotype performance and crop improvement. The cross over type exemplifies the instability of genotype performance (Hallauer et al., 2010). The cross over type limits breeding progress due the alterations in constitution of selection at every environment and represents the genotypes' specific adaptation across environments. However, non-crossover type represents stability of performance across the unfavourable environments, where cultivars are ranked consistently across environments resulting in

analogous selection in all environments. The capacity of the new testcross hybrids to produce higher grain yield may be attributed to their ability to adapt to the biotic or abiotic stress conditions (Butrón et al., 2004; Carena et al., 2010). In the current study, experiments were set up in different mega environments because the genotype x environment interactions were an important consideration; because insect infestation also depends on whether favourable conditions prevail for insect feeding, fertility and development.

1.11 Conclusions from the literature review

From the review of literature, the two stem borers, *B. fusca* and *C. partellus* are identified as one of the most devastating insect pests limiting maize production in tropical environments. Suitable maize germplasm should have resistance to *B. fusca* and *C. partellus* borers where they occur. There is a need to breed and promote genotypes with *B. fusca* resistance, and to encourage wide adoption across maize agro-ecologies of the competitive hybrids with *B. fusca* resistance. Breeding for resistance to *Busseola fusca* and *Chilo partellus* requires a good understanding of heritability of resistance, gene action, and combining ability effects in relation to heterosis among the testcrosses. The S1 progeny recurrent selection was considered relevant for the current study and useful given the low heritability of the polygenic traits that constitute stem borer resistance, because a larger portion of additive genetic variance would contribute towards breeding progress. The line x tester mating scheme using single cross testers was preferred since in generating information on gene action, the products formed from the testcrosses would be three way crosses which will be deployed immediately into the national performance trials for further testing. The majority of the farmers in SSA use three way cross products since the cost of seed is less compared to that of single crosses. Screening for resistance to stem borers is an important component of breeding for resistance. Quick screening methods for borer resistance should be found because current approaches are time consuming and labour intensive. The use of detached leaf disk bioassay and whole plant assays methods for screening for *B. fusca* and *C. partellus* resistance maize in the laboratory and greenhouse trials would provide a rapid technique that would enable breeders to screen and make decisions faster towards breeding progress. Therefore the need to carry out an appraisal of the leaf disk bioassay and whole plant assays in both the greenhouse and laboratory.

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Chapter 2

Evaluation of tropical maize inbred lines for resistance to two stem borers, *Busseola fusca* and *Chilo partellus*

Abstract

Stem borers, *Busseola fusca* and *Chilo partellus*, are serious insect pests of maize. However, the response of genotypes showing exclusive resistance to each of these borers, and with combined resistance to both has not been studied in maize breeding programmes in Kenya. The objective of this study was to evaluate tropical maize inbred lines for resistance to two stem borers *B. fusca* and *C. partellus*. One hundred and twelve maize inbred lines were artificially screened in three locations in Kenya. Each inbred line was sown in one row of 6.75 m, divided into three parts namely, *B. fusca* and *C. partellus* larvae infested on either side, while the middle part was protected using the insecticide *beta cyfluthrin 25g/L.* Data was collected on leaf feeding damage rating, cumulative stem tunnel length, tunnel length to plant height ratio, number of exit holes, number of dead-hearts, and stalk strength and selected agronomic traits. Data were analyzed using PROC GLM of SAS statistical package. There were significant differences among the test genotypes, ($p<0.01$) for resistance to *B. fusca* and *C. partellus,* for all the traits measured. Leaf damage scores, cumulative stem tunnel length and number of exit holes were the most effective parameters in discriminating the test genotypes for resistance to the two borers. Twenty one entries showed resistance to both *B. fusca and C. partellus* in at least two sites, and only four entries showed resistance to both species across the locations. Among all the test genotypes, twenty-six entries showed resistance to *C. partellus* only, while five entries had resistance to *B. fusca* only. Furthermore, 84 and 28 entries showed susceptibility to *B. fusca* and *C. partellus* respectively. The remainder were categorized as either moderately resistant or moderately susceptible to either *B. fusca* or *C. partellus*. The results showed that most of the test genotypes were susceptible to *B. fusca* and less so to *C. partellus*. The observed responses to *B. fusca* and *C. partellus* stem borers showed that the maize genotypes identified with resistance may be used as parents in hybrid breeding programmes that emphasize stem borer resistance.

Keywords: *Busseola fusca*, *Chilo partellus*, combined resistance, tropical maize

2.1 Introduction

In sub-Saharan Africa (SSA), maize is the key food crop grown by a vast majority of rural households. In the region, maize is both a subsistence and a commercial crop for both small and large-scale farmers (Brooks et al., 2009; Sasson, 2012). Plant breeding in combination with other tools has led to the formation of new maize varieties with tolerance to biotic and abiotic stresses and with better agronomic traits. The spotted stem borer (*Chilo partellus* Swinhoe) and the African stem borer (*Busseola fusca* Fuller), are among the most damaging insect pests that greatly reduce maize grain yield in tropical environments. However, there is limited available germplasm with resistance to these insect pests in most maize breeding programmes in the tropical environments, including combined resistance for both pests where they both occur. The two pests cause up to 13.5% yield losses in maize in Kenya. The distribution and occurrence of *B. fusca* and *C. partellus* stem borers in different locations and crop ecosystems is diverse (Mailafiya et al., 2009). The environments in SSA are favourable for insect development and accelerate the formation of numerous generations of the insect pests per season leading to severe crop yield losses (Mailafiya et al., 2011). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tons or about $72 million (De Groote et al., 2003; De Groote et al., 2005). This amount represents an average of 13.54% of the farmers' total annual harvest of maize.

Several options for managing maize stem borers have potential to mitigate their damaging effects, but each option has its own limitations. Host plant resistance forms an important part of integrated pest management as it provides inherent control without environmental issues and is compatible with other pest management approaches (Singh et al., 2012). Effective breeding methods for resistance to borer damage could therefore be designed by plant breeders using both improved and new sources of stem borer resistance.

Development of effective methods requires a better understanding of the genetic basis of the resistances among the germplasm used. Suitable maize germplasm should have resistance to both *B. fusca* and *C. partellus*. Recent reports indicate that climate change has led to *C. partellus* increasingly displacing *B. fusca* from the high altitude areas in Kenya (Mailafiya et al., 2011; Tefera et al., 2011). Furthermore, farmers exchange maize germplasm across agro-ecologies, therefore the need to investigate the reaction of these tropical maize inbred lines for resistance to these borers becomes paramount. The objective of this study was to evaluate tropical maize inbred lines for resistance to two *B. fusca and C. partellus* stem borers.

2.2 Materials and Methods

2.2.1 Germplasm

One hundred and twelve (112) maize inbred lines used in the study were sourced from the International Maize and Wheat Improvement Center (CIMMYT), Mexico and the Kenya Agricultural Research Institute (KARI) breeding programmes. Two elite but stem borer resistant and susceptible maize lines from CIMMYT and KARI were included as checks. These maize inbred lines have not been tested for resistance to *B. fusca* and *C. partellus* stem borers. The lines were developed from recombination and recurrent selection in multiple borer resistance (MBR) populations under artificial infestation with Southern corn borer (SWCB), sugarcane borer (SCB), (*Diatrae sacharalis*), European corn borer (ECB), *Ostrinia nubilalis* and fall armyworm (FAW), (*Spodoptera* spp) in various locations globally (Smith et al., 1989). The full list of maize inbred lines used in the study and their pedigree information is presented in Appendix 2, Table 2.1.

2.2.2 Experimental sites

Experiments were established at Kakamega, Kiboko, and Embu locations in Kenya (Figure 2.1). KARI Kakamega ($37^0$75'E 2^0 15'S, 1585m asl) centre is located in the moist transitional mid altitude agro-ecological zone of western Kenya and experiences mean annual temperatures of 25^oC. Kakamega lies within a high potential agro-ecological zone and receives a bimodal mean annual rainfall of approximately 1850 to 1916 mm. The soils in Kakamega are well drained, moderately deep to very deep, red to dark in colour and in some places shallow over petroplinthite (Jaetzold et al., 1982).

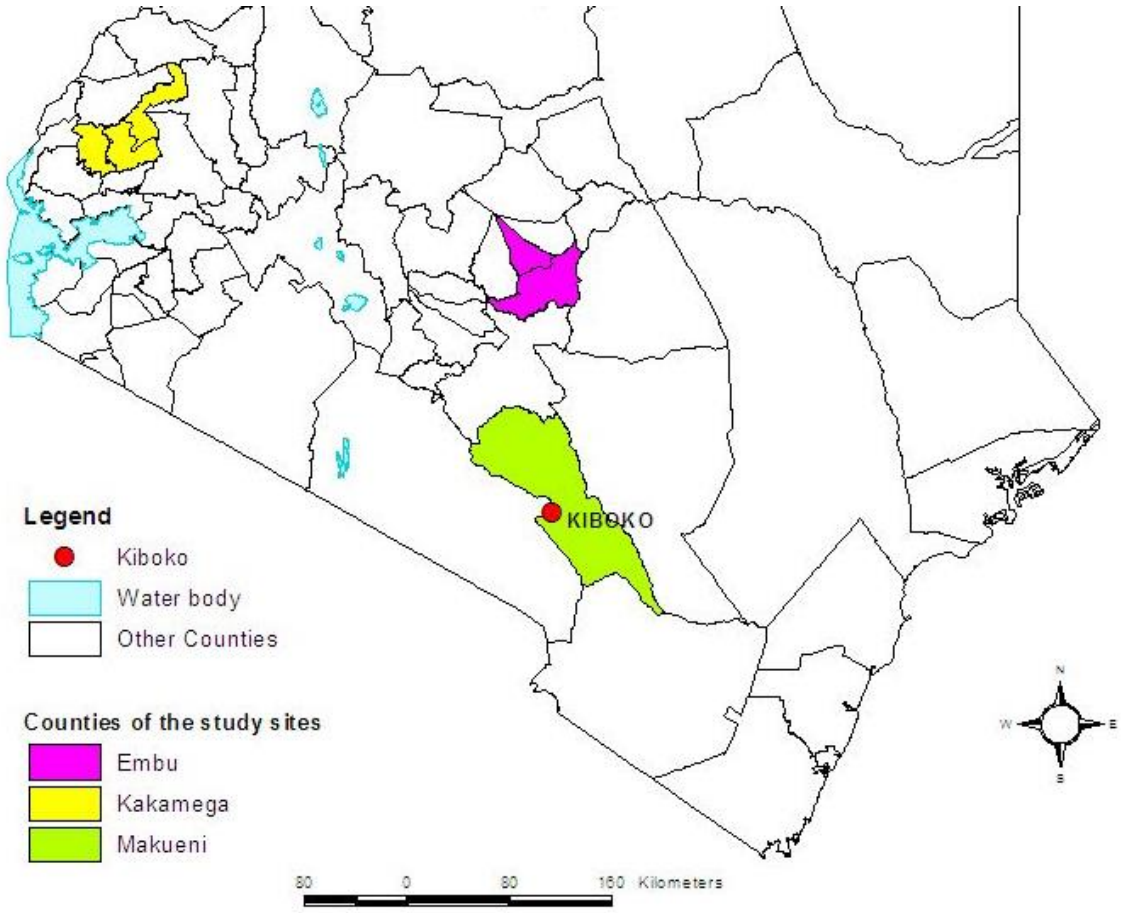

Figure 2.1. Map of Kenya showing the locations Embu, Kakamega and Kiboko

Source: KARI Land Resources and Analytical Services, 2013

KARI-Kiboko (2°15'S 37°75' E, 975 m asl) is located in the dry mid altitude agro-ecological zone of eastern Kenya and experiences mean annual temperature ranges of 28 to 37^{o}C, with February and October being the hottest months. Kiboko receives a mean annual rainfall of approximately 530 mm. The soils are well drained, Fluvisols, Ferralsols, and Luvisols with soil pH of about 7.9 (Jaetzold et al., 1982; KARI Land Resources and Analytical Services, 2007).

KARI-Embu centre (03°56' 44'S and 39°46' 00'E, 1510 m asl) is located in the moist transitional mid altitude agro-ecological zone of eastern slopes of Mt. Kenya and experiences mean annual temperature ranges of 14-25°C. Embu lies within a high potential agro-ecological zone. Rainfall received is bi-modal ranging between 800-1400mm annually. The soils are deep (about 2 m); well weathered Humic Nitisols with moderate to high inherent fertility (Jaetzold et al., 1982).

2.2.3 Experimental design and treatments

The maize inbred lines were evaluated in a 28 x 4 α-lattice design with three replications in each location. Each inbred line was sown in one row plot of 6.75 m each per replication (Figure 2.2). Two seeds were sown per hill and later thinned to one. Each plot consisted of one row with inter-row spacing of 0.75 m and inter-hills spacing of 0.25 m within the rows.

Fertilizers were applied to give 60kg N and 60kg P_2O_5 ha^{-1} as recommended for each location. Nitrogen was applied in two splits, while supplementary irrigation was applied when needed. The fields were kept free of weeds by hand weeding throughout the growth cycle.

2.2.3.1 Artificial infestation with insects

Each 6.75m plot was divided into three parts namely, *B. fusca* and *C. partellus* infested on either side of the plot at Embu and Kakamega, while the middle part was protected using insecticide Bulldock® (active ingredient, *beta cyfluthrin 25g/L*) (Figure 2.2). At Kiboko, 5 m row plots were used, and were infested with *C. partellus* on one half of the plot while the remaining part was protected using the insecticide. Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and the Kenya Agricultural Research Institute at Katumani stem borer insect pests mass rearing facility (Tefera et al., 2010; Tefera et al., 2011). Plants were artificially infested in a controlled and uniform manner with the respective stem borer species (Figure 2.2) by placing ten larvae in the maize whorl using a camel brush at two weeks after planting.

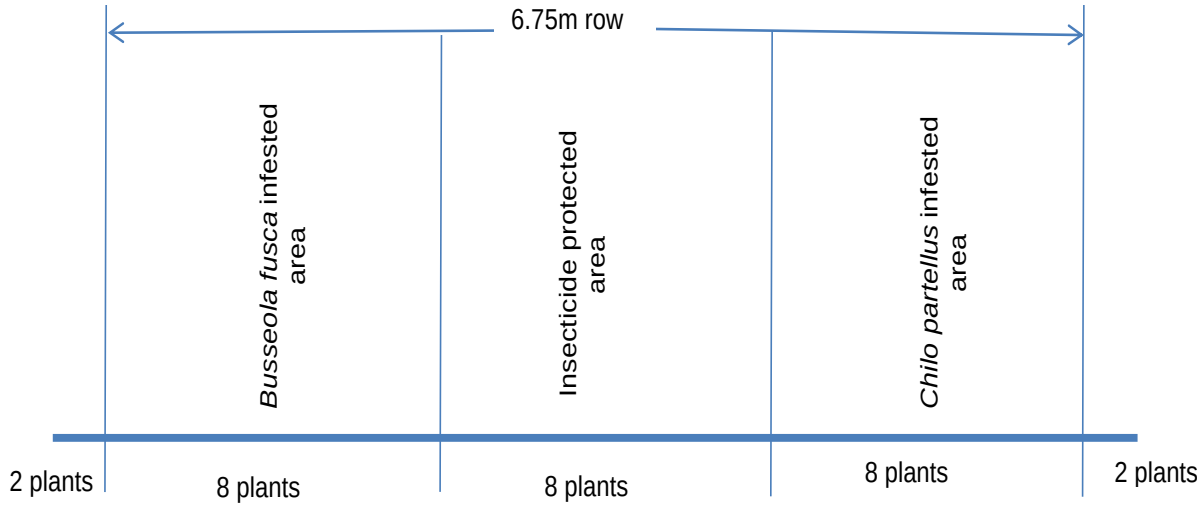

Figure 2.2. Schematic presentation of rows and treatments at Embu and Kakamega

2.2.4 Data collection and analysis

Plants were evaluated for leaf damage scores using a scale of 1 (resistant) to 9 (susceptible) (Table 2.1) (CIMMYT, 1989) at the V3 stage of maize growth.

Table 2.1. Scale for scoring stem borer leaf damage from seedling to whorl-stage in maize

Numerical scores	Visual ratings of plant damage	Reaction to resistance
0	No damage	Probable escape
1	Few pin holes	Highly resistant
2	Few shot holes on a few leaves	Resistant
3	Several shot holes on leaves (<50%)	Resistant
4	Several shot holes on leaves (>50%) or small lesions (<2 cm long)	Moderately resistant
5	Elongated lesions (>2 cm long) on a few leaves	Moderately resistant
6	Elongated lesions on several leaves	Susceptible
7	Several leaves with long lesions with leaf tattering	Susceptible
8	Several leaves with long lesions with severe leaf tattering	Highly susceptible
9	Plant dying due to death of growing points (dead-hearts)	Extensively sensitive to damage

Source: Adapted and modified from CIMMYT (1989).

Other plant damage parameters were measured at harvest namely; cumulative tunnel length (measured as the total length of tunneling along the maize stalk), tunnel length to plant height ratio, number of exit holes, number of dead-hearts, stalk strength, and number of larvae recovered per plant. Agronomic traits were measured following standard protocols used at CIMMYT (CIMMYT, 1989).

The traits measured were; number of days to anthesis, and to silking; plant height (cm); ear height (cm); ear position (ratio of plant height to ear placement); number of ears harvested; stem and root lodging; grain weight (Kg) and moisture content (%) at harvest; plant stand (number of plants per row at harvest); number of rotten ears; plant and ear aspect (where 1= good and uniform plants/ears with the stature, colour and strength preferred in the area, 5=ugly plants/ears with the undesirable features in the area); stem diameter (measurement across the stalk) (cm); internode length (four below the upper-most ear), and leaf damage. A rank summation index (RSI) was constructed to determine the ranking of each line within the population for suitable response. The index was obtained by the sum of the means of each of the leaf feeding damage score; number of dead-hearts; number of exit holes; and cumulative stem tunnel length for each line, to get its mean performance compared with other lines within the same population. An entry with the least value was ranked higher for the resistance traits. The rank selection index (Mulamba et al., 1978; Mutinda et al., 2013) was determined as follows;

$$RSI=\sum Ri's$$

Where Ri is the rank of mean of each of the desired traits. Rank summation index is the mean performance of each of the desired traits of each genotype using the ranking of leaf feeding damage score, number of dead-hearts, number of exit holes, and cumulative stem tunnel length.

Least square means for insect damage parameters and agronomic traits were calculated using plot data for each location separately. All analysis of variance using PROC GLM of SAS was performed for individual as well as for combined environments, considering environments as random effects and genotypes as fixed effects(SAS Institute. Inc., 2012). Genotypic and phenotypic correlation coefficients were determined using variance-covariance matrix and estimates of genotypic and phenotypic variances (Falconer et al., 1996).

Genotypic correlation was calculated as follows;

$$r_{G} = \sigma_{G\,(X,\,Y)} / \sqrt{\sigma^2_{G(X)}, \sigma^2_{G(Y)}}$$

Where r_G is the genetic correlation between traits X and Y, $\sigma_{G\,(X,\,Y)}$ is the genotypic covariance between trait X and Y, and $\sigma^2_{G(X)}$ is the genotypic variance of trait X and the $\sigma^2_{G(Y)}$ is the genotypic variance of trait Y.

Phenotypic correlation was calculated as follows;

$$r_{P} = \sigma_{P\,(X,\,Y)} / \sqrt{\sigma^2_{P(X)}, \sigma^2_{P(Y)}}$$

Where r_P is the phenotypic correlation between traits X and Y, $\sigma_{P\ (X,\ Y)}$ is the phenotypic covariance between trait X and Y, and $\sigma_{P\ (X)}$ is the genotypic variance of trait X and the $\sigma^2_{P(Y)}$ is the phenotypic variance of trait Y.

Correlation coefficients based on plant damage and some agronomic traits for *B. fusca* and *C. partellus* were also computed. Broad-sense heritability was estimated using the following formulae; H^2=Vg/Vp, where Vg is the genotypic variance while the Vp is the phenotypic variance.

2.3 Results

2.3.1 Mean performance of maize inbred lines

There were highly significant differences for resistance to both *B. fusca* and *C. partellus* (p≤0.01). At Embu and Kakamega; significant differences were observed for leaf feeding damage (p≤0.01), number of exit holes (p≤0.03 to 0.04), and number of dead hearts (p≤0.01) for *B. fusca and C. partellus* (Table 2.1 and 2.3), except for cumulative stem tunnel length for both sites. The first instar larvae of *B. fusca* feed and produce a distinguishing pattern of small holes where leaf tissues have been consumed. However, larvae of *C. partellus* feeding damage occur as series of small pin holes on leaves along the leaves (Figure 2.3).

Figure 2.3. Differences in the leaf feeding damage patterns for *B. fusca* (*left*) and *C. partellus* (*right*)

At Embu and Kakamega; the genotypic variances ranged from 0.01 to 0.36, while the environmental variance ranged from 0.02 to 0.59 for traits for all sites under *B. fusca* and *C. partellus* infestation (Table 2.2 and 2.3). The rank summation index ranged from 0.47 to 2.96. Mean performance of individual entries at Embu and Kakamega where plot infestation was carried out using *B. fusca* and *C. partellus* showed a wide range for dead hearts (0 to 3.31), leaf feeding damage (1 to 6.76)

(Figure 2.4), number of exit holes (0 to 11.4) (Figure 2.5), and cumulative tunnel length (0.08 to 5.48 cm) (Figure 2.6). The rank summation indices ranged from 0.75 to 2.57) for the top *B. fusca* resistant inbred lines at both Embu and Kakamega. There was a varied range for heritability estimates (0.18 to 0.58) for all traits among *B. fusca* resistant inbred lines (Table 2.4 and 2.5). The estimates for means for individual entries for *C. partellus* treatment, in Embu and Kakamega, indicated a wide range for dead hearts (0-1.33), number of exit holes (0.1 to 6.93), leaf feeding damage (1.4 to 6.65), and cumulative stem tunneling (0 to 2.18 cm). The rank summation index ranged 0.45 to 7.12 for the top *C. partellus* resistant inbred lines in Embu and Kakamega. There was a varied range for heritability estimates (0.11 to 0.78) for all traits among *C. partellus* resistant inbred lines (Table 2.4 and 2.5).

Similarly, at Kiboko where there was exclusive *C. partellus* treatment, there were significant differences for leaf feeding damage ($p \leq 0.01$), number of exit holes ($p \leq 0.05$), and cumulative stem tunnel length (0.02), except for number of dead hearts ($p \leq 0.009$ to 0.01) for *C. partellus* (Table 2.6). The genotypic variances ranged from 0.05 to 0.24, while the environmental variance ranged from 0.02 to 5.59 for all traits (Table 2.6). The rank summation index ranged from 0.47 to 2.96. There was a varied range for heritability estimates (0.18 to 0.69) for all traits among *C. partellus* resistant inbred lines (Table 2.6).

There were *C. partellus* only resistant entries at Embu (8), Kiboko (9), and Kakamega (4), and 6 each for *B. fusca* only resistant entries at Embu and Kakamega. Twenty one entries showed combined resistance to both *B. fusca* and *C. partellus* in at least two sites. Entries CKSBL10026, CKSBL10028, CKSBL10040, and CKSPL10028 showed resistance to both species across sites (Table 2.7). Out of the 112 test entries, 28 were categorized as susceptible since they showed a RSI score of above 5.1 (Mulamba et al., 1978; CIMMYT, 1989). Most of the CML lines showed susceptibility to both pests. Inbred line CML395, the susceptible check showed the highest damage besides the highest rank summation index for all traits in all sites (Table 2.7, Figures 2.7 and 2.8).

Table 2.2. Mean performance of top 19 maize inbred lines for selected stem borer resistance traits under *B. fusca* infestation at Embu (averaged over two seasons)

Entry	Genotype	No. of dead hearts	No. of exit holes	stem borer leaf damage scores (1-9)	cumulative tunnel length (cm)	Rank Selection Index	Rank
91	CKSBL10040	0.01	1.20	1.69	0.11	0.75	1
90	CKSBL10045	0.01	0.80	2.20	0.08	0.77	2
85	CKSBL10039	0.03	3.50	1.67	0.98	1.55	3
82	CKSBL10042	0.02	2.40	2.32	0.44	1.30	4
81	CKSBL10038	0.02	6.90	1.44	0.87	2.31	5
16	CKSBL10206	0.02	4.70	2.52	0.16	1.85	6
10	CKSBL10026	0.28	8.80	2.25	0.12	2.86	7
61	CKSPL10090	0.03	8.10	1.63	1.00	2.69	8
73	CKSBL10016	0.19	6.50	2.11	0.92	2.43	9
75	CKSBL10028	0.08	3.10	2.46	0.82	1.62	10
41	CKSBL10157	0.00	5.10	2.02	1.12	2.06	11
13	CKSBL10203	0.00	0.00	2.31	0.79	0.78	12
70	CKSBL10013	0.02	7.90	1.80	1.23	2.74	13
24	CKSBL10165	0.03	3.50	1.96	1.32	1.70	14
95	CML312	0.02	11.40	2.33	0.60	3.59	15
21	CKSBL10213	0.00	10.40	2.13	0.86	3.35	16
65	CKSPL10229	0.07	5.10	2.12	1.22	2.13	17
49	CKSPL10028	0.02	8.30	2.50	0.76	2.90	18
63	CKSPL10146	0.75	6.90	1.97	0.66	2.57	19
96	**CML395 (sus. check)**	**3.05**	**8.71**	**6.76**	**5.48**	**6.00**	**92**
	Genotype Variance	0.01	0.05	0.18	0.22		
	Residual Variance	0.06	0.31	0.39	3.06		
	Grand Mean	0.21	4.90	2.55	2.08		
	LSD	0.42	0.99	1.13	3.12		
	CV	23.65	28.69	22.43	25.75		
	Heritability	0.21	0.32	0.58	0.18		
	P-value	0.01	0.03	<0.0001	0.16		

sus. check-susceptible check

Table 2.3. Mean performance of top 18 maize inbred lines for selected stem borer resistance traits under *B. fusca* infestation at Kakamega (averaged over two seasons)

Entry	Genotype	No. of dead hearts	No. of exit holes	stem borer leaf damage scores (1-9)	cumulative tunnel length (cm)	Rank Selection Index	Rank
22	CKSBL10250	0.07	2.22	1.53	0.57	1.10	1
79	CKSBL10043	0.87	2.15	2.38	0.5	1.48	2
90	CKSBL10045	0.24	2.55	2.24	0.42	1.36	3
80	CKSBL10035	0.01	3.39	1.91	0.06	1.34	4
25	CKSBL10169	0.02	2.91	3.12	0.55	1.65	5
75	CKSBL10028	0.08	3.51	1.83	0.15	1.39	6
91	CKSBL10040	0.01	4.18	1.26	0.05	1.38	7
85	CKSBL10039	0.05	5.13	1.13	0.04	1.59	8
49	CKSPL10028	0.01	5.33	1.53	0.03	1.73	9
56	CKSPL10081	0.02	5.53	1.82	0.04	1.85	10
100	CKSBL10026	0.28	5.68	0.96	0.2	1.78	11
29	CKSBL10286	0.03	5.61	2.02	0.02	1.92	12
7	CKSBL10194	0.03	5.61	2.08	0.45	2.04	13
38	CKSBL10321	0.00	5.15	2.35	2.27	2.44	14
92	CML264	0.01	5.8	1.93	0.78	2.13	15
111	CML489	0.07	7.05	1.16	0.23	2.13	16
95	CML312	0.02	7.03	1.52	0.4	2.24	17
60	CKSPL10089	0.25	6.96	2.14	0.64	2.50	18
102	CML334	0.33	9.08	1.38	0.13	2.73	89
96	**CML395 (susceptible check)**	**3.31**	**8.3**	**4.03**	**0.21**	**3.96**	**102**
	Genotypic variance	0.01	0.27	0.06	0.36		
	Residual variance	0.06	33.14	0.26	1.52		
	Grand Mean	0.07	6.73	2.55	2.08		
	LSD	0.42	0.99	1.13	3.12		
	CV	25.65	23.69	22.43	25.73		
	Heritability	0.41	0.35	0.58	0.28		
	P-value	0.009	0.04	<0.0001	0.36		

Table 2.4. Mean performance of top maize inbred lines for selected stem borer resistance traits under *C. partellus* infestation at Embu (averaged over two seasons)

Entry	Genotype	No. of dead hearts	No. of exit holes	stem borer leaf damage scores (1-9)	cumulative tunnel length (cm)	Rank Selection Index	Rank
100	CKSBL10026	1.33	1.98	1.72	0.20	0.45	1
91	CKSBL10040	0.00	0.30	1.72	0.05	0.47	2
49	CKSPL10028	1.32	1.65	1.80	0.04	0.48	3
73	CKSBL10016	0.02	0.93	2.16	0.25	0.48	4
90	CKSBL10045	0.33	0.57	2.17	0.39	0.68	5
97	CKSBL10001	0.01	1.02	2.19	0.27	0.68	6
79	CKSBL10043	1.28	3.66	2.20	0.50	0.7	7
41	CKSBL10157	0.21	2.04	2.21	0.02	0.74	8
82	CKSBL10042	0.01	1.20	2.39	0.27	0.75	9
25	CKSBL10169	0.03	2.52	2.46	0.09	0.76	10
80	CKSBL10035	0.07	2.25	2.54	0.1	0.78	11
109	LPSC7-F86-3-1-1-1-BB-#-B-B	0.34	2.28	2.58	0.02	0.81	12
70	CKSBL10013	0.03	4.86	2.59	0.23	0.83	13
9	CKSBL10197	0.03	6.12	2.60	0.07	0.84	14
13	CKSBL10203	0.01	2.61	2.60	0.52	0.85	15
81	CKSBL10038	0.38	6.84	2.78	0.10	0.86	16
101	CML444	0.01	6.93	2.97	0.02	0.88	17
53	CKSPL10070	0.01	5.22	3.23	0.03	0.89	18
93	CML202	0.36	4.50	6.51	0.09	0.89	19
96	**CML395 (susceptible check)**	**1.02**	**6.21**	**6.65**	**0.23**	**3.75**	**90**
	Genotype Variance	0.05	0.08	0.29	0.36		
	Residual Variance	0.25	0.50	0.31	1.52		
	Grand Mean	0.26	1.09	3.23	0.79		
	LSD	0.85	1.27	1.1	2.07		
	CV	24.14	28.58	17.17	23.14		
	Heritability	0.38	0.31	0.74	0.41		
	P-value	0.49	0.001	<0.0001	0.01		

Table 2.5. Mean performance of top maize inbred lines for selected stem borer resistance traits under *C. partellus* infestation at Kakamega (averaged over two seasons)

Entry	Genotype	No. of dead hearts	No. of exit holes	stem borer leaf damage scores (1-9)	cumulative tunnel length (cm)	Rank Selection Index	Rank
100	CKSBL10026	0.07	0.17	2.75	0.59	3.58	1
91	CKSBL10040	0.03	0.31	3.26	0.14	3.74	2
80	CKSBL10035	0.01	1.09	2.46	0.24	3.80	3
79	CKSBL10043	0.31	1.34	2.16	0.14	3.95	4
4	CKSBL10073	0.31	1.38	2.16	0.14	3.99	5
12	CKSBL10200	0.23	1.29	2.03	0.67	4.22	6
47	CKSPL10280	0.04	1.25	3.03	0.06	4.38	7
49	CKSPL10028	0.01	0.74	3.00	0.85	4.60	8
45	CKSPL10256	0.70	1.52	2.57	0.00	4.79	9
38	CKSBL10321	1.32	1.79	1.80	0.04	4.95	10
37	CKSBL10155	0.03	1.16	3.31	0.47	4.97	11
56	CKSPL10081	0.30	1.32	2.98	0.43	5.03	12
5	CKSBL10107	0.74	1.61	2.61	0.14	5.10	13
83	CKSBL10008	1.04	1.65	2.41	0.03	5.13	14
60	CKSPL10089	0.34	1.40	2.37	1.07	5.18	15
15	CKSBL10205	0.68	1.50	3.02	0.00	5.20	16
6	CKSBL10195	0.66	1.42	2.00	1.23	5.31	17
92	CML264	1.28	1.71	2.85	0.19	6.03	18
48	CKSPL10309	0.68	1.43	2.66	2.18	6.95	19
96	**CML395 (susceptible check)**	**0.99**	**1.62**	**3.47**	**1.04**	**7.12**	**86**
	Genotype Variance	0.05	0.09	0.05	0.05		
	Residual Variance	0.25	17.72	0.38	1.19		
	Grand Mean	0.26	4.54	2.3	0.77		
	LSD	0.85	8.72	1.12	1.85		
	CV	19.14	19.53	24.58	20.67		
	Heritability	0.58	0.78	0.65	0.11		
	P-value	0.50	<0.0001	<0.0001	0.27		

Table 2.6. Mean performance of top maize inbred lines for selected stem borer resistance traits under *C. partellus* infestation at Kiboko (averaged over two seasons)

Entry	Genotype	No. of dead hearts	No. of exit holes	stem borer leaf damage scores (1-9)	cumulative tunnel length (cm)	Rank Selection Index	Rank
91	CKSBL10040	0.00	0.10	2.00	0.11	0.55	1
82	CKSBL10042	0.01	0.29	2.08	0.79	0.79	2
90	CKSBL10045	0.01	0.40	1.40	0.44	0.56	3
49	CKSPL10028	0.01	0.58	1.52	1.88	1.00	4
79	CKSBL10043	0.01	0.77	1.79	1.48	1.01	5
4	CKSBL10073	0.02	0.31	1.76	0.92	0.75	6
13	CKSBL10203	0.02	0.90	2.12	0.89	0.98	7
81	CKSBL10038	0.03	0.54	2.01	1.23	0.95	8
85	CKSBL10039	0.03	0.65	2.31	1.00	1.00	9
80	CKSBL10035	0.03	0.84	1.49	1.27	0.91	10
99	CKSBL10004	0.04	0.49	1.97	1.32	0.96	11
70	CKSBL10013	0.05	0.84	1.49	1.22	0.90	12
24	CKSBL10165	0.06	0.55	1.68	0.66	0.74	13
32	CKSBL10178	0.06	0.86	1.54	1.56	1.01	14
53	CKSPL10070	0.21	0.33	1.99	0.16	0.67	15
7	CKSBL10194	0.31	0.53	1.73	0.98	0.89	16
111	CML489	0.33	0.19	1.87	0.08	0.62	17
103	CML254	0.38	0.76	1.49	0.87	0.88	18
61	CKSPL10090	0.89	1.46	2.69	2.29	1.83	19
96	**CML395 (susceptible check)**	**1.02**	**6.21**	**6.65**	**0.23**	**3.53**	**95**
	Genotype Variance	0.05	0.08	0.24	0.22		
	Residual Variance	0.25	0.5	0.33	3.06		
	Grand Mean	0.26	1.09	2.29	2.08		
	LSD	0.85	1.27	1.01	3.12		
	CV	27.14	28.35	20.16	35.73		
	Heritability	0.38	0.34	0.69	0.18		
	P-value	0.16	0.05	0.01	0.02		

Table 2.7. Distribution of maize inbred lines for resistance to under *B. fusca* and *C. partellus* infestation at Embu, Kiboko and Kakamega

		Species and location				
		Chilo partellus			***Busseola fusca***	
Entry	**Genotype**	**Embu**	**Kakamega**	**Kiboko**	**Embu**	**Kakamega**
13	CKSBL10203	+	-	+	-	-
49	CKSPL10028	+	+	+	+	+
53	CKSPL10070	+	-	+	-	-
75	CKSBL10028	-	+	-	+	+
79	CKSBL10043	+	+	+	-	-
80	CKSBL10035	-	-	+	-	-
81	CKSBL10038	+	-	+	-	-
85	CKSBL10039	-	-	-	+	+
91	CKSBL10040	+	+	+	+	+
95	CML312	+	-	-	+	+
100	CKSBL10026	+	-	-	+	+
101	CML444	-	-	+	-	-
96	**CML395 (susceptible check)**	+	+	+	+	+
Total		**9**	**9**	**9**	**7**	**7**

Key: + = Present and - = Absent

2.3.1.1 Stem borers resistance traits in different environments

Across the sites, most test genotypes were more susceptible to *B. fusca* leaf feeding damage than to *C. partellus* (Figure 2.4).

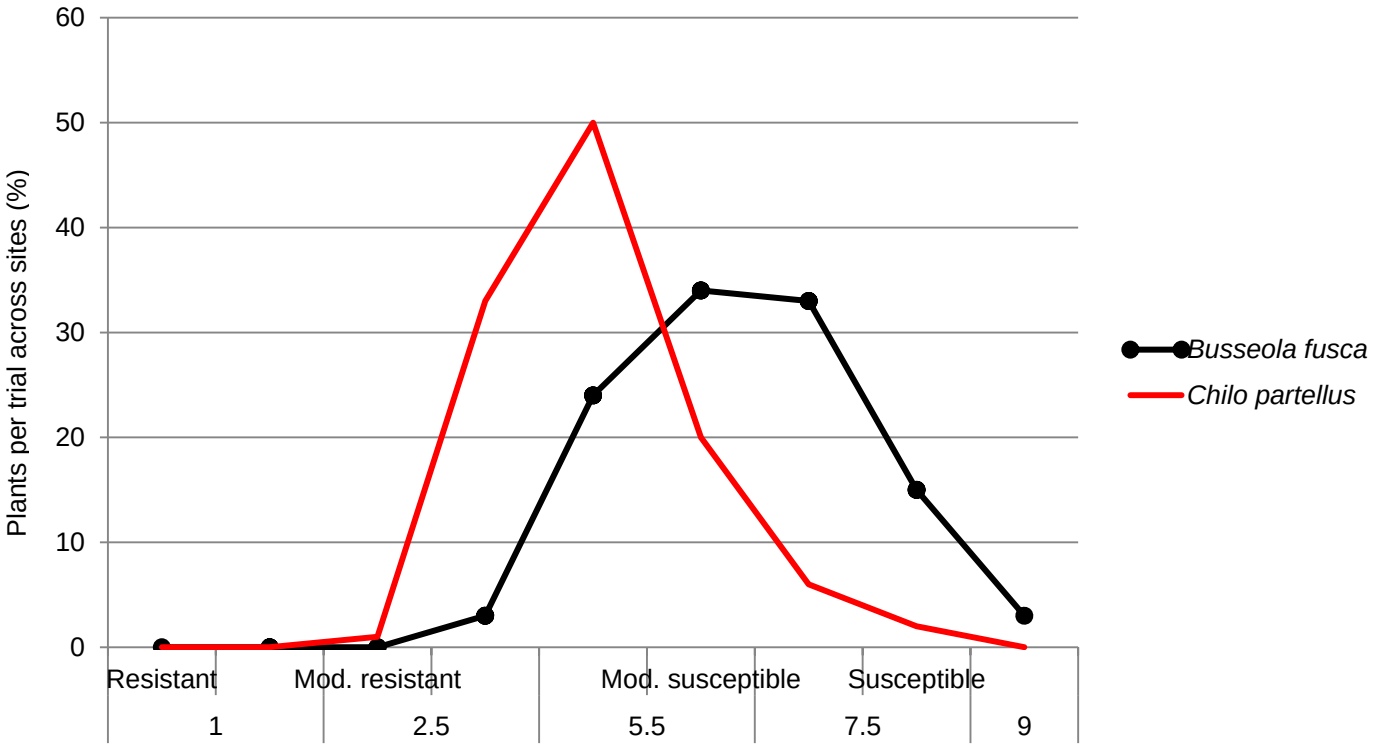

Figure 2.4. Overall number of plants per trial showing leaf feeding damage score due to *B. fusca* and *C. partellus* at Embu, Kakamega and Kiboko

The number of exit holes among the test genotypes indicated a wide range (0-25) for *B. fusca* and *C. partellus* averaged over Kakamega, Kiboko and Embu (Figure 2.5). Across the locations, *B. fusca* appeared to cause a higher number of exit holes per plant per trial than *C. partellus*.

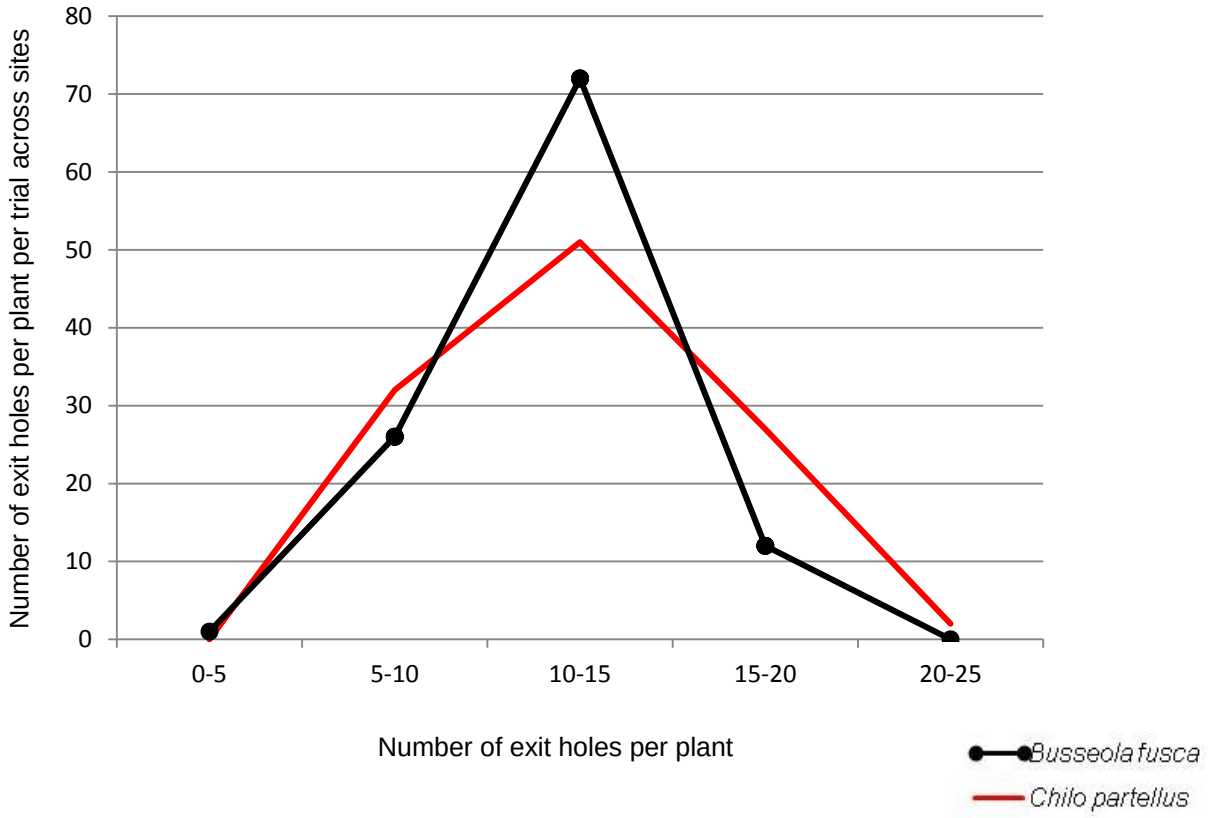

Figure 2.5. Overall number of plants showing number of exit holes per plant per trial due to *B. fusca* and *C. partellus* at Embu, Kakamega and Kiboko

Cumulative stem tunneling among the test genotypes indicated a wide range (0-50) for *B. fusca* and *C. partellus* averaged over Embu, Kakamega and Kiboko (Figure 2.6). Across the locations, *B. fusca* appeared to cause more cumulative stem tunneling per plant than *C. partellus*. There were more genotypes that were resistant to *C. partellus* than *B. fusca*.

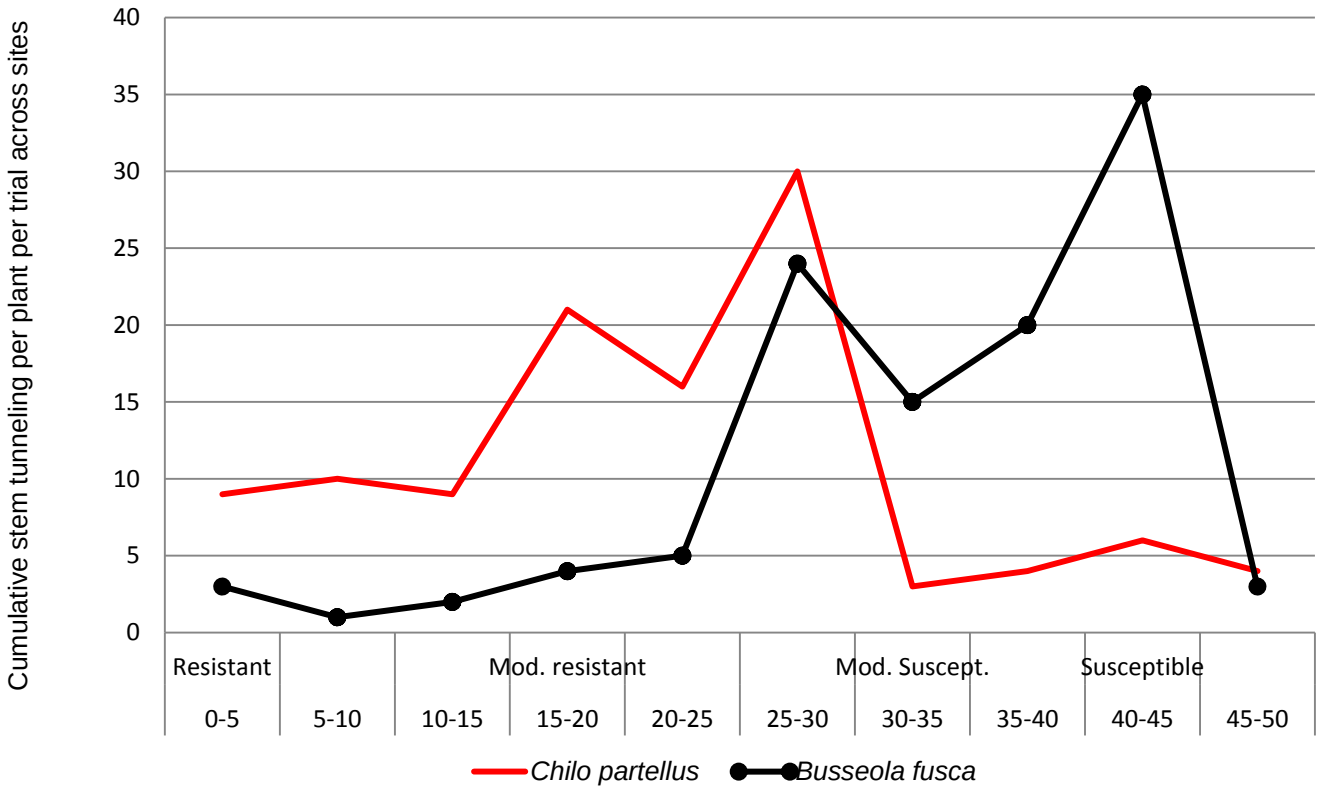

Figure 2.6. Overall numbers of plants showing cumulative stem tunneling due to *B. fusca* and *C. partellus* at Embu, Kakamega and Kiboko

There were differences in the means of stem borer damage traits per trial due to *B. fusca* and *C. partellus* at Embu, Kakamega and Kiboko (Figures 2.7 and 2.8).

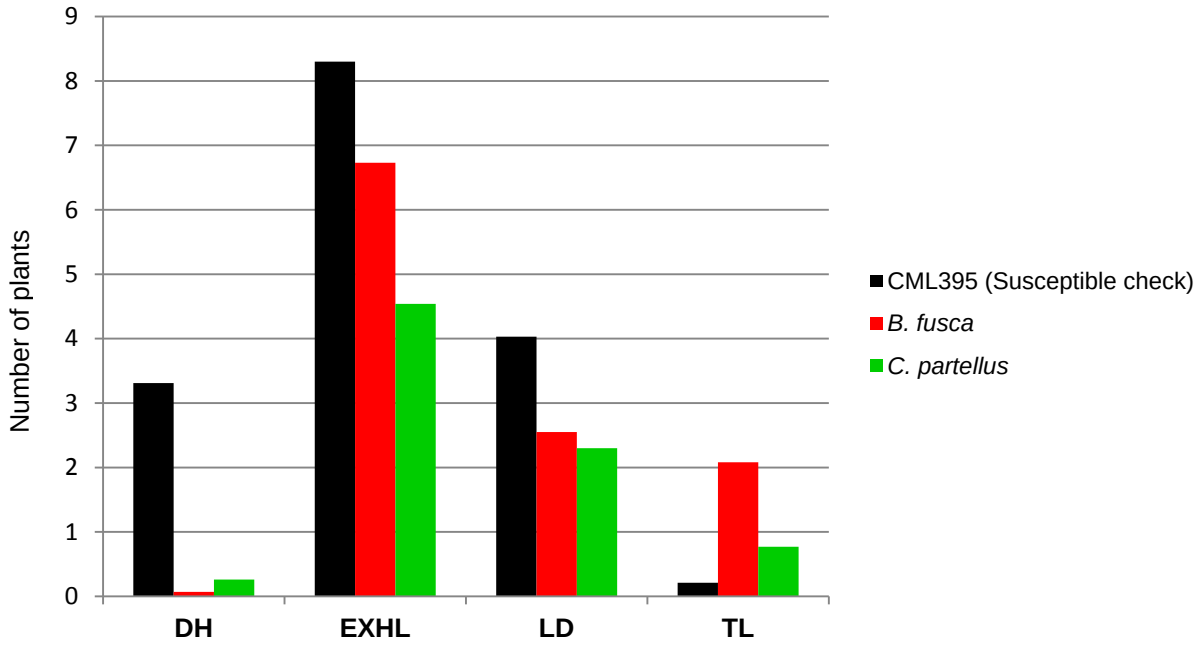

Figure 2.7. Means of selected stem borer damage traits per trial under *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

DH-number of dead hearts, EXHL-Number of exit holes, LD-leaf feeding damage score and TL-cumulative stem tunneling

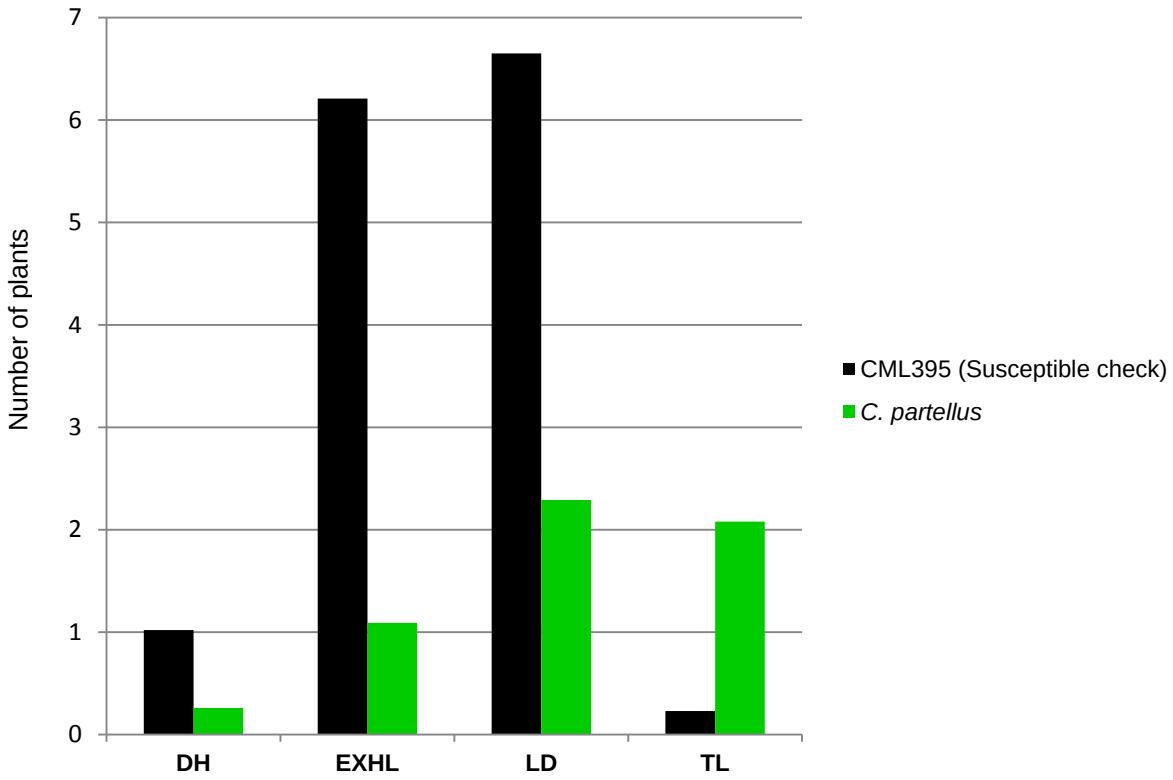

Figure 2.8. Mean of stem borer damage traits per trial under *C. partellus* infestation at Kiboko

NB: DH= number of dead hearts, EXHL= number of exit holes, LD= stem borer leaf damage scores, TL= cumulative tunnel length

2.3.2 Correlations for stem borer resistance and agronomic traits

There were highly significant correlations among the lines for resistance to both *B. fusca* and *C. partellus* and agronomic traits in all sites. The correlation coefficients were positive and significant for the number of exit holes and stem diameter for *B. fusca* r=0.83, (p≤0.01) while *C. partellus* was r=0.39, (p≤0.01). There were no significant correlation between leaf feeding damage and the number of exit holes for *B. fusca,* but a negative r=-0.46, (p≤0.01) for *C. partellus* was detected. Both *B. fusca* and *C. partellus* had negative significant correlation coefficients for number of exit holes r=-0.68, (p≤0.01) and plant aspect r=-0.62, (p≤0.01), plant height r=-0.22, (p≤0.01) and leaf feeding damage r=-0.49, (p≤0.01) respectively. In addition, both *B. fusca* and *C. partellus* showed negative significant correlation coefficients for plant height r=0.53, (p≤0.01) and plant aspect r=-0.53, (p≤0.01) respectively. However, no significant differences were observed for leaf feeding damage relative to the cumulative tunneling for both stem borers (Table 2.8).

Table 2.8. Correlation coefficients for selected stem borer resistance traits under *B. fusca* and *C. partellus* infestation at Kakamega, Kiboko and Embu

		Chilo partellus					
		EXHL	**LD**	**NE**	**PA**	**PH**	**TL**
Busseola fusca	**DIAM**	0.39**	0.22	0.17	0.24	0.40*	0.03
	EXHL	1	-0.46**	0.61**	-0.62**	0.89**	0.14
	LD	0.83**	1	-0.45**	0.29*	-0.49**	-0.06
	NE	0.14	-0.17	1	-0.61**	0.65**	0.11
	PA	0.38**	0.54**	-0.20*	1	-0.53**	-0.23
	PH	0.69**	-0.68**	0.06	-0.50**	1	0.11
	TL	0.68**	0.89**	-0.22*	0.51**	-0.53**	1
		0.34**	0.46**	0.26	0.12	-0.30**	0.47**

DIAM = plant diameter, EXHL = number of exit holes, LD = leaf damage scores, NE = number of ears harvested, PA = plant aspect, PH = plant height, TL = cumulative stem tunneling; and *, ** = significant (p≤0.05), highly significant (p≤0.01), ns = non-significant

2.3.3 Correlations between *B. fusca* and *C. partellus* borer resistance traits

Various stem borer resistance and agronomic traits were correlated between *B. fusca* and *C. partellus* (Table 2.9). There were significant correlations between traits due to *B. fusca* and *C. partellus (*r≥0.70 p≤0.05) for number of exit holes, number of ears harvested, plant aspect, plant height and internode length (Table 2.9).

Table 2.9. Correlation coefficients for selected stem borer resistance traits between *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

Parameter	Correlation coefficient (r)
Number of dead hearts	0.09**
Number of exit holes	0.75**
Leaf feeding damage score	0.55*
Cumulative stem tunneling	0.26*
Number of rotten ears	0.47*
Number of ears harvested	0.72*
Plant aspect	1.00*
Root lodging	0.50*
Stem lodging	0.56*
Plant height	0.81**
Stem diameter	0.40
Internode length§	0.70

§Four internodes below the uppermost ear

2.3.4 Heritability and genotypic and phenotypic correlations

Grain yield showed significant and a high positive phenotypic (0.65) and genotypic (0.88) correlation coefficients and high heritability estimates under *B. fusca* (0.68) and under *C. partellus* (0.80), respectively. A similar trend was detected for traits and their heritability under *B. fusca* infestation (Table 2.10). The genotypic correlation coefficients were higher than the phenotypic correlation coefficients for both *B. fusca* and *C. partellus* stem borers, and for both stem borer resistance and agronomic traits measured (Table 2.10).

Table 2.10. Heritability and genotypic and phenotypic correlation coefficients for selected stem borer resistance traits under *B. fusca* and *C. partellus* infestation at Embu, Kakamega, and Kiboko

	Heritability value (h^2)		Correlation coefficients	
Trait	***B. fusca***	***C. partellus***	**Phenotypic (r_P)**	**Genotypic (r_G)**
Grain yield (t ha^{-1})	0.68	0.80	0.65	0.88
Number of dead hearts	0.21	0.38	0.09	0.32
Number of exit holes	0.71	0.78	0.35	0.47
Leaf feeding damage	0.47	0.58	0.45	0.86
Cumulative stem tunneling (cm)	0.25	0.11	0.12	0.72
Stem diameter (cm)	0.68	0.68	0.40	0.62
Internode length (cm)[§]	0.76	0.74	0.70	0.81
Plant aspect	0.86	0.87	1.00	0.88
Ear aspect	0.18	0.47	0.25	0.86
Ear height (cm)	0.82	0.85	0.70	0.84
Plant height (cm)	0.74	0.83	0.73	0.93
Ear position	0.54	0.19	0.29	0.91
Number of ears per plant	0.61	0.65	0.54	0.86
SE	**0.22**	**0.24**	**0.26**	**0.18**

[§]Four internodes below the uppermost ear

2.4 Discussion

The analysis of variance revealed significant variation among the genotypes for all characters examined. The partitioning of the phenotypic variance and genotypic variance provided a better understanding of the variation patterns among *B. fusca* and *C. partellus* and their response to the test genotypes across different environments. For example, the number of dead hearts exhibited the least genotypic variance (0.01), while the number of exit holes had the highest (0.27) in Kakamega for *B. fusca*, compared to Embu which had 0.01 and 0.05 respectively (Tables 2.2 and 2.3). Kiboko had the least genotypic variance for all traits measured for *C. partellus* (Tables 2.4, 2.5 and 2.6). Observations on the number of dead hearts and number of exit holes may imply that trait variations for borer resistance are not completely under genetic control. The higher genotypic variances than the

environmental variances suggest that selection for particular stem borer resistance trait can be carried out, and that progress can be made.

The suggestions may apply to observations on the moderate to high broad sense heritability values for borer resistance traits. In both maize and sorghum, the role of leaf resistance and other traits in conferring resistance to stem borers *C. partellus* (Swinhoe), *Ostrinia nubilalis* (Hubner), *S. nonagrioides*, and *Diatreae* spp is well documented (Butrón et al., 2009; Singh et al., 2012). Even though heritability estimates indicate the relative values of selection based on the phenotypic expression, it is not definitive unless genetic gain under selection is considered together with heritability (Akinwale et al., 2011). The low to moderate broad sense heritability estimates (H^2<0.50) for characters such as number of dead hearts, number of exit holes, leaf feeding damage and cumulative stem tunneling may be due to environmental influence on the traits (Tables 2.4, 2.5 and Table 2.6).

Since selection indices for stem borer resistance traits provide efficiency in the improvement of quantitatively inherited traits such as stem borer resistance in maize (Mulamba et al., 1978; Mutinda et al., 2013), a rank selection index was used to identify genotypes with resistance for both *B. fusca* and *C. partellus*. The response of tropical maize inbred lines for resistance to two *B. fusca* and *C. partellus*, stem borers showed that resistance may be exclusive for *B. fusca* only or *C. partellus* only or for both borers where they exist. It was observed that five entries had resistance to *B. fusca* only, 26 entries showed resistance to *C. partellus* only, and 21 entries showed combined resistance to both *B. fusca* and *C. partellus* in at least two sites. Four entries CKSBL10025, CKSBL10039, CKSBL10040, and CKSBL10028 showed resistance to both species across the sites. Eighty four and 28 entries respectively showed susceptibility to *B. fusca and C. partellus* in all test genotypes (Table 2.7). Most of the genotypes were found to be susceptible to *B. fusca* and less so for *C. partellus.* These may be attributed to its (*B. fusca*) fitness and adaptation in Africa because it is indigenous unlike *C. partellus*. These findings suggest that genotypes with the specific borer resistance can be deployed directly as parent lines in the formation of hybrids with resistance to *B. fusca* and *C. partellus* to areas where these borers occur in league or exclusively.

The knowledge on genetic correlations between borer resistance traits is important in creating selection criteria (Sujiprihati et al., 2003). Since grain yield is a result of interrelationships of yield components (Schnable et al., 2013; Udaykumar et al., 2013), to maintain grain yield, breeding for stem borer resistance should be based on multi-trait selection. To do this, several correlations for stem borer resistance traits for *B. fusca* and *C. partellus* were examined to understand their relationships. There were highly significant differences for correlations among the lines for resistance to both *B. fusca* and *C. partellus* and agronomic traits in all sites. The correlation coefficients were positive and significant for

the number of exit holes and stem diameter for *B. fusca* r=0.83, (p≤0.01) while that for *C. partellus* was r=0.39, (p≤0.01). The findings from the current study, corroborate with previous studies that have shown that most cultivated grass species have large stem diameters that support a higher larval survival and more larvae have been recovered per plant unlike wild grass species (Akinwale et al., 2011; Hosseini et al., 2011). However, there were no significant correlations between leaf feeding damage and the number of exit holes for *B. fusca,* but a negative significant correlations r=-0.45, (p≤0.01) for *C. partellus*.

For both borers, besides the length of the life cycles for the two borers, morphological characteristics such as trichome density, leaf pubescence, leaf glossiness, thorns, spines, cuticles, and waxes may hinder insect development (Munyiri et al., 2013; Santamaria et al., 2013). These may in turn affect the observed differences in resistance traits due to leaf feeding and larval survival on hosts.

Similarly, both *B. fusca* and *C. partellus* had negative significant correlations for number of exit holes r=-0.68, (p≤0.01) and plant aspect r=-0.62, (p≤0.01), plant height r=-0.22, (p≤0.01) and leaf feeding damage r=-0.49, (p≤0.01) respectively. In addition, both *B. fusca* and *C. partellus* showed negative significant correlation for plant height r=-0.53, (p≤0.01) and plant aspect r=-0.53, (p≤0.01) respectively. Leaf feeding damage relative to the cumulative tunneling for both stem borers indicated no significant correlations (Table 8). Based on stem borer resistance trait rank selection indices; leaf feeding damage, cumulative stem tunneling and number of exit holes were found to be reliable parameters that may be used in discriminating genotypes for resistance to the two borers. The findings may imply that both *B. fusca* and *C. partellus* affect plants negatively in a similar manner. For example, stem tunneling disrupts nutrients and water uptake, leaf feeding damage reduces the photosynthetic area, exit holes may cause weakened stems which may result in susceptible to stem lodging and other plant deformities thus result in increased losses to grain yield.

Previous studies showed that stem tunneling damage had a significant influence on maize plant growth, and that the direct effect of stem tunneling on loss in maize grain yield was greater than the effect of leaf feeding (Kumar, 1997; Singh et al., 2012). The results from the current study agree with the findings of Ajala et al. (2010), Akinwale et al. (2011), and Mailafiya et al. (2011) reported that leaf damage and cumulative tunneling were positively correlated. These may show differences among *B. fusca* and *C. partellus* nature of feeding, stem tunneling, oviposition, and exit from host plants. Other studies found that *B. fusca* and *C. partellus* stem borer damage reduced the number of ears harvested per plant and plant height (Sujiprihati et al., 2003; Sharma et al., 2007; Akinwale et al., 2011).

Further trait correlations between *B. fusca and C. partellus* revealed positive and significant correlations for the for both borers for number of exit holes (r=0.75, (p≤0.01), leaf feeding damage score (r=0.55, (p≤0.05), cumulative stem tunneling (r=0.26, (p≤0.01), number of rotten ears (r=0.47, (p≤0.05), number of ears harvested (r=0.72, (p≤0.05), number of plants per plot (r=0.73, (p≤0.05), plant aspect (r=0.99, (p≤0.05), plant height (r=0.81, (p≤0.01), root lodging (r=0.50, (p≤0.05), and stem lodging (r=0.56, (p≤0.05). However, no significant differences were observed for trait correlations between *B. fusca and C. partellus* for number of dead hearts, stem diameter and internode length across the sites (Table 9).

For successful selection of useful genotypes, an understanding of the genotypic and phenotypic inter-trait correlations is essential. The magnitude of genotypic and phenotypic correlations and their use in selection has been reported in literature (Ali et al., 2008; Al Tabbal et al., 2012). For example, in this study genotypic correlations were greater for most of the traits than the phenotypic correlation coefficient values (Table 9). Grain yield showed significant and high positive genotypic (1.13) and phenotypic (0.83) correlation coefficients and high heritability values for both *B. fusca* (0.68) and *C. partellus* (0.80). Similarly, high genotypic correlations were observed for number of exit holes (1.01), leaf feeding damage (1.06), and cumulative stem tunneling (1.56) for both *B. fusca* and *C. partellus*. These may indicate a heritable correlation of these traits (Sahoo et al., 2011; Al Tabbal et al., 2012). However, stem borer resistance traits had low heritability for number of dead hearts (0.21), leaf feeding damage (0.47), and cumulative stem tunneling (0.25), except for the number of exit holes (0.71); and correspondingly low phenotypic correlation values of less than 0.60. Most agronomic traits had high phenotypic and genotypic correlations (0.58-1.68) and a wide range for heritability estimates for both *B. fusca* (0.18-0.86) and *C. partellus* (0.19-0.87). Despite the high genotypic variability revealed by the genetic coefficients of variation for the various stem borer resistance and agronomic traits, it may not provide information on the heritable variation that is useful for genetic improvement (Akinwale et al., 2011; Singh et al., 2012). Expected genetic advance may be achieved through phenotypic selection when the genotypic coefficients of variation are coupled with heritable estimates (Sahoo et al., 2011; Al Tabbal et al., 2012). Correlation coefficients may be useful as indicators of trait association among the borers, for example, the high number of exit holes and cumulative tunnel length shows the probability that either may be a useful selection criterion for resistance to *B. fusca* and *C. partellus* in maize. Similar results have been reported indicating that selection based on these traits may lead to improvement in stem borer resistance (Munyiri et al., 2013). Low to moderate heritability values were observed for stem *B.* fusca and *C. partellus* stem borers' resistance traits in the test germplasm suggesting that suggests that those traits are under genetic control. Previous studies have shown low

heritability for various stem borer resistance traits due to compromised experimental procedures, low frequency for resistance genes in the reference populations (Singh et al., 2012; Chaudhary, 2013), or due to environmental influence or due to few sites used for evaluations (Falconer et al., 1996).

2.5 Conclusion

The overall results suggest that a high variability of germplasm for resistance to *B. fusca* and *C. partellus* stem borers exists. Since both *B. fusca* and *C. partellus* stem borers are serious insect pests of maize, the identification of germplasm with resistance to these pests is key. The high heritability, genotypic and phenotypic correlations values showed the presence of inherent association between some stem borer resistance traits for both borers. Further genetic improvement may be explored for number of exit holes, cumulative stem tunneling alongside the agronomic traits in selection for the resistance to either or both *B. fusca* and *C. partellus* in maize. Leaf feeding damage scores, ccumulative stem tunnel length and number of exit holes were the most effective parameters in discriminating the test genotypes for resistance to the two borers. Genotypes identified for resistance to *C. partellus* only may be deployed in breeding programmes in zones where *C. partellus* exclusively occurs and likewise for regions with *B. fusca* only. Genotypes that showed combined resistance to both borers may be deployed to areas where these borers exist in league. However, breeding for resistance to these borers should continue besides deployment of these stem borer resistant hybrids. The observed responses to either or both *Busseola fusca* and *Chilo partellus*, stem borers where they occur exclusively or in league helped to identify resistant maize inbred lines, and showed their possible use in hybrid breeding programmes in tropical maize that emphasize stem-borer resistance especially in eastern and southern Africa.

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Appendix 2

Table 2.11. List of pedigree¥ information of maize inbred lines used in the study

Entry	Pedigree code	Entry	Pedigree code	Entry	Pedigree code	Entry	Pedigree code
1	CKSBL10105	29	CKSBL10286	57	CKSPL10086	85	CKSBL10039
2	CKSBL10108	30	CKSBL10170	58	CKSPL10087	86	CKSPL10341
3	CKSBL10138	31	CKSBL10168	59	CKSPL10088	87	CKSPL10344
4	CKSBL10073	32	CKSBL10178	60	CKSPL10089	88	CKSPL10035
5	CKSBL10107	33	CKSBL10307	61	CKSPL10090	89	CKSBL10025
6	CKSBL10195	34	CKSBL10154	62	CKSPL10136	90	CKSBL10045
7	CKSBL10194	35	CKSBL10153	63	CKSPL10146	91	CKSBL10040
8	CKSBL10196	36	CKSBL10158	64	CKSPL10212	92	CML264
9	CKSBL10197	37	CKSBL10155	65	CKSPL10229	93	CML202
10	CKSBL10201	38	CKSBL10321	66	CKSBL10060	94	CML204
11	CKSBL10202	39	CKSBL10160	67	CKSBL10014	95	CML312
12	CKSBL10200	40	CKSBL10155	68	CKSBL10034	96	CML395 (susceptible check)
13	CKSBL10203	41	CKSBL10157	69	CKSPL10177	97	CKSBL10001
14	CKSBL10204	42	CML442	70	CKSBL10013	98	CKSBL10004
15	CKSBL10205	43	CKSBL10020	71	CKSBL10007	99	CKSBL10004
16	CKSBL10206	44	CKSBL10082	72	CKSBL10015	100	CKSBL10026
17	CKSBL10209	45	CKSPL10256	73	CKSBL10016	101	CML444
18	CKSBL10208	46	CKSPL10273	74	CKSBL10030	102	CML334
19	CKSBL10207	47	CKSPL10280	75	CKSBL10028	103	CML254
20	CKSBL10210	48	CKSPL10309	76	CKSBL10029	104	CKSBL10046
21	CKSBL10213	49	CKSPL10028	77	CKSBL10033	105	CML144
22	CKSBL10250	50	CKSPL10035	78	CKSPL10343	106	CML159
23	CKSBL10254	51	CKSPL10036	79	CKSBL10043	107	CML445
24	CKSBL10165	52	CKSPL10042	80	CKSBL10035	108	CML511
25	CKSBL10169	53	CKSPL10070	81	CKSBL10038	109	LPSC7-F86-3-1-1-1-BB-#-B-B
26	CKSBL10171	54	CKSPL10074	82	CKSBL10042	110	P300C5S1B-2-3-2-#-#-1-2-B-B-#-B-B
27	CKSBL10150	55	CKSPL10080	83	CKSBL10008	111	CML489
28	CKSBL10212	56	CKSPL10081	84	CKSBL10041	112	CML488

¥ - The full pedigree information is available, Resistant check - MBR C5 BC F1-13-3-2-1-B-4-2-B

CHAPTER 3

Combining ability for Stem Borer Resistance and Heterotic Orientation of Maize inbred lines using CIMMYT single cross testers under *Busseola fusca* infestation

Abstract

The African stem borer, *Busseola fusca* Fuller, is one of the most devastating insect pests of maize in tropical environments. Understanding of combining ability and heterosis may be useful for designing insect resistant hybrids. However, among the adapted maize inbred lines used in this study, the combining ability and heterotic orientation for grain yield and borer resistance is not known. The objective of this study was to determine combining ability and heterotic orientation of maize inbred lines under *B. fusca* infestation. Sixty six inbred lines were crossed to two single cross testers from CIMMYT in accordance with the line x tester mating scheme. The 132 three-way testcross hybrids and four checks were evaluated at two locations in Kenya. Data were analysed using PROC GLM of SAS statistical package. The genotypes x environment interactions were significant, therefore the two test locations were considered as ideal environments for genotype comparison. General combining ability effects were significant for *B. fusca* stem borer resistance and grain yield, suggesting a preponderance of the additive gene effects for borer resistance traits. Specific combining ability effects were significant for *B. fusca* borer resistance traits and grain yield indicating that non-additive effects were also influential. Based on grain yield heterosis data at Embu, 22 lines were allocated to group A, 18 to group B and 8 to group AB, while at Kakamega, 24 lines were oriented to group A, 13 to group B and 9 to group AB, whilst the remainder could not be classified. Based on the SCA effects, at Embu, 20 lines revealed positive SCA effects with both testers and were considered to be AB-oriented; while 12 and 7 lines were oriented towards A and B, respectively. A similar trend was observed at Kakamega but only one line exhibited positive SCA effects for grain yield with both testers, while the remainder had negative SCA effects. The identified lines and heterotic groups may be used by maize programmes that emphasize stem borer resistance in hybrids.

Keywords: combining ability, *Busseola fusca*, stem borer resistance, heterosis, heterotic orientation, tropical maize

3.1 Introduction

Maize is a principal crop grown for its economic importance as foodstuff and alternative energy source by a majority of rural households in sub-Saharan Africa (SSA). Maize is both a staple food and a source of income to millions of small-scale farmers, all over the world (Brooks et al., 2009; Sasson, 2012). Through plant breeding, new maize lines and hybrids have been formed with improved traits for biotic and abiotic stress tolerance. Stem borers are one of the biotic stresses limiting maize production. For example, *B. fusca* stem borers' whose distribution and occurrence in different locations and crop ecosystems is diverse (Mailafiya et al., 2011; Ong'amo et al., 2012). Its population dynamics may be affected by numerous factors namely; host availability, location and suitability, mate location, success of oviposition, larval survival and establishment, temperature and altitude (Mailafiya et al., 2011; Ong'amo et al., 2012). In SSA, the environmental conditions favour the insect pest development such that more generations of insect pests per season occur. These in turn leads to higher high levels of crop losses (Ong'amo et al., 2012; Tefera, 2012).

The African stem borer, *Busseola fusca,* Fuller (Lepidoptera, Noctuidae), indigenous to Africa, is among the major insect pests that greatly reduce maize grain yield in tropical environments. It causes maize production losses in the high yielding mid-altitude transitional and highlands tropic zones in Kenya (De Groote et al., 2004). In addition, *B. fusca* accounts for approximately 82% of the losses associated with stem borers (Ong'amo et al., 2012). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al., 2003). This amount represents an average of 13.54% of the farmers' total annual harvest of maize and prompts breeding investigations.

Various alternatives for managing maize stem borers have potential to alleviate their damaging effects, but each option has its own limitations. Host plant resistance forms an important part of integrated pest management as it provides inherent control without environmental concerns and is compatible with other pest management approaches (Mugo et al., 2005). Currently varieties with host plant resistance are limited in most tropical environments. Therefore, effective breeding methods for resistance to *B. fusca* damage should be designed by plant breeders using both improved and new sources of stem borer resistance. Development of effective methods requires a better understanding of the genetic basis of the resistances among the germplasm used.

Suitable maize germplasm should have resistance to *B. fusca* borers where they occur. There is a need to breed and promote genotypes with *B. fusca* resistance, and to encourage wide adoption of the competitive hybrids with *B. fusca* resistance across maize agro-ecologies. Therefore the necessity to

identify resistance to *B. fusca* stem borers in tropical maize inbred lines becomes paramount (Adijah et al., 2011).

An appropriate hybrid maize breeding programme must encompass and exploit the knowledge of general combining ability (GCA) of lines and specific combining ability (SCA) of their crosses, heterosis and its accompanying patterns. Combining ability of germplasm, the type of gene action controlling the inheritance economic traits and heterosis are a precondition in fixing the appropriate parent lines, and in designing successful hybrid breeding programmes (Liberatore et al., 2013; Schnable et al., 2013).

Heterosis in maize has been reported in literature since 1900's through investigations carried out by Shull (1908) and East (1909). It has been defined as the superiority of highly heterozygous F1-hybrids in relation to the mid-parent performance of their genetically distinct homozygous parents (Sanghera et al., 2012). A high genetic diversity in maize inbred lines strongly determines the levels of heterosis exhibited by the single cross hybrids and vice versa (Hallauer et al., 1988), and may be useful in hybrid development. Heterotic orientation of lines can be based on both the heterosis and specific combining ability data.

The line x tester mating design provides consistent information on the general and specific combining ability effects of parents and their hybrid combinations (Kempthorne, 1957). The design has been applied in many previous quantitative genetic studies in maize (Kanagarasu et al., 2010; Udaykumar et al., 2013). The design is mainly used to generate data on nature and magnitude of gene action, combining ability effects, heritability and nature and extent of heterosis for different traits (Udaykumar et al., 2013). For example, Sprague and Tatum, (1942) on studies in maize yield detected that general combining ability is mainly due to the additive gene effects while specific combining ability is attributed to dominance or epistatic gene effects. The line x tester mating design has been applied for determining the pattern of gene action for stem borer resistance potential in maize (Sharma et al., 2007). Line x tester mating scheme in the early generations of breeding mostly S_2 or S_3 generations reduces the amount of germplasm carried forward. Populations and inbred lines or single cross hybrids have been used as testers in the identification of hybrids for yield performance (Sanghera et al., 2012). The line x tester continues to be applied in determination of the maize heterotic orientations using different testers (Sanghera et al., 2012). The design was therefore used in this study to evaluate testcross hybrids in the target environments.

The objective of this study was to determine combining ability for resistance and heterotic orientation of maize inbred lines under *Busseola fusca* infestation. The knowledge generated was important in the selection of favourable maize inbred lines and testcrosses for manipulation in a hybrid breeding program with emphasis on *B. fusca* stem borer resistance.

3.2 Materials and Methods

3.2.1 Germplasm

The experimental material comprised of three-way cross hybrids derived from crosses of 66 stem borer resistant lines (as female parents) with two single cross testers (as male parents) (CML312/442 and CML395/444). The sixty six inbred lines have a wide genetic base formed from various nurseries at CIMMYT, Kiboko, and the Kenya Agricultural Research Institute (KARI) breeding programmes. Known elite but stem borer resistant and susceptible maize lines from CIMMYT and KARI were included as checks. The 66 lines were selfed for 5 generations and selected from the previous study on responses of tropical maize inbred lines for resistance to two stem borers *B. fusca* and *C. partellus* (Chapter 2). Four commercial varietiesincluding two single cross testers CML312/442 and CML395/444 were used as checks in the study. CML312/CML442 and CML395/CML444 were used as testers A and B, respectively, classified according to the heterotic group system at CIMMYT (CIMMYT, 2001). Both testers were resistant to *B. fusca* stem borers. Single cross testers were used because the programme aims at releasing three-way cross hybrids that can be nominated directly into the national performance trials for additional evaluation and use. The pedigree information on the lines used is presented in Appendix 3, Table 3.14.

3.2.2 Experimental sites

Experiments were established at Kakamega and Embu in Kenya (Figure 3.1). KARI Kakamega ($37^0$75'E 2^0 15'S, 1585m asl) centre is located in the moist transitional mid altitude agro-ecological zone of western Kenya and experiences mean annual temperatures of 25^oC. Kakamega lies within a high potential agro-ecological zone and receives a bimodal mean annual rainfall of approximately 1850 to 1916 mm. The soils in Kakamega are well drained, moderately deep to very deep, red to dark in colour and in some places shallow over petroplinthite (Jaetzold et al., 1982).

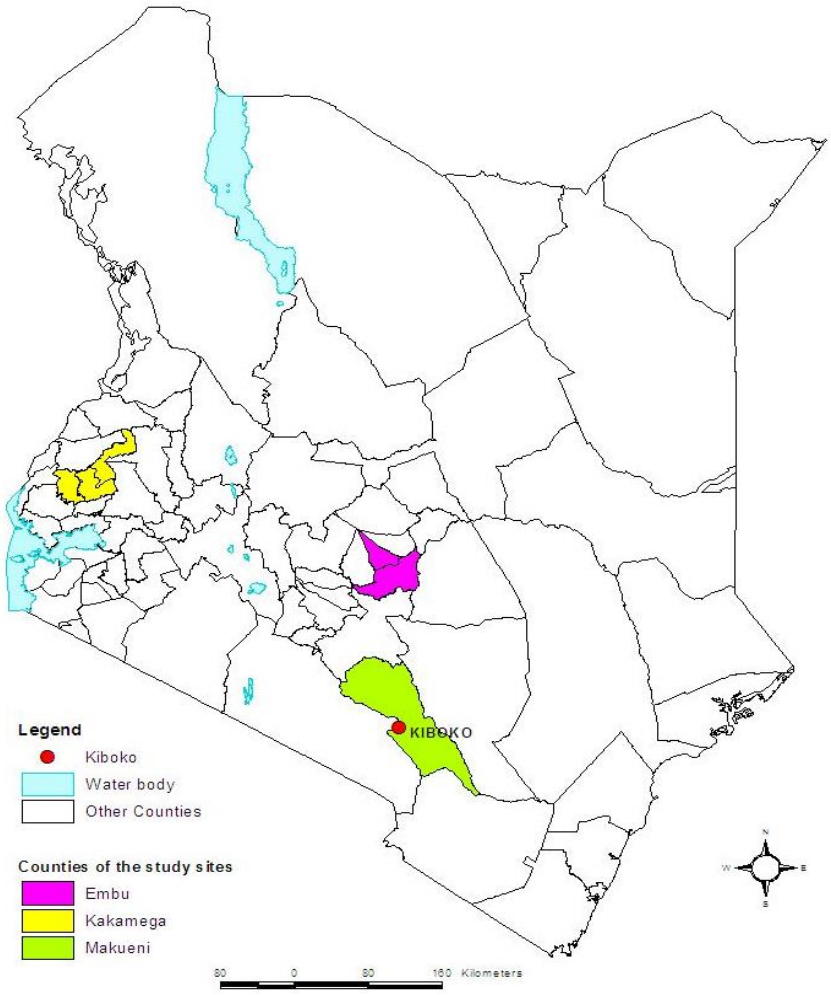

Figure 3.1. Map of Kenya showing Embu, Kakamega and Kiboko locations of the studies

Source: KARI Land Resources and Analytical Services (KARI Land Resources and Analytical Services, 2013)

KARI-Embu centre (03°56' 44'S and 39°46' 00'E, 1510m asl) is located in the moist transitional mid altitude agro-ecological zone of eastern slopes of Mt. Kenya and experiences mean annual temperature ranges of 14-25°C. Embu lies within a high potential agro-ecological zone. Rainfall received is bi-modal ranging between 800-1400 mm annually. The soils are deep (about 2 m); well weathered Humic Nitisols with moderate to high inherent fertility (Jaetzold et al., 1982).

3.2.3 Experimental design and infestation

The three way testcross hybrids were evaluated in a 10 x 7 α-lattice design with three replications in each location. Each testcross hybrid was sown in one row plot of 6.75 m. Two seeds were sown per hill

and later thinned to one. Inter-row spacing of 0.75 m and inter-hills spacing of 0.25 m within the rows was used.

Recommended fertilizer application of nitrogen (60 kg N ha^{-1}) and phosphate (60 kg P_2O_5 ha^{-1}) and irrigation were applied as recommended for each location to ensure healthy and vigorous plants. Nitrogen was applied in two splits, while supplementary irrigation was applied when needed. The fields were kept free of weeds by hand weeding throughout the growth cycle.

3.2.3.1 Artificial infestation with insects

Eight plants per plot were infested with *B. fusca* at Embu and Kakamega. Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and the Kenya Agricultural Research Institute at Katumani stem borer insect pests mass rearing facility. Plants were artificially infested in a controlled and uniform manner with the respective stem borer species by placing 10 larvae in the maize whorl using a camel brush at two weeks after planting.

3.2.4 Data collection and analysis

Plants were evaluated for leaf damage scores using a scale of 1 (resistant) to 9 (susceptible) (CIMMYT, 1989). The numbers of dead-hearts were assessed as a proportion of plants in the plot indicating death of the growing points. Other plant damage parameters were measured at harvest namely; cumulative tunnel length (measured as the total length (cm) of tunneling along the maize stalk), tunnel length to plant height ratio, number of exit holes, number of dead hearts, stalk strength, and number of larvae recovered per plant. Stalk strength was measured using a rind penetrometer 8 weeks after planting (Figure 3.2).

Figure 3.2. Measurement of stalk strength of maize stalks at KARI Embu

Agronomic traits were measured following standard protocols used at CIMMYT (CIMMYT, 1989). Grain yield (kg $plot^{-1}$) was obtained as grain weight adjusted for moisture content at 13%, and converted to t ha^{-1}. Data on number of dead-hearts and cumulative stem tunnel length were transformed into arcsine values before subjecting them to analysis of variance (ANOVA).

The analysis of variance (ANOVA) across environments for all data was performed using PROC GLM procedures in SAS computer package, version 9.2 following a linear model:

$Y_{ijk} = \mu + r(e_k) + e_k + l_i + t_j + (l \times t)_{ij} + (l \times e)_{ik} + (t \times e)_{jk} + (l \times t \times e)_{eijk} + Є_{ijk}$

Where: Y_{ijk} is measured trait of the genotype of i^{th} line crossed to j^{th} tester evaluated in r replications across k environments; μ is grand mean; r (e_k) = effect of replication nested within the *k* environments; l and t represent average effects of lines and of testers, respectively, which is equivalent to GCA effects of lines and testers, respectively; l x t = line x tester interaction effects that is equivalent to the SCA effects of the crosses; *e* is the environmental main effects; l x *e*, t x *e* and l x t x *e* are the interactions of the lines, testers and the lines x testers with the environments, and e_{ijk} = random experimental error.

The GCA of lines (GCA_l) and testers (GCA_t), and SCA of crosses (SCA) and their standard errors were estimated (Dabholkar, 1992). Relative standard heterosis (SH) was calculated using the following formula: SH = ((F1- Mean of tester)/ Mean of tester) * 100, where: F1 = F1 hybrid mean performance; MoT = mean of tester (A), mean of tester (B).

Heterosis was estimated from mean values according to Fehr (1987) t-test was performed. Heterosis was calculated for each testcross relative to the two testers as follows:

Heterosis $=\left[\frac{F1-Tester}{Tester}\right] \times 100$, while the SE for heterosis was calculated as $\sqrt{\sigma^2_e}/2$

Clustering of lines into heterotic group A (CML312/CML442) and B (CML395/CML444) depended on the direction of the specific combining ability such that lines exhibiting positive SCA with tester A were allocated to the opposite heterotic group B, and vice versa, whereas lines displaying positive SCA to both were designated as AB group.

3.3 Results

3.3.1 Genotype x environment interactions

There were significant genotypes x environment interactions for grain yield, therefore the two test locations were considered as representative environments. Therefore the results are presented for individual locations.

3.3.2 Trait variations under *Busseola fusca* infestation

The mean squares of the testcrosses from the combined analysis of variance for selected stem borer resistance traits for *B. fusca* at Embu and Kakamega were significant ($p \leq 0.05$) for all traits (Table 3.1). Environments were defined as site x season combination.

The site, lines and testers showed highly significant differences for most traits. The testers showed significant ($p \leq 0.05$) differences for grain yield and internode length. The environment x line interaction effects were significant ($p \leq 0.01$) for grain yield, cumulative stem tunneling, number of exit holes and leaf feeding damage. The environment x tester interaction effects were significant ($p \leq 0.05$) for cumulative stem tunneling, number of exit holes and leaf feeding damage. The line x tester interaction effects were highly significant ($p \leq 0.05$) for grain yield, cumulative stem tunneling, number of exit holes, leaf feeding damage, plant height and ear aspect (Table 3.1). The environment x line x tester interaction effects were significant ($p \leq 0.05$) for grain yield, cumulative stem tunneling, number of exit holes, ear aspect and plant height (Table 3.1). Under *B. fusca* infestation the mean for the following traits was: grain yield (2.08 t ha^{-1}), cumulative stem tunneling (8.57 cm), leaf feeding damage score (2.52) and number of exit holes (5.02). Means for the other agronomic traits had similar trends (Table 3.1).

Table 3.1. Mean squares of testcrosses for selected stem borer resistance and agronomic traits for hybrids under *B. fusca* infestation averaged over four environments

Source	DF	GY	TL	EXH	LD	PA	EA	AD	SD	PH	IL	DIAM
Rep	2	307.73**	43.5	9.74	40.00**	39.88**	5.91**	5626.54**	5768.90**	2740.47**	12526.98**	23.08**
Env.	3	269.69**	71.99	63.82**	26.34**	34.86**	16.50**	7607.19**	7829.04**	1672.48*	177.59	26.14**
Line	65	7.70*	151.48*	56.48**	11.29**	1.77**	2.38**	63	58.42	8968.81*	2323.56**	2.79**
Tester	1	10.09*	2.91	5.42	4.25	0.5	0.7	5.02	1.74	244.52	7682.26**	0
Env.*Line	195	1.03**	32.83**	29.36**	4.00**	0.9	0.91	20.51	20.16	1367.80**	1367.61**	0.5
Env.*Tester	3	0.36	72.15**	31.47**	13.68**	0.78	2.07	9.6	3.4	506.65	357.63	1.07
Line*Tester	65	0.95***	42.64**	14.60**	6.70**	0.96	1.22**	13.22	10.93	2429.89**	808.93	0.67
Env.*Line*Tester	195	0.55*	36.51**	14.15**	3.17	0.77	0.97*	7.3	9.48	1186.21**	558.39	0.55
Error	1053	2.1	24.81	7.86	3.08	0.99	0.79	64.73	66.11	675.13	567.83	0.62
Trial mean		**2.08**	**8.57**	**5.02**	**2.52**	**2.66**	**2.56**	**73.97**	**75.08**	**201.25**	**27.55**	**2.37**
(%) R		**46.87**	**53.11**	**65.03**	**44.75**	**38.25**	**47.74**	**32.49**	**32.37**	**67.51**	**54.7**	**47.94**

GY - grain yield, TL-cumulative stem tunneling (cm), EXH-number of exit holes, LD-leaf feeding damage, PA-Plant aspect, EA-Ear aspect, AD-days to anthesis, SD-days to silking, PH-plant height, IL-internode length, and DIAM-stem diameter, *, ** = significant (p≤0.05), highly significant (p≤0.01).

3.3.3 General combining ability effects

Results of general combining ability effects of top 20 lines and their corresponding two testers for *B. fusca* stem borer resistance traits and grain yield are presented for Embu and Kakamega (Table 3.2 and Table 3.3).

At Embu, grain yield had positive significant ($p \leq 0.05$) GCA effects for grain yield were detected for all top 20 lines except for lines 52, 53, 54, 58, and 28. Negative significant ($p \leq 0.05$) GCA effects were detected for cumulative stem tunneling for all lines except 7 lines; similarly, for number of exit holes. For leaf feeding damage, 9 lines showed negative significant ($p \leq 0.05$) GCA effects. The top 20 lines showed positive significant GCA effects for days to anthesis for lines except 9 lines. All lines had negative significant ($p \leq 0.05$) GCA effects except line 21 for plant height. The lines had varied trends for plant and ear aspects (Table 3.2).

At Kakamega, positive significant ($p \leq 0.05$) GCA effects were detected for grain yield for all top 20 lines except lines 5 lines. Negative significant ($p \leq 0.05$) GCA effects were detected for cumulative stem tunneling for all lines except 4 lines. For the number of exit holes lines all showed negative significant ($p \leq 0.05$) GCA effects except lines 5 lines. Leaf feeding damage score showed negative significant ($p \leq 0.05$) GCA effects for 11 lines (Table 3.3). All top 20 lines showed significant negative ($p \leq 0.05$) GCA effects for days to anthesis except lines 4 lines, while for days to silking significant negative effects were revealed for all top 20 lines except lines 39, 58, and 60 (Table 3.3). Other agronomic traits showed varied significant negative ($p \leq 0.05$) GCA effects for plant height and ear aspect.

The testers (CML395/CML444 and CML312/CML442) had diverse trends for the various *B. fusca* stem borer resistance and agronomic traits at the two locations (Table 3.2 and Table 3.3).

Table 3.2. General combining ability effects of top 20 maize inbred lines for selected stem borer resistance traits and grain yield under *B. fusca* infestation at Embu (averaged over 2 seasons)

	Line	GY	Line	TL	Line	EXH	Line	LD	Line	AD	Line	SD	Line	PH	Line	PA	Line	EA
	40	1.23**	51	-3.29**	66	-0.92**	54	-0.58	40	-0.98**	31	-2.57	4	-35.23**	34	-0.68	1	-0.71
	47	0.82	17	-2.73**	61	-0.28	39	0.49	31	0.29	40	-3.07	5	-31.06**	41	-0.78	2	-0.8
	48	1.89**	57	-3.16**	28	0.99**	6	0.01	3	-1.88**	-	-	16	-25.23	44	-0.95	6	-1.05
	49	1.89**	54	0.94	65	-0.21	13	0.06	62	-0.54**	-	-	21	-31.89	46	-0.78	7	-0.71
	50	1.89**	25	0.84	64	-0.31	11	0.14	8	-0.38	-	-	22	-32.73	47	-0.88	20	-0.71
	51	1.31**	20	0.96	29	0.12	7	-1.33**	52	-0.38	-	-	23	-31.89	51	-0.95	24	-0.8
	52	-0.39	26	4.06	63	-1.7	15	-1.31**	23	-0.38	-	-	38	-34.39	52	-0.95	27	-0.96
	53	-0.44	19	1.71	31	2.57	23	-0.59	38	-1.88**	-	-	48	-37.73	53	-0.78		-
	54	-0.39	48	-1.09**	32	-0.90**	48	0.07	48	-0.21	-	-	49	-51.06	55	-0.78		-
	55	0.87**	15	0.11	30	0.85**	49	-0.57	58	0.29	-	-	55	-46.89	56	-0.78		-
	56	1.89**	53	-1.39	7	-0.65	25	-1.32**	64	0.62	-	-	56	-32.73	60	-0.78		-
	57	1.89**	3	1.28	50	1.71	41	0.27	56	0.79	-	-	-	-	64	-0.78		-
	58	-0.42	2	-2.19	62	-0.50	45	-1.33**	1	0.02	-	-	-	-	65	-0.95		-
	63	1.47**	47	-3.42	34	-0.21	8	-0.21	60	-0.54	-	-	-	-	66	-0.95		-
	28	-1.04	59	-0.51	27	1.44	1	-1.25**	25	0.46	-	-	-	-	-	-		-
	-	-	14	-5.49**	33	-0.71	10	0.97	14	0.79	-	-	-	-	-	-		-
	-	-	23	-1.39	18	-1.30	17	-0.56	4	1.62	-	-	-	-	-	-		-
	-	-	22	-3.67**	54	-0.58	46	0.85	42	0.62	-	-	-	-	-	-		-
	-	-	41	-4.04**	37	2.47	43	0.04	5	-0.04	-	-	-	-	-	-		-
	-	-	28	-3.02**	35	2.87	56	0.02	63	-0.04	-	-	-	-	-	-		-
Testers																		
CML395/CML444		-0.1		-0.01		-0.05		-0.12		-0.06		2.18		0.02		-0.03		-0.3
CML312/CML442		0.1		0.01		0.05		0.12		0.06		-2.18		-0.02		0.03		0.3
SE		**0.33**		**0.01**		**0.08**		**0.01**		**0.03**		**2.14**		**0.04**		**0.02**		**0.08**

TL – cumulative stem tunneling, LD-leaf feeding damage, , EXH-number of exit holes, AD - days to anthesis, SD- days to silking, PH-plant height, PA - plant aspect and EA - ear aspect, GY - grain yield, *, ** = significant (p≤0.05), highly significant (p≤0.01), The mean yield of CML395/CML444 and CML312/CML442 was 1.24 and 1.32 t ha^{-1}

Table 3.3. General combining ability effects of top 20 maize inbred lines for selected stem borer resistance traits and grain yield under *B. fusca* infestation at Kakamega (averaged over 2 seasons)

Line	GY	Line	TL	Line	EXH	Line	LD	Line	AD	Line	SD	Line	PH	Line	PA	Line	EA
4	1.42**	23	-2.40**	65	0.29**	7	-0.25**	47	-1.08	58	-1.44	4	-28.07**	9	-0.81**	5	-0.78**
5	1.31**	17	-1.08**	27	0.09**	48	0.58**	39	-0.75	39	-1.44	5	-38.07**	17	-0.72**	15	-0.86**
6	1.41**	49	-2.55**	30	-0.32**	44	0.14	57	-1.75**	47	-1.77**	6	-36.41**	-	-	19	-0.86**
11	1.22**	45	2.78	61	-0.16**	46	-0.80**	35	-1.58	57	-2.60**	15	-30.57**	-	-	-	-
12	1.41**	50	-1.54**	32	0.23**	35	-0.06**	58	0.92	61	-1.60**	16	-26.41**	-	-	-	-
13	1.51**	26	-2.22**	33	2.33**	9	0.78	36	-1.25	38	-2.60**	20	-27.02**	-	-	-	-
14	1.41**	15	-0.12**	62	2.23**	25	-1.43**	55	-1.75**	60	-1.44	21	-28.91	-	-	-	-
20	1.05**	14	1.61**	64	2.09**	4	-0.67**	61	-1.75**	66	-2.44**	22	-31.41**	-	-	-	-
53	-1.01	51	-4.50**	29	0.74	49	-0.81**	62	-1.08	46	-3.10**	38	-32.24**	-	-	-	-
28	-1.41	52	-4.89**	66	0.89	45	0.63	63	-1.58	35	-1.77**	39	-28.07**	-	-	-	-
29	1.71**	48	-3.85**	31	0.19	53	0.00*	41	-2.42**	36	-2.27**	47	-23.07**	-	-	-	-
52	0.58	53	-6.37**	63	-1.22**	42	0.11	49	-1.75**	53	-1.44	48	-38.91	-	-	-	-
31	0.73*	16	-0.94**	28	0.89	16	-0.11**	66	0.75	62	-1.77**	49	-47.24**	-	-	-	-
58	0.94*	59	-3.67**	50	0.51	43	-0.50**	51	0.92		-	55	-36.41**	-	-	-	-
33	-1.06	54	-3.35**	60	0.01	8	0.21	54	-1.58		-	56	-35.57**	-	-	-	-
36	-0.66	19	0.32**	12	-0.07**	22	-0.72**	60	-1.58	-	-	-	-	-	-	-	-
47	-1.42	47	-3.19**	53	-0.62**	58	0.6	65	-1.25	-	-	-	-	-	-	-	-
39	1.49**	20	-7.30**	17	2.64	52	-0.26**	43	0.75	-	-	-	-	-	-	-	-
40	1.40**	58	1.05**	49	0.91	47	-0.17	38	-2.25**	-	-	-	-	-	-	-	-
41	1.41**	22	-1.47**	42	1.28	14	-0.17	45	-2.25**	-	-	-	-	-	-	-	-
Testers																	
CML395/CML444	-0.1		-0.01		-0.05		-0.12		-0.06		2.18		0.02		-0.03		-0.3
CML312/CML442	0.1		0.01		0.05		0.12		0.06		-2.18		-0.02		0.03		0.3
SE	**0.33**		**0.01**		**0.08**		**0.01**		**0.03**		**2.14**		**0.04**		**0.02**		**0.08**

TL – cumulative stem tunneling, LD-leaf feeding damage, EXH-number of exit holes, AD-days to anthesis, SD- days to silking, PH-plant height, PA - plant aspect and EA - ear aspect, GY - grain yield, *, ** = significant (p≤0.05), highly significant (p≤0.01)

3.3.4 Specific combining ability effects

Orientations of lines into heterotic group A (CML312/CML442) and B (CML395/CML444) depended on the direction of the specific combining ability such that lines exhibiting positive SCA with tester A were allocated to the opposite heterotic group B, and vice versa, whereas lines displaying positive SCA to both testers were designated as AB group.

Results of SCA effects of top 20 testcrosses and their corresponding two testers for *B. fusca* stem borer resistance traits and grain yield are presented for Embu and Kakamega (Table 3.4 and Table 3.5). At Embu, all testcrosses revealed significant and desirable SCA effects ($p \leq 0.05$) for grain yield, cumulative stem tunneling, number of exit holes, and leaf feeding damage. Eleven entries out of twenty were crosses with CML395/CML444, while the remaining were testcrosses with CML312/CML442 (Table 3.4). Similar interpretations were made at Kakamega, where 20 top testcrosses with significant and desirable SCA effects ($p \leq 0.05$) were crosses with CML395/CML444, while the rest with CML312/CML442 (Table 3.5). Testcrosses that showed resistance to *B. fusca* at Embu and Kakamega were the following; 16 x 1, 16 x 2, 18 x 1, 18 x 2, 30 x 1, 30 x 2, 38 x 1 and 40 x 1 (Table 3.4 and Table 3.5).

Table 3.4. Specific combining ability effects of testcrosses for selected stem borer resistance traits and grain yield under *B. fusca* infestation at Embu (averaged over 2 seasons)

Embu Testcross	Grain yield (t ha^{-1})	Cumulative stem tunneling	No. of exit holes	Leaf damage score (1-9)
9 x 1	0.19*	-0.02	-2.95**	-1.82**
16 x 1	0.24**	-0.02**	-2.57**	-1.34*
11 x 1	0.21**	-0.04**	-2.23**	-1.35*
18 x 2	0.24*	-0.02**	-1.43*	-1.39*
27 x 2	0.28*	-0.04**	-1.68*	-1.35
28 x 1	0.21*	-0.01	-1.68*	-1.49**
30 x 1	0.27*	-0.02*	-2.50**	-1.48**
37 x 2	0.19*	-0.02*	-2.37**	-1.54**
38 x 1	0.24*	-0.02*	-2.35**	-1.90**
40 x 1	0.65**	-0.02*	-2.22**	-1.45**
41 x 2	0.51**	-0.01	-3.07**	-2.03**
45 x 2	0.23**	-0.01	-1.43	-1.49**
46 x 2	0.29**	-0.03**	-4.15**	-1.38*
47 x 1	1.06**	-0.03*	-1.97	-1.87**
51 x 2	0.59**	-0.03*	-2.50**	-1.58**
52 x 1	0.22**	-0.12**	-10.25**	-1.52**
54 x 1	0.38*	-0.02	-1.77	-2.16**
55 x 1	0.63**	-0.03**	-1.37	-1.29*
58 x 2	0.73**	-0.01	-2.72**	-1.31
63 x 2	0.43**	-0.01	-2.28**	-1.73**
SE	**0.19**	**0.02**	**1.72**	**0.71**

Tester 1 = CML312/CML442 and Tester 2=CML395/CML444, SE for heterosis of grain yield = 0.30

Table 3.5. Specific combining ability effects of testcrosses for selected stem borer resistance traits and grain yield under *B. fusca* infestation at Kakamega (averaged over 2 seasons)

Kakamega Testcross	Grain yield (t ha^{-1})	Cumulative stem tunneling (cm)	No. of exit holes	Leaf damage score (1-9)
10 x 1	0.23	-0.02*	-2.14*	-1.91*
10 x 2	0.26	-0.03**	-1.49	-1.53*
16 x 2	0.25	-0.03**	-2.96*	-2.08*
18 x 1	0.22	-0.02	-2.91*	-1.54*
22 x 1	0.29	-0.01	-2.07	-1.37
21 x 2	0.33	-0.01	-1.81	-1.33
30 x 2	0.80**	-0.01	-2.54*	-1.78*
31 x 2	0.65**	-0.02	-1.93	-1.51*
32 x 2	0.45*	-0.02*	-1.34	-1.40
33 x 2	0.32	-0.01	-1.36	-1.65*
34 x 1	0.41*	-0.02*	-1.54	-1.35
36 x 1	0.77**	-0.02	-1.57	-2.43*
38 x 1	0.48*	-0.02*	-2.81*	-1.45
40 x 1	0.24	-0.02	-1.23	-1.3
43 x 1	0.26	-0.01	-2.02	-1.31
44 x 1	0.42**	-0.02*	-1.46	-2.34*
59 x 1	0.24	-0.02	-1.34	-1.35
61 x 2	0.60**	-0.02*	-1.23	-1.82*
62 x 1	0.70**	-0.02	-1.51	-1.59*
66 x 2	0.21	-0.03**	-2.01	-1.50*
SE	**0.18**	**0.01**	**1.35**	**0.75**

Tester 1 = CML312/CML442 and Tester 2=CML395/CML444, SE for heterosis of grain yield = 0.30

At Embu, among the top 20 testcrosses, there were positive significant and desirable SCA effects ($p \leq 0.05$) for 9 testcrosses for days to anthesis and 8 testcrosses for days to silking (Table 3.6). At Kakamega, comparable interpretations were made for days to anthesis where all testcrosses with both testers and twelve testcrosses for days to silking showed positive significant and desirable SCA effects (Table 3.7).

Table 3.6. Specific combining ability effects of testcrosses for selected agronomic traits under *B. fusca* infestation at Embu (averaged over 2 seasons)

Embu					
Testcross	**AD**	**SD**	**PH**	**PA**	**EA**
9 x 1	-1.56*	-1.71*	-16.30	-0.50	-0.47
16 x 1	-1.84*	-2.22*	-27.03*	-0.42	-0.44
11 x 1	-1.84*	-2.06*	-28.80*	-0.67*	-0.64
18 x 2	-2.34*	-1.44	-13.70	-0.42	-0.44
27 x 2	-1.66	-1.11	-14.53	-0.50	-0.53
28 x 1	-1.18	-2.89*	-33.80*	-0.42	-0.44
30 x 1	-1.18	-1.78	-19.53	-0.75*	-0.72*
37 x 2	-2.18	-1.11	-19.53	-0.50	-0.53
38 x 1	-1.32	-1.28*	-32.86*	-0.42	-0.64
40 x 1	-1.49*	-1.28	-19.64	-0.42	-0.69*
41 x 2	-2.49*	-1.28	-13.80	-0.33	-0.63
45 x 2	-1.26	-2.28*	-33.70	-0.67*	-0.61
46 x 2	-1.34*	-1.11	-19.64	-0.67*	-0.56
47 x 1	-1.49	-1.22*	-17.97	-0.33	-0.53
51 x 2	-1.34	-1.28	-16.53	-0.83*	-0.72*
52 x 1	-2.16	-1.56	-37.86*	-0.50	-0.44
54 x 1	-2.82*	-2.61	-21.30	-0.50	-0.78*
55 x 1	-1.34	-1.11*	-37.14*	-0.50	-0.44
58 x 2	-1.34	-1.72	-16.4	-0.40	-0.89*
63 x 2	-2.18*	-2.06*	-20.15	-0.67*	-0.69*
SE	**1.07**	**1.20**	**12.65**	**0.35**	**0.34**

NB: AD - days to anthesis, SD- days to silking, PH - plant height, PA - plant aspect and EA - ear aspect, 1 = CML312/CML442 and 2 = CML395/CML444

Table 3.7. Specific combining ability effects of testcrosses for selected agronomic traits under *B. fusca* infestation at Kakamega (averaged over 2 seasons)

Kakamega					
Testcross	**AD**	**SD**	**PH**	**PA**	**EA**
10 x 1	-0.58*	-0.06	-24.52*	-0.44	-0.80*
10 x 2	-0.58*	-0.06*	-14.68	-0.4	-0.63
16 x 2	-1.08*	-0.06	-20.36	-0.31	-0.46
18 x 1	-0.75*	-0.06	-18.84	-0.44	-0.38
22 x 1	-0.75*	-0.06	-16.34	-0.35	-0.55
21 x 2	-0.58*	-0.06	-16.19	-0.35	-0.55
30 x 2	-0.92*	-0.06	-12.18	-0.56	-0.59
31 x 2	-0.92*	-0.61*	-23.24*	-0.65	-0.44
32 x 2	-1.08*	-0.61*	-22.18	-0.48	-0.80*
33 x 2	-1.25*	-1.06*	-18.02	-0.77*	-1.04**
34 x 1	-1.08*	-1.78**	-21.98	-0.31	-1.04**
36 x 1	-1.25*	-0.44	-20.51	-0.40	-1.46**
38 x 1	-1.42*	-0.78*	-34.36*	-0.60	-0.49
40 x 1	-1.08*	-1.78**	-24.52*	-0.73*	-0.45
43 x 1	-0.92*	-0.61*	-25.36*	-0.69	-0.62
44 x 1	-1.92*	-0.72*	-14.36	-0.81*	-0.84**
59 x 1	-1.25*	-1.06*	-15.18	-0.81*	-0.91**
61 x 2	-0.75*	-0.89*	-15.51	-0.40	-0.54
62 x 1	-1.08*	-0.72*	-25.36*	-0.44	-0.39
66 x 2	-0.75*	-0.94*	-13.01	-0.73	-1.54**
SE	**0.60**	**0.43**	**11.56**	**0.35**	**0.36**

NB: AD - days to anthesis, SD- days to silking, PH - plant height, PA - plant aspect and EA - ear aspect, Tester 1 = CML395/CML444 and Tester 2 = CML312/CML442

3.3.5 Heterotic orientations based on specific combining ability

Below are results of the heterotic orientations of lines based on specific combining ability data for grain yield under *B. fusca* infestation (Table 3.8 and Table 3.9). Lines were clustered into two groups A and B depending on the direction of the SCA estimate. At Embu, 12 lines showed positive SCA effects for grain yield with CML395/CML444 therefore were assigned to B, while 9 lines revealed positive SCA effects with CML312/CML442 therefore were allocated to A. Line 30 showed positive SCA effects for grain yield with both testers and was oriented towards AB (Table 3.8).

At Kakamega, 11 lines showed positive SCA effects for grain yield with CML395/CML444, hence belong to B, while 9 lines exhibited positive SCA effects with CML312/CML442, thus belong to A. Lines 10, 30, and 32 showed positive SCA effects with both testers and were oriented towards group AB.

Across environments, lines 16, 18, 38, and 40 showed positive SCA effects with both testers CML312/CML442 and CML395/CML444. In addition, they showed both *B. fusca* resistance and high grain yielding; and were therefore oriented to group AB (Table 3.9).

Table 3.8. Heterotic orientation of maize inbred lines based on specific combining ability effects for grain yield for under *B. fusca* infestation at Embu (averaged over 2 seasons)

Line	SCA effects for grain yield		Heterotic orientation
Embu	CML395/CML444	CML312/CML442	
9	0.19*	-0.18	A
16	0.24**	-0.25	A
11	0.21**	-0.20**	A
18	0.24*	-0.23*	A
27	-0.29*	0.28*	B
28	0.21*	-0.23	A
30	0.27*	0.26*	A/B
37	-0.18*	0.19*	B
38	0.24*	-0.23*	A
40	0.65**	-0.66**	A
41	-0.50**	0.51**	B
45	-0.22**	0.23**	B
46	-0.28**	0.29**	B
47	1.06**	-1.04**	A
51	-0.58	0.59**	B
52	0.22**	-0.21**	A
54	0.38*	-0.37*	A
55	0.63**	-0.64**	A
58	-0.72**	0.73**	B
63	-0.44**	0.43**	B

*, ** = significant ($p \leq 0.05$), highly significant ($p \leq 0.01$)

Table 3.9. Heterotic orientation of maize inbred lines based on specific combining ability effects for grain yield for under *B. fusca* infestation at Kakamega (averaged over 2 seasons)

Line	SCA effects for grain yield		Heterotic orientation
Kakamega	CML395/CML444	CML312/CML442	
10	0.23*	0.26*	A/B
16	0.24*	-0.25*	A
18	0.22*	-0.23*	A
22	0.29*	-0.28*	A
21	-0.33	0.32	B
30	0.79**	0.80**	A/B
31	-0.66**	0.65**	B
32	0.44*	0.45*	A/B
33	-0.32*	0.32*	B
34	0.41*	-0.40*	A
36	0.77**	-0.78**	A
38	0.48*	-0.47*	A
40	0.24*	-0.23*	A
43	0.26	-0.26	A
44	0.42**	-0.43	A
59	0.24	-0.25	A
61	-0.61**	0.60**	B
62	0.70**	-0.69	A
66	-0.21	0.21	B

*, ** = significant (p≤0.05), highly significant (p≤0.01)

3.3.6 Heterosis of testcrosses relative to testers

Heterosis of testcrosses was estimated relative to CML312/CML442 and CML395/CML444. The degree of heterosis varied from testcrosses to testcrosses. At Embu, inbred lines 1, 2, 3, 4, 5, 52, 53, 54 and 55, revealed positive heterosis with both testers for grain yield and were oriented towards to heterotic group AB under *B. fusca* infestation (Table 3.10). Heterosis for grain yield ranged from -91.9% to 98.9% relative to both testers under *B. fusca* infestation (Figure 3.3). In total among the all test genotypes 22 lines were allocated to A, 18 to B and 8 to AB, while the remainder could not be classified with both testers (Figure 3.4).

Table 3.10. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *B. fusca* infestation at Embu (averaged over 2 seasons)

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
54	98.9	87.1	AB
51	69.9	59.8	AB
53	62.7	53.0	AB
55	50.6	41.6	AB
52	37.7	29.5	AB
1	20.8	13.6	AB
2	20.8	13.6	AB
3	20.8	13.6	AB
4	20.8	13.6	AB
5	20.8	13.6	AB
CML395/CML444	**1.24**		
CML312/CML442		**1.32**	

Mean yield of CML395/CML444 and CML312/CML442 was 1.24 and 1.32 t ha^{-1}, SE for heterosis of grain yield = 0.30

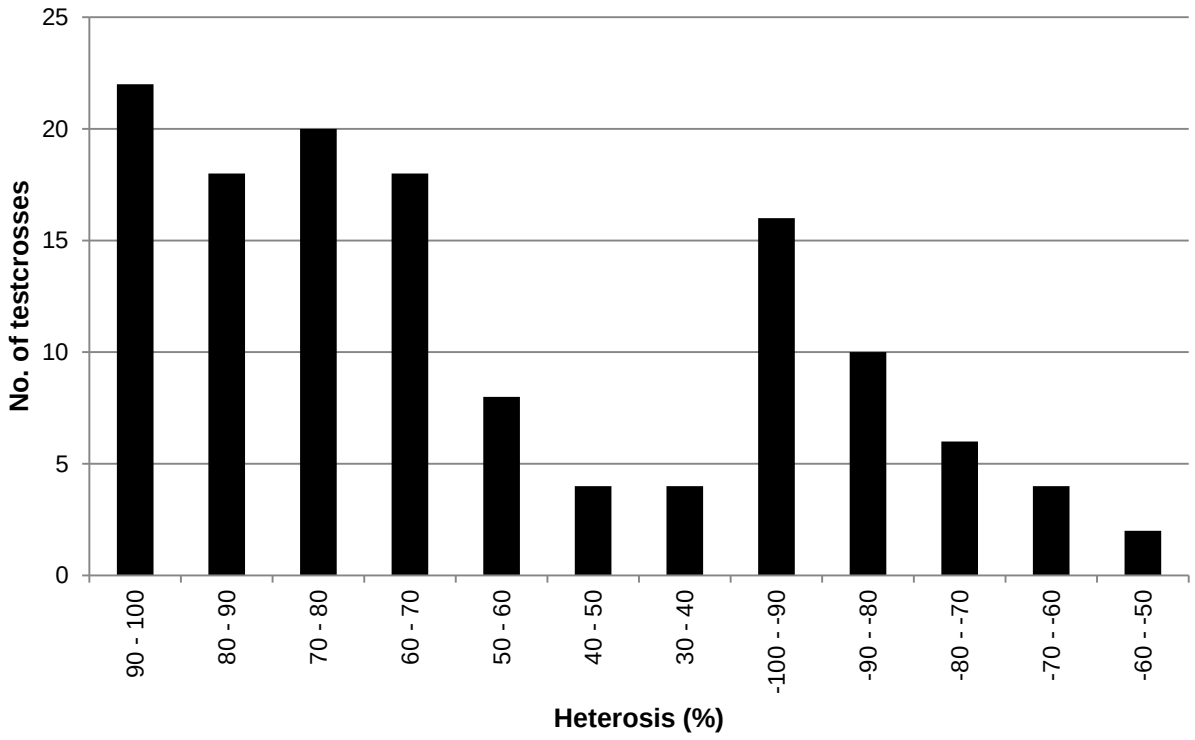

Figure 3.3. Distribution of heterosis (%) for 132 testcrosses under *B. fusca* infestation at Embu

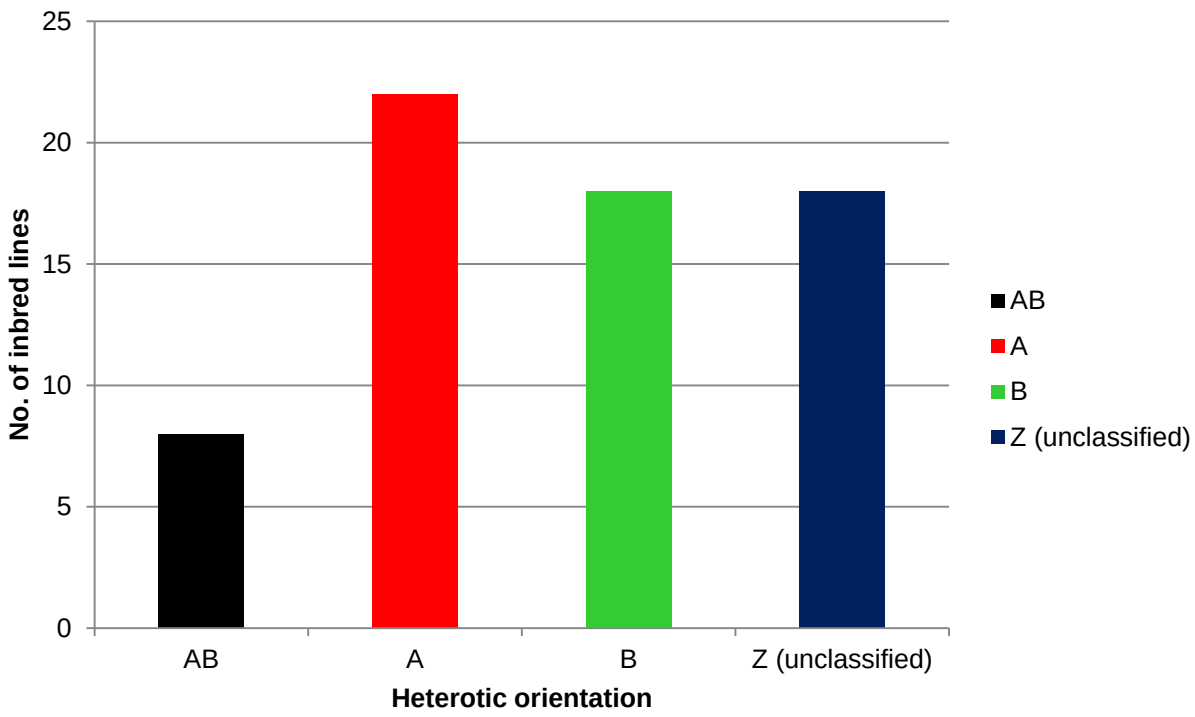

Figure 3.4. Classes of the heterotic orientations for 66 maize inbred lines under *B. fusca* infestation at Embu

At Kakamega, lines 62, 11, 33, 20, 32, 42, 66, 10, 7, and 43, revealed positive heterosis for grain yield with both testers and were oriented towards to heterotic group AB under *B. fusca* infestation (Table 3.11). Heterosis (%) ranged from -87.3% to 80.2% relative to both testers under *B. fusca* infestation (Figure 3.5). Overall the among the test genotypes 24 lines were oriented towards group A, 13 to B and 9 to AB, while the remainder could not be classified with both testers (Figure 3.6).

Table 3.11. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *B. fusca* infestation at Kakamega

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
62	80.2	73.6	AB
11	66.9	60.8	AB
33	46.8	41.5	AB
20	46.4	41.1	AB
32	31.4	26.6	AB
42	29.5	24.8	AB
66	22.4	17.9	AB
10	21.5	17.1	AB
7	17.9	13.6	AB
43	16.5	12.2	AB
CML395/CML444	**1.58**		
CML312/CML442		**1.64**	

SE for heterosis of grain yield = 0.30

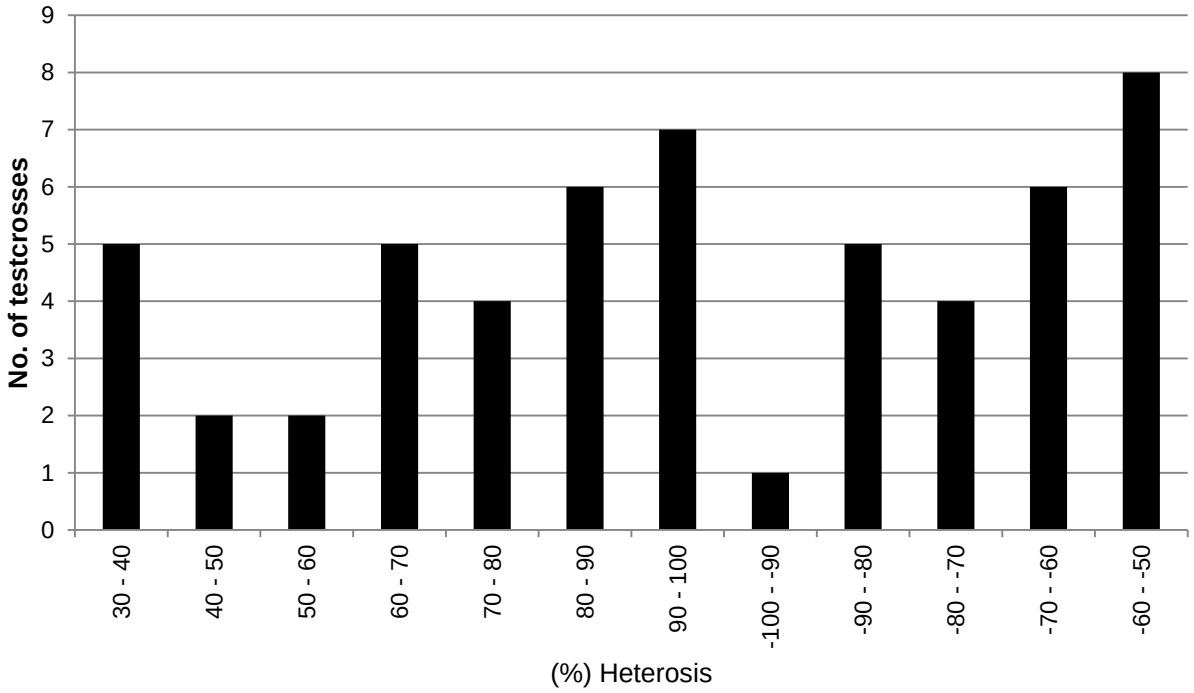

Figure 3.5. Distribution of heterosis (%) for 132 testcrosses under *B. fusca* infestation at Kakamega

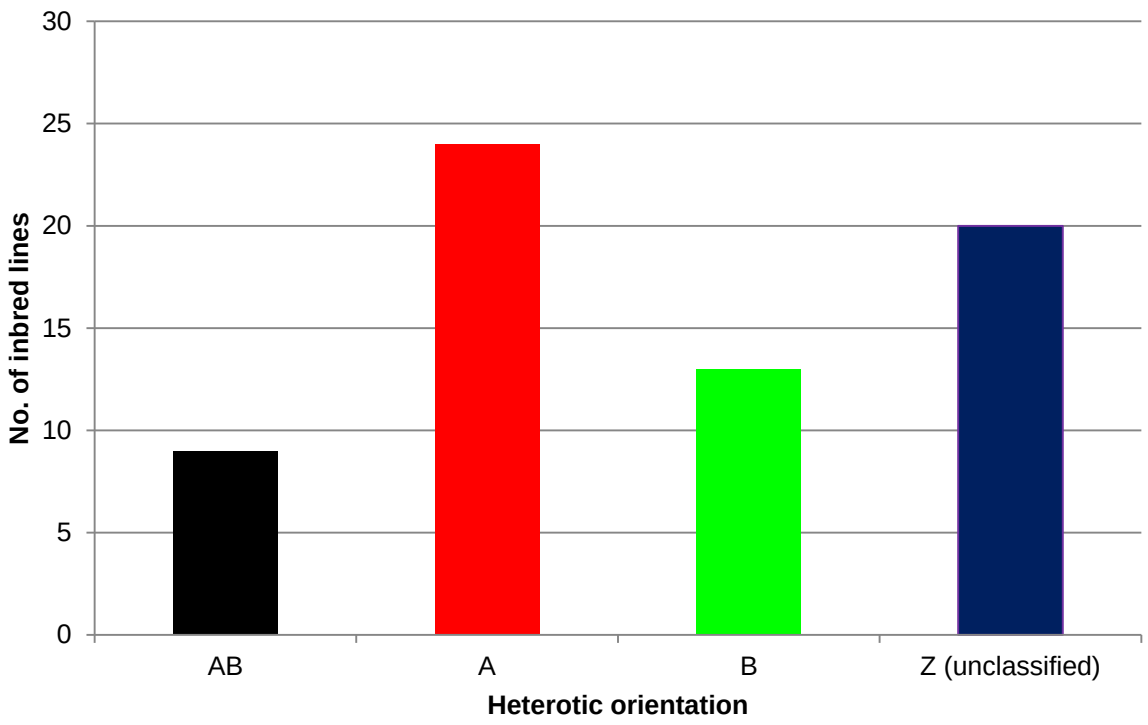

Figure 3.6. Classes of the heterotic orientations for 66 maize inbred lines under *B. fusca* infestation at Kakamega

3.3.7 Relative standard heterosis

At Embu, lines 54, 51, 53, 55, 52, 1, 2, 3, 4, and 5 revealed positive heterosis with both testers for grain yield and were considered as best for grain yield (Table 3.12). The range for grain yield was to -47.2% to -13.2% compared to the best check and trial mean under *B. fusca* infestation.

Table 3.12. Percent grain yield of testcrosses relative to the trial mean, best check hybrid and mean of hybrids under *B. fusca* infestation at Embu

Line	%yield relative to trial mean	%yield relative to best check	%yield relative to mean of checks
54	30.0	-13.0	-1.20
51	11.1	-25.7	-15.6
53	6.30	-28.9	-19.2
55	-1.60	-34.2	-25.2
52	-10.0	-39.8	-31.6
1	-21.1	-47.2	-40.0
2	-21.1	-47.2	-40.0
3	-21.1	-47.2	-40.0
4	-21.1	-47.2	-40.0
5	-21.1	-47.2	-40.0
Mean	**1.90**		
Best Hybrid check		**2.84**	
Check mean			**2.50**

SE for heterosis of grain yield = 0.30

At Kakamega, lines 62, 11, 33, 20, 32, 42, 66, 10, 7, and 43 revealed positive heterosis with both testers for grain yield and were categorized as the best for grain yield under *B. fusca* infestation (Table 3.13). The best 10 hybrids had heterosis ranging from 79% to 176% compared with the best hybrid checks for grain yield under *B. fusca* infestation (Table 3.13).

Table 3.13. Percent grain yield of testcrosses relative to the trial mean, best check hybrid and mean of hybrids under *B. fusca* infestation at Kakamega

Line	%yield relative to trial mean	%yield relative to best check	%yield relative to mean of checks
62	76.2	176.4	278.3
11	63.2	156.0	250.4
33	43.6	125.2	208.3
20	43.2	124.6	207.4
32	28.5	101.6	176.0
42	26.7	98.7	172.0
66	19.7	87.7	156.9
10	18.8	86.4	155.1
7	15.3	80.9	147.6
43	13.9	78.6	144.5
Mean	**1.62**		
Best hybrid check		**1.03**	
Check mean			**0.75**

SE for heterosis of grain yield = 0.30

3.4 Discussion

3.4.1 Variations among lines and testcross hybrids

The African stem borer, *Busseola fusca,* Fuller, (Lepidoptera, Noctuidae) occurs at Embu and Kakamega. The highly significant differences detected among the lines and their testcrosses for the various stem borer resistance and agronomic traits indicated the existence of considerable variation among the genotypes that allows for selection of preferred inbred lines and hybrids. The separation of lines, testers and environments and their interactions into variances provided a better understanding of the different patterns among lines and their reaction to *B. fusca* infestation across different environments.

Across locations combined analysis of variance for *B. fusca* treatment revealed highly significant differences among lines, testers, lines x testers, and the line x tester x environment interactions for all the characters studied. The mean squares for the testcrosses, testers and interactions under *B. fusca* infestation at Embu and Kakamega showed highly significant differences for grain yield and borer resistance traits. The results showed that the testcrosses, testers and interactions had more dissimilarity in the expression of the stem borer resistance and agronomic traits studied. The findings may probably suggest that all testcrosses showed variable performance in two mega-environments.

There was a higher mean for cumulative stem tunneling, leaf feeding damage, and the number of exit holes under *B. fusca* infestation. These observations may be attributed to the longer life cycle of *B. fusca*.

3.4.2 General and specific combining ability

General and specific combining abilities as well as gene action for different stem borer resistance and agronomic traits have been estimated by many researchers (Butrón et al., 2009; Beyene et al., 2011; Sanghera et al., 2012; Wegary et al., 2013). In the current study, the significant difference of mean squares between lines, testers, lines x testers for stem borer resistance traits and grain yield showed their suitability for combining ability studies. Further, significant mean squares of lines, testers, lines x testers' revealed good possibility for manifestation of heterosis in all the traits studied. It is desirable that stem borer resistance traits namely; leaf feeding damage, cumulative stem tunneling, number of exit holes, and number of dead hearts to obtain negative GCA and SCA effects (Morais et al., 2012). Equally, positive GCA and SCA effect are necessary for grain yield, number of plants and ears per plant. The genetic variations due to lines and testers were significantly different at Embu and Kakamega for stem borer resistance parameters and grain yield, and other agronomic traits. This revealed a preponderance of the additive effects for these traits. Both additive and non-additive gene effects have been reported in the literature for stem borer resistance, and grain yield and yield components for various crops (Udaykumar et al., 2013). Similar results were reported in rice (Sanghera et al., 2012). Some SCA effects were not significant for some of the parameters measured, indicating that non-additive effects were not important in stem borer resistance, grain yield and other agronomic traits.

Maize inbred lines with high GCA also revealed hybrids with high SCA values for grain yield. For example, comparison of the lines and their responses to *B. fusca* at Embu and Kakamega only lines 16, 18, 30, 38 and 40 showed positive significant GCA effects for grain yield across locations for *B. fusca*. For *B. fusca* resistance different lines showed significant and negative GCA and SCA effects for cumulative tunneling, number of exit holes and the leaf feeding damage. This implies that these lines possess favourable alleles with additive genetic effects for resistance traits. The detected significant SCA effects suggest a deviation of a specific cross from the mean performance of the inbred parents (Hallauer, 1988). In hybrid formation and deployment to mega environments, there is a need for the targeted *B. fusca* specific varieties that combine high grain yield and resistance. The lines that showed positive significant GCA effects for grain yield and negative GCA and SCA effects across locations and borer resistance should be subjected to further testing and possible exploitation as parents in hybrid breeding. From this study, additive gene effects were shown to control cumulative stem tunneling,

number of exit holes, leaf feeding damage and the related agronomic characters. These results corroborate with previous findings in studies on stem borer resistance and grain yield (Beyene et al., 2011; Schnable et al., 2013; Udaykumar et al., 2013).

3.4.3 Heterotic orientations of lines under *B. fusca* infestation

Data on the relative heterosis was the basis of evaluation of the testcross performance for grain yield relative to the mean of the testers. For *B. fusca*, at Embu, 10 lines (1, 2, 3, 4, 5, , 51, 52, 53, 54, and 55), and at Kakamega, 10 lines (7, 10, 11, 20, 32, 33, 42, 43, 62, and 66) that showed positive heterosis for grain yield with both testers could be used in formation of high grain yielding and stem borer resistant hybrids. Relative heterosis was highest for grain yield, which is in tandem with other reports on maize (Sanghera et al., 2012; Liberatore et al., 2013). These results suggest that selected lines in the two locations represent breeding progress with higher grain yields exhibited compared to the commercial hybrids in the market. The differences in genotype performance may be due to the genotype x environment interactions. However, the magnitudes of the SCA effects for the lines were used in the clustering groups and the identification of response patterns for *B. fusca* in the various locations.

Lines were clustered into two groups A and B depending on the direction of the SCA estimate. At Embu, 12 and 8 lines revealed positive SCA estimates for grain yield with CML395/CML444 and CML312/CML442 respectively. Line 30 showed positive SCA estimates for grain yield with both testers. Similarly, at Kakamega, 11 and 9 lines exhibited positive SCA estimates with CML395/CML444 and CML312/CML442 respectively. Lines 10, 30, and 32 showed positive SCA estimates with both testers. At Embu, 22 lines were allocated to group A, 18 to group B and 8 to group AB, while the rest could not be classified since they showed negative heterosis for grain yield with both testers, while at Kakamega, 24 lines were oriented to group A, 13 to group B and 9 to group AB, while the rest could not be classified since they showed negative heterosis for grain yield with both testers. The implication of the findings on heterotic orientation of lines is such that if a line is designated to group A or B, it may be crossed to form new lines (A x B) that have a higher heterosis for *B. fusca* resistance and higher grain yield.

3.3.4 Heterosis relative to testers

At Embu, relative standard heterosis for grain yield grain yield was to -47.2% to -13.2% compared to the best check mean and trial mean probably due to strong genotypes x environment interaction effects. However, at Kakamega, the best 10 hybrids had heterosis for grain yield ranging from 79% to 176% in comparison to the best hybrid checks for grain yield under *B. fusca* infestation. Significant

correlations (r=0.29 p≤ 0.05) were detected between SCA and heterosis data, demonstrating that the two data sets may be used in identification of similar lines. In this study, lines 16, 18, 38 and 40 showed positive SCA with both testers, and were grouped similarly based heterosis and SCA data sets across locations akin to other studies (Fato et al., 2012).

The high genetic variability for *B. fusca* resistance detected in the testcross hybrids clustering with the two testers are desirable characteristics for a good maize tester. However, some lines did not show heterotic orientation to both testers. Theoretically, these lines may be useful in breeding; however, in practice they would be disposed. In addition, these findings suggest that various lines and groups identified may be useful in *B. fusca* specific hybrid breeding programmes across the tropical environments where these borers occur. However, the results also showed that some lines may have had good general combining ability, but probably require all new testers with new genetic constitution to distinguish them for *B. fusca* resistance (Guimaraes et al., 2012). The challenge is occasioned by the many lines that were not classified by both testers at the two locations. In this study, the unclassified heterotic orientations suggest need for new testers with new inherent structures, since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive to discriminating materials.

3.5 Conclusions

General combining ability effects were significant for *B. fusca* stem borer resistance. The results suggest that additive gene effects were more important than non-additive in the control of resistance for both borers. It is possible to identify specific lines that may be useful for hybrid breeding for specific ecologies where *B. fusca* stem borers occur exclusively or in league. In this study, lines 16, 18, 38 and 40 showed positive SCA effects with both testers, and were grouped similarly based on the two data sets across locations. The testers CML312/CML442 and CML395/CML444 were able to discriminate these materials based on the general combining ability for stem borer resistance, agronomic characters and grain yield. This implied that the testers can be used for line evaluations in breeding programmes for the identification of heterotic orientations in a hybrid-based stem borer resistance breeding programme.

In maize agroecologies where these *B. fusca* stem borers occur exclusively different lines were identified based on heterosis for grain yield data. The products from the line x tester evaluations are three way crosses that can be nominated directly into the national performance trials for further evaluation and deployment into maize growing areas where these borers occur exclusively. The implication is that these advanced lines may be used in hybrid breeding with emphasis on *B. fusca*

resistance breeding programmes in the tropics. Similarly, using specific combining ability estimates, various heterotic orientations identified testcrosses that showed positive significant SCA effects for *B. fusca* resistance and grain yield. These testcrosses may be evaluated further for *B. fusca* stem borer resistance and grain yield to confirm their stability. Finally, under artificial infestation conditions, it is desirable to consider grain yield in a hybrid breeding programme that emphasises *B. fusca* stem borer resistance.

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Appendix 3.

Table 3.14. List of germplasm used in the study

Entry No.	Pedigree code[¥]	Entry No.	Pedigree code
1	CKSBL10001	34	CKSBL10157
2	CKSBL10004	35	CKSBL10157
3	CKSBL10007	36	CKSBL10158
4	CKSBL10008	37	CKSBL10165
5	CKSBL10013	38	CKSBL10168
6	CKSBL10014	39	CKSBL10169
7	CKSBL10015	40	CKSBL10170
8	CKSBL10020	41	CKSBL10171
9	CKSBL10025	42	CKSBL10178
10	CKSBL10027	43	CKSBL10194
11	CKSBL10028	44	CKSBL10195
12	CKSBL10028	45	CKSBL10196
13	CKSBL10030	46	CKSBL10197
14	CKSBL10033	47	CKSBL10200
15	CKSBL10034	48	CKSBL10201
16	CKSBL10035	49	CKSBL10202
17	CKSBL10038	50	CKSBL10203
18	CKSBL10040	51	CKSBL10204
19	CKSBL10041	52	CKSBL10205
20	CKSBL10042	53	CKSBL10206
21	CKSBL10043	54	CKSBL10207
22	CKSBL10045	55	CKSBL10208
23	CKSBL10060	56	CKSBL10209
24	CKSBL10073	57	CKSBL10210
25	CKSBL10107	58	CKSBL10211
26	CKSBL10108	59	CKSBL10212
27	CKSBL10138	60	CKSBL10213
28	CKSBL10153	61	CKSBL10248
29	CKSBL10154	62	CKSBL10250
30	CKSBL10155	63	CKSBL10254
31	CKSBL10155	64	CKSBL10286
32	CKSBL10155	65	CKSBL10307
33	CKSBL10155	66	CKSBL10321

[¥]- full pedifgree information is available.

CHAPTER 4

Combining ability for Stem Borer Resistance and Heterotic Orientation of Maize inbred lines towards CIMMYT testers under *Chilo partellus* infestation

Abstract

The spotted stem borer, *Chilo partellus,* Swinhoe (Lepidoptera, Crambidae) is one of the most destructive insect pests of maize in tropical environments. However, the combining ability and heterotic orientation of the germplasm for grain yield and borer resistance is limited. The objective of this study was to determine combining ability and heterotic orientation of new maize inbred lines under *C. partellus* infestation. The 66 inbred lines were crossed to two single cross testers from CIMMYT in accordance with the line x tester mating scheme. The 132 testcross hybrids and four checks were evaluated at three locations in Kenya in a line x tester mating design under *C*. *partellus* infestation. Data were analysed using PROC GLM of SAS statistical package. The genotype x location interactions were highly significant, consequently the three test locations were considered as mega environments in assigning genotypes to heterotic groups. General combining ability effects were significant for *C. partellus* stem borer resistance and grain yield, suggesting a predominance of the additive gene effects for stem borer resistance traits. Specific combining ability effects were significant for *C. partellus* resistance traits and grain yield signifying that non-additive effects were also crucial for borer resistance and grain yield. Heterotic classification of lines was done based on both heterosis and specific combining ability data. Based on heterosis for grain yield data at Embu, 15 lines were allocated to group A, 18 to group B and 12 to group AB. At Kakamega, 26 lines were oriented towards group A, 19 to group B and 9 to group AB. At Kiboko, 15 lines were inclined towards group A, 18 to group B and 11 to group AB, whilst the remainder could not be classified. Based on the SCA estimates, at Embu, 10 lines revealed positive SCA effects with both testers and were considered to be AB-oriented while 8, 1 and 1 lines were oriented towards A, B and AB, respectively. A similar trend was detected at Kakamega and Kiboko. The identified lines and heterotic groups would be used by maize programmes that emphasize stem borer resistance in hybrids. In maize agroecologies where *C. partellus* stem borers occur exclusively or in league with other stem borers, the lines identified may be used as parents in maize hybrid breeding programmes with focus on borers.

Keywords: combining ability, *Chilo partellus*, stem borer resistance, heterosis, heterotic orientation, tropical maize

4.1 Introduction

Maize is the principal crop grown by the mainstream of rural families in Sub-Saharan Africa (SSA). Maize is both a staple food and a cash crop through consumption and income generation for small-scale farmers (Brooks et al., 2009; Sasson, 2012), respectively. The progress made in breeding plants for improved quality and tolerance to both biotic and abiotic stresses has led to development of new maize hybrids with better agronomic characteristics. In SSA, several generations of the insect pests occur per season, leading to high pest incidences that result into high levels of crop losses because of the friendly environmental conditions that enable insect development (Kfir et al., 2002; Tefera, 2012). Stem borers attacking cereal crops are considered one of the devastating biotic stress factors limiting production of maize in tropical Africa.

The spotted stem borer, *Chilo partellus,* Swinhoe (Lepidoptera, Crambidae), is one of the serious borer species affecting maize in SSA. It consists of 41 pestiferous species that are significant in Africa and Asia. *C. partellus* occupied Africa from Asia before 1930's and accounts for 90% of the stem borers in the lowland tropics, mid altitude and the moist transitional areas of East Africa (Ong'amo et al., 2012). The distribution and occurrence of *C. partellus* stem borers in different locations and crop ecosystems is varied (Ong'amo et al., 2012). Several factors affect *C. partellus* population dynamics specifically; host availability, location and suitability, mate location, success of oviposition, larval survival and establishment, temperature and altitude (Mailafiya et al., 2011; Ong'amo et al., 2012). Although *C. partellus* is absent in the highland tropics, it is progressively intensifying its range to higher altitudes, and currently, it is the most widely distributed stem borer in the maize growing zones in Kenya (Kfir et al., 2002; Tefera et al., 2011).

C. partellus in combination with other stem borer species greatly reduce maize grain yield in tropical environments ranging from 10% to total loss (Ajala et al., 2010). In Kenya, grain yield loss due to stem borers in maize is estimated annually at about 400,000 metric tonnes or about $72 million (De Groote et al., 2003). This amount represents approximately 13.54% of the farmers' total annual harvest of maize and prompts breeding investigations.

Various management options exist for alleviating the damaging effects due to maize stem borers, but each opportunity has its own limitations. For example, host plant resistance forms an important part of integrated pest management since it provides inherent control without environmental concerns, and it is compatible with other pest management methods (Mugo et al., 2005). Currently varieties with host plant resistance are limited in most tropical environments. Therefore, effective breeding methods for resistance to *C. partellus* stem borer damage may be designed by plant breeders using both improved

and new sources of resistance. A better understanding of the genetic basis of the resistances among the germplasm used may contribute towards the development of effective approaches against these *C. partellus* borers.

Appropriate maize germplasm with resistance to *C. partellus* borers should be deployed where they occur. Identification and improvement of maize germplasm for resistance to *C. partellus* may be useful against other borers where they exist. Farmers exchange maize germplasm across agro-ecologies, therefore the requisite to identify resistance to these borers in tropical maize inbred lines becomes key (Adijah et al., 2011). There is a need to breed and promote genotypes with *C. partellus* resistance, and to support their widespread acceptance across maize growing areas.

Heterosis in maize has been reported in literature since 1900's through studies carried out by Shull (1908) and East (1909). It refers to the superiority of highly heterozygous F1-hybrids in relation to the mid parent performance of their genetically distinct homozygous parents (Avinashe et al., 2013). For stem borer resistance, hybrid maize breeding programmes must encompass and exploit the knowledge of general combining ability (GCA) of lines and specific combining ability (SCA) of their testcrosses, and heterosis and heterotic orientation. The knowledge of combining ability, type of gene action controlling economic traits, and heterosis is useful in fixing the appropriate parent lines, and in designing successful hybrids (Liberatore et al., 2013). Maize inbred lines with a high genetic diversity, strongly determine the levels of heterosis exhibited by the single cross hybrids and vice versa (Hallauer et al., 1988), and may be useful in hybrid development. Unfortunately such information is limited which affects the efficacy of stem borer resistance programs.

The line x tester mating design provides reliable information on the general and specific combining ability effects of parents and their hybrid combinations (Kempthorne, 1957), and has been effectively applied in various previous quantitative genetic investigations in maize (Kanagarasu et al., 2010). The line x tester mating scheme is mainly used to generate data on the nature and magnitude of gene action, combining ability effects, heritability and nature and extent of heterosis for different traits (Sanghera et al., 2012). For example, Sprague and Tatum, (1942) on studies in maize yield found that general combining ability is mainly due to the additive gene effects while specific combining ability is due to dominance or epistatic effects. This mating scheme has been applied for determining the possible gene action configuration for stem borer resistance in maize (Sharma et al., 2007). Its application in the early generations of breeding mostly S_2 or S_3 generations reduces the amount of germplasm for advancing with positive budgetary implications. Populations and inbred lines or single cross hybrids have been used as testers in the identification of hybrids for yield performance (Sanghera et al., 2012). This mating design continues to be applied in determination of the maize heterotic

orientations using different testers (Morais et al., 2012). The design was therefore used in the study to evaluate testcross hybrids in the target locations. Single cross testers were used in the current study because the end product would be a three way cross. The three way cross is the most appropriate for majority of the farmers in SSA due to the low price of seed compared to the single cross hybrids.

The objective of this study was to determine combining ability and heterotic orientation of maize inbred lines under *C. partellus* infestation. The information generated was important in the allocation of inbred lines and testcrosses into heterotic clusters as a basis for possible exploitation in a hybrid breeding program with focus on *C. partellus* stem borer resistance.

4.2 Materials and Methods

4.2.1 Germplasm

The experimental materials used in this study are described in Chapter 4.

4.2.2 Experimental sites

Experiments were established at Embu, Kakamega and Kiboko locations in Kenya. Features of KARI Kakamega and KARI-Embu are described in Chapter 4. KARI-Kiboko (2°15'S 37°75' E, 975 m asl) is located in the dry mid altitude agro-ecological zone of eastern Kenya and experiences mean annual temperature ranges of 28 to 37^{o}C, with February and October being the hottest months. Kiboko receives a mean annual rainfall of approximately 530mm. The soils are well drained, Fluvisols, Ferralsols, and Luvisols with soil pH of about 7.9 (Jaetzold et al., 1982; KARI Land Resources and Analytical Services, 2007).

4.2.3 Experimental design and Treatments

The experimental design and treatments are as described in Chapter 3.

4.2.4 Artificial infestation with insects

Artificial infestations with insects are as described in Chapter 3.

4.2.5 Data collection and analysis

Data collection and analysis are as described in Chapter 3.

4.3 Results

4.3.1 Genotype x environment interactions

There was a highly significant genotype x environment interactions for grain yield, therefore the three test locations were treated as mega environments. Consequently, the results are presented on a site by site basis.

4.3.2 Trait variations under *Chilo partellus* infestation

The mean squares of the test cross from the combined analysis of selected stem borer resistance and agronomic traits for *C. partellus* at Embu, Kakamega and Kiboko were significant (p≤0.05) for most traits (Table 4.1). The site and the lines showed highly (p≤0.01) significant differences for all traits. The testers showed highly significant (p≤0.05) differences for gain yield and ear aspect. The line x tester interaction effects were highly significant (p≤0.05) for grain yield, cumulative stem tunneling, leaf feeding damage and plant aspect (Table 4.1). The sites x line interaction effects were significant (p≤0.05) for all traits except leaf feeding damage. The sites x tester, and the sites x line x tester interaction effects were not significant for all traits except for grain yield (p≤0.05) (Table 4.1). The mean each for the following traits was detected under *C. partellus* infestation; grain yield (1.17 t ha^{-1}), cumulative stem tunneling (9.06 cm), number of exit holes (3.99), leaf feeding damage score (2.21) and ear aspect (2.53), plant aspect (2.46), days to anthesis (74.02), days to silking (75.13) and stem diameter of (2.28 cm) (Table 4.1).

Table 4.1. Mean squares of combined analysis for selected stem borer resistance and agronomic traits for hybrids over six environments under *C. partellus* infestation

Source	DF	GY	TL	EXH	LD	EA	PA	AD	SD	DIAM
Rep	2	0.37	139.48	29.73	8.92	2.35	1.40	7.44	3.52	1.48*
Env.	5	280.46**	304.16**	451.42**	144.31**	23.21**	61.37**	11652.58**	11876.82**	37.71**
Line	65	6.77**	69.24**	33.68**	6.38**	1.84**	1.39**	64.64*	59.71*	2.08**
Tester	1	0.13	11.68	54.31	11.48	5.42**	2.10	3.97	9.80	3.12*
Env.*Line	325	4.79**	46.27**	15.23**	4.04	0.95	1.62**	63.76**	66.25**	1.31**
Env.*Tester	5	1.50*	34.76	6.17	5.51	0.45	2.33**	8.82	7.70	0.87
Line*Tester	65	1.55**	34.57	20.99**	4.64	0.85	1.29*	9.57	10.97	0.69
Env.*Line*Tester	325	0.83**	25.65	9.24	2.13	0.79	0.71	10.62	10.89	0.34
Error	1581	0.56	30.54	11.05	3.75	0.80	0.91	47.46	48.24	0.49
Cp Mean		**1.17**	**9.06**	**3.99**	**2.21**	**2.53**	**2.46**	**74.02**	**75.13**	**2.28**
(%) R^2		**80.96**	**39.94**	**44.32**	**37.14**	**40.70**	**46.52**	**53.76**	**54.02**	**54.36**

GY - grain yield, TL- cumulative stem tunneling, EXH-number of exit holes, LD-leaf feeding damage, EA- ear aspect, AD-days to anthesis, SD-days to silking, DIAM-stem diameter, *, ** = significant (p≤0.05), highly significant (p≤0.01)

4.3.3 General combining ability effects

Results of general combining ability effects of top 20 lines and their corresponding two testers for *C. partellus* stem borer resistance traits and grain yield are presented for Embu, Kakamega and Kiboko (Table 4.2).

At Embu, Kakamega and Kiboko, for grain yield, positive significant ($p \leq 0.05$) GCA effects were detected for all top 20 lines. Negative significant ($p \leq 0.05$) GCA effects were detected for cumulative stem tunneling ranging from -7.47 to -4.69 and number of exit holes from -3.04 to 2.25. However, no significant GCA effects were found for leaf feeding damage for *C. partellus* in all environments. The number of days to anthesis and days to silking showed negative significant ($p \leq 0.05$) GCA effects for all top lines across locations. The testers; CML395/444 and CML312/442 had varied trends for GCA effects for the various stem borer resistance and agronomic traits for *C. partellus* at the three locations (Table 4.2).

Table 4.2. General combining ability estimates of top 20 maize inbred lines for selected stem borer resistance traits and grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko

	Site	Line	GY	Site	Line	TL	Site	Line	EXH	Site	Line	LD	Site	Line	SD	Site	Line	AD
	A	1	0.99**	A	30	-6.24**	A	41	-2.85**	A	14	-1.16	A	13	-2.68**	A	13	-2.31**
	A	2	0.75**	A	31	-6.34**	A	42	-2.71**	A	34	-1.29	A	16	-1.68*	A	16	-2.14**
	A	3	0.72**	A	36	-6.69**	A	43	-2.73**	A	35	-1.29	A	17	-1.35*	A	28	-1.48*
	A	10	0.32*	A	37	-4.87**	A	44	-3.04**	A	41	-1.29	A	28	-2.02	A	38	-1.48*
	A	18	0.79**	A	38	-5.07**	A	45	-2.41**	A	54	-1.14	A	38	-1.52*	A	45	-2.98**
	A	19	0.89**	A	40	-5.92**	A	49	-2.36**	A	55	-1.13	A	45	-1.68*	A	53	-1.31*
	A	20	0.93**	A	42	-5.94**	A	60	-2.71**	A	57	-1.12	A	50	-1.52*	A	66	-2.31**
	A	21	0.74**	A	46	-5.79**	A	63	-2.54**	A	58	-1.13	A	59	-1.85*	B	28	-1.75*
	A	22	0.91**	A	48	-6.12**	A	66	-2.26**	A	59	-1.19	A	60	-1.52*	B	56	-1.25*
	A	23	0.92**	A	50	-6.39**	B	42	-3.39**	A	61	-1.13	A	66	-2.85**	B	66	-1.38*
	A	24	0.48	B	36	-7.09**	B	44	-2.89**	A	62	-1.13	B	63	-1.47*	C	4	-1.44*
	A	25	0.76**	B	37	-6.40**	B	45	-2.34**	A	63	-1.16	C	9	-1.99*	C	11	-2.28*
	A	26	0.39*	B	38	-5.87**	B	46	-3.29**	A	64	-1.15	C	11	-2.66**	C	24	-2.28**
	A	28	0.40*	B	43	-7.47**	B	47	-2.75**	A	65	-1.10	C	16	-2.16**	C	33	-2.44**
	A	30	0.17	B	44	-6.24**	B	49	-2.25**	A	66	-1.17	C	18	-2.16**	C	39	-1.61*
	A	40	0.29*	B	46	-5.35**	B	51	-2.47**	B	34	-1.13	C	24	-3.33**	C	40	-1.28*
	B	8	0.27*	B	49	-5.99	B	54	-2.33**	B	35	-1.12	C	32	-1.49*	C	43	-2.11**
	B	29	0.24*	B	50	-5.79**	B	55	-2.59**	B	53	-1.08	C	33	-1.83*	C	48	-3.11**
	B	37	0.19*	B	51	-4.69**	B	59	-2.49**	B	54	-1.01	C	39	-1.66*	C	58	-1.31*
	B	47	0.25*	B	52	-5.67**	B	60	-2.74**	B	55	-1.01	C	48	-2.99**	C	62	-1.28*
	C	25	0.15*	C	3	-5.19**	B	63	-2.55**	B	58	-1.02	C	58	-1.56**	C	63	-2.44**
	C	53	0.15*	C	63	-6.30**	B	64	-2.29**	B	61	-1.02	C	62	-1.76*	C	66	-2.91**
Standard Error-A			**0.23**			**2.19**			**1.24**		**-**	**-**			**1.06**			**0.94**
Standard Error- B			**0.23**			**2.25**			**1.51**		**-**	**-**			**0.82**			**0.88**
Standard Error -C			**0.23**			**2.50**			**1.24**		**-**	**-**			**1.81**			**1.71**

Site	Tester	GY	TL	EXH	LD	AD	SD	PH	PA	EA
Embu	CML395/CML444	0.24	7.37	0.05	0.01	0.99	-0.08	-3.56	0.53	-0.07
	CML312/CML442	-0.24	-7.37	-0.05	-0.01	-0.99	0.08	3.56	-0.53	0.07
Kakamega	CML395/CML444	-0.17	8.05	0.26	-0.12	-	1.36	-0.24	-0.09	-
	CML312/CML442	0.16	8.05	-0.26	0.12	-	-1.35	0.24	0.09	-
Kiboko	CML395/CML444	-0.25	11.00	-0.94	1.19	-	1.71	-1.88	-0.59	-
	CML312/CML442	0.25	-11.00	0.94	-1.19	-	-1.71	1.88	0.59	-
Standard Error		**0.03**	**0.64**	**0.12**	**0.08**	**0.50**	**0.22**	**1.57**	**0.05**	**0.12**

Sites A=Embu, B=Kakamega and C=Kiboko, GY - grain yield, TL-cumulative stem tunneling, EXH-number of exit holes, LD-leaf feeding damage, PA-plant aspect, EA – ear aspect, AD-days to anthesis, SD-days to silking, *, ** = significant (p≤0.05), highly significant (p≤0.01), SE for heterosis for grain yield = 0.33

4.3.4 Specific combining ability effects

Results of SCA effects of top 20 lines and their corresponding two testers for *C. partellus* stem borer resistance, grain yield and agronomic traits are presented for Embu, Kakamega and Kiboko (Table 4.3 and Table 4.4). The SCA data was averaged over seasons at each site.

At Embu, all the testcrosses revealed significant and desirable SCA effects (P≤0.05) for grain yield except testcrosses with CML395/CML444. However, the same testcrosses had significant and desirable SCA effects (P≤0.05) for the following traits; cumulative stem tunneling ranging from -7.77 to -2.26, number of exit holes from -2.91 to -1.03 and leaf damage score from -2.75 to -1.43. Similarly, there were significant and desirable SCA effects (P≤0.05) for the following agronomic traits and testcrosses; days to anthesis and days to silking, plant height, and plant and ear aspects (Table 4.3 and Table 4.4).

At Kakamega, significant and desirable SCA effects (P≤ 0.05) for grain yield were detected for the following 10 testcrosses. Six out of 10 testcrosses that showed desirable SCA effects grain yield were crosses with CML312/CML442. These testcrosses had significant and desirable SCA effects (P≤0.05) for the following borer resistance characters; cumulative stem tunneling ranging from -6.21 to -2.57, number of exit holes from -3.61 to -1.26 and leaf damage score from -2.71 to -1.32 (Table 4.3 and Table 4.4). Twelve testcrosses displayed significant and desirable SCA effects (P≤0.05) for days to anthesis and days to silking and 5 testcrosses for plant height and 6 testcrosses for plant aspects. There were no testcrosses that showed significant SCA effects for ear aspects (Table 4.3 and Table 4.4).

At Kiboko, significant and desirable SCA effects (P≤0.05) for grain yield were detected for all testcrosses with CML312/CML442. Only testcross 65 involving CML395/CML444 showed significant desirable SCA effects for grain yield at Kiboko (Table 4.3 and Table 4.4). Similarly, these testcrosses had significant and desirable SCA effects (P≤0.05) for cumulative stem tunneling, number of exit holes, and leaf damage (Table 4.3 and Table 4.4).

Table 4.3. Specific combining ability effects of top 20 testcrosses for selected stem borer resistance traits and grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site)

Embu					Kakamega					Kiboko				
TC	**GY**	**TL**	**EXH**	**LD**	**TC**	**GY**	**TL**	**EXH**	**LD**	**TC**	**GY**	**TL**	**EXH**	**LD**
11 x 2	-0.02	-7.77**	-2.91**	-2.75**	4 x 1	-0.01	-3.63**	-1.54	-1.43	3 x 2	0.02**	-3.46**	-1.19	-0.71
11 x 1	0.02**	-5.56**	-2.87**	-2.58**	4 x 2	0.01**	-2.57	-1.56	-1.58*	3 x 1	-0.02	-2.45	-2.18*	-0.48
16 x 2	-0.04	-5.09**	-2.73**	-2.50**	11 x 1	0.01**	-2.70	-1.71	-1.69*	5 x 2	0.02**	-2.57	-2.38*	-1.17
16 x 1	0.04**	-4.94**	-2.35	-2.19**	11 x 2	0.01**	-2.81	-1.56	-1.84*	5 x 1	-0.02	-2.68	-2.87*	-0.60
26 x 2	-0.04	-4.74**	-2.08	-2.06**	15 x 1	-0.01	-5.60**	-1.94	-1.67*	13 x 2	0.05**	-5.33**	-0.92	-0.55
26 x 1	0.04**	-4.32**	-1.87	-1.80**	15 x 2	0.01**	-4.36**	-4.10**	-1.90*	13 x 1	-0.05	-4.15**	-1.61	-0.48
28 x 2	-0.05	-4.29**	-1.80	-1.68**	19 x 1	-0.04	-4.18	-8.04**	-2.17	14 x 2	0.03**	-3.98**	-2.39*	-0.95
28 x 1	0.05**	-4.17	-1.79	-1.68**	21 x 1	-0.04	-2.49	-2.22	-1.46	31 x 2	0.05**	-2.37	-2.47*	-0.95
39 x 2	-0.06	-4.08	-1.74	-1.63**	26 x 1	0.01*	-5.88**	-1.59	-2.61*	31 x 1	-0.05	-5.60**	-2.59*	-0.72
39 x 1	0.06**	-3.99	-1.64	-1.60**	26 x 2	-0.01	-3.31	-2.28	-2.71*	39 x 2	0.05**	-3.15**	-0.87	-0.63
46 x 2	0.06**	-3.51	-1.62	-1.60**	30 x 1	-0.03	-2.94	-2.28	-1.55	39 x 1	-0.05	-2.80	-1.01	-0.60
46 x 1	0.06**	-3.36	-1.60	-1.55**	43 x 1	-0.01	-3.02	-1.74	-1.33	43 x 2	0.05**	-2.88	-0.92	-0.65
9 x 2	-0.07	-3.19	-1.54	-1.52**	43 x 2	0.01*	-2.71	-3.61**	-1.36	43 x 1	-0.05	-2.58	-1.14	-0.61
9 x 1	0.07**	-3.01	-1.39	-1.51**	46 x 1	-0.04	-2.57	-2.66	-1.40	60 x 2	0.05**	-2.45	-1.01	-0.56
40 x 2	-0.1	-2.88	-1.38	-1.50**	50 x 1	-0.04	-2.70	-3.24**	-1.32	60 x 1	-0.05	-2.57	-0.98	-0.62
40 x 1	0.11**	-2.66	-1.37	-1.49**	51 x 1	-0.03	-2.92	-1.51	-1.38	61 x 2	0.05**	-2.78	-0.89	-0.48
29 x 2	0.10**	-2.44	-1.20	-1.48**	51 x 2	0.03*	-5.93**	-3.01*	-1.38	61 x 1	-0.05*	-5.65**	-2.74*	-0.76
29 x 1	0.11**	-2.44	-1.10	-1.45	64 x 1	-0.03	-6.21**	-1.34	-1.64*	65 x 1	0.05**	-5.91**	-0.91	-0.57
60 x 2	-0.11	-2.36	-1.08	-1.44	64 x 2	0.03**	-4.00	-1.91	-1.42	65 x 2	-0.05	-3.81	-1.22	-0.49
60 x 1	0.11**	-2.26	-1.03	-1.43	66 x 2	0.01**	-4.12	-1.26	-1.56	66 x 2	0.06*	-3.92	-1.21	-0.86
SE	**0.23**	**2.18**	**1.24**	**0.74**		**0.13**	**2.25**	**1.51**	**0.68**		**0.14**	**2.50**	**0.97**	**0.71**

TC=testcross, GY- grain yield, TL-cumulative stem tunneling, EXH-number of exit holes, LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01), 1=CML395/CML444 and 2=CML312/CML442, SE for heterosis for grain yield = 0.33

Table 4.4. Specific combining ability effects of top 20 testcrosses for selected agronomic traits under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site)

Embu						Kakamega						Kiboko					
T/cross	**AD**	**SD**	**PH**	**PA**	**EA**	**T/cross**	**AD**	**SD**	**PH**	**PA**	**EA**	**T/cross**	**AD**	**SD**	**PH**	**PA**	**EA**
11 x 2	-2.29**	-3.08**	-15.60	-0.44*	-0.38	4 x 1	-0.82	-0.81	-14.41	-0.59	-0.90	3 x 2	-0.82	-0.81	-14.41	-0.59	-0.90*
11 x 1	-2.12**	-2.59**	-14.80	-0.32	-0.72**	4 x 2	-0.69	-0.65	-15.24	-0.42	-0.88	3 x 1	-0.69	-0.65	-15.24	-0.42	-0.88*
16 x 2	-2.06**	-2.09**	-28.11**	-0.35	-0.38	11 x 1	-0.69	-0.65	-39.76**	-0.76**	-0.76	5 x 2	-0.69	-0.65	-39.76**	-0.76**	-0.76
16 x 1	-2.05**	-1.91*	-13.60	-0.39	-0.28	11 x 2	-0.85*	-0.65*	-31.42**	-0.58	-0.73	5 x 1	-0.85*	-0.65*	-31.42**	-0.58	-0.73
26 x 2	-2.05**	-1.91*	-49.41**	-0.32	-0.45	15 x 1	-0.82*	-1.36*	-13.09	-0.59	-0.73	13 x 2	-0.82*	-1.36*	-13.09	-0.59	-0.73
26 x 1	-1.88**	-1.91*	-12.70	-0.35	-0.37	15 x 2	-0.82*	-0.65*	-30.09**	-0.58	-0.72	13 x 1	-0.82*	-0.65*	-30.09**	-0.58	-0.72
28 x 2	-1.88**	-1.91*	-11.90	-0.40*	-0.30	19 x 1	-1.19*	-0.65*	-18.09	-0.66	-0.71	14 x 2	-1.19*	-0.65*	-18.09	-0.66	-0.71
28 x 1	-1.88*	-1.90*	-27.74**	-0.32	-0.28	21 x 1	-0.82*	-0.65*	-21.76	-0.51	-0.65	31 x 2	-0.82*	-0.65*	-21.76*	-0.51	-0.65
39 x 2	-1.79*	-1.76*	-28.30*	-0.52*	-1.03**	26 x 1	-0.69	-0.65	-20.76	-0.58	-0.61	31 x 1	-0.69	-0.65	-20.76*	-0.58	-0.61
39 x 1	-1.79*	-1.76*	-11.90	-0.60*	-0.47	26 x 2	-0.67	-0.65	-17.73	-0.76**	-0.6	39 x 2	-0.82*	-0.65*	-17.73	-0.76**	-0.60
46 x 2	-1.71	-1.74	-21.40*	-0.35	-0.55	30 x 1	-0.82*	-0.65*	-14.76	-0.66	-0.56	39 x 1	-0.82*	-0.65*	-14.76	-0.66	-0.56
46 x 1	-1.62	-1.59	-15.80	-0.60*	-0.33	43 x 1	-0.99*	-0.81*	-26.26**	-0.49	-0.52	43 x 2	-0.99*	-0.81*	-26.26**	-0.49	-0.52
9 x 2	-1.62	-1.59	-13.10	-0.40*	-0.45	43 x 2	-1.02*	-0.65*	-12.26	-0.99**	-0.52	43 x 1	-1.02*	-0.65*	-12.26	-0.99**	-0.52
9 x 1	-1.55	-1.59	-23.10*	-0.32	-0.30	46 x 1	-0.69	-0.56	-27.74**	-0.67	-0.48	60 x 2	-0.69	-0.56	-27.74**	-0.67	-0.48
40 x 2	-1.55	-1.58	-12.40	-0.51	-0.53	50 x 1	-0.99*	-0.48*	-23.57	-0.76**	-0.48	60 x 1	-0.99*	-0.48*	-23.57*	-0.76**	-0.48
40 x 1	-1.38	-1.58	-21.10	-0.35	-0.55	51 x 1	-0.69*	-0.81*	-11.91	-0.49	-0.47	61 x 2	-0.69	-0.81	-11.91	-0.49	-0.47
29 x 2	-1.38	-1.42	-23.90*	-0.43*	-0.45	51 x 2	-0.82*	-0.81*	-14.41	-0.49	-0.41	61 x 1	-0.82	-0.81	-14.41	-0.49	-0.41
29 x 1	-1.38	-1.42	-12.70	-0.57*	-0.37	64 x 1	-0.69	-0.65	-24.41	-0.84**	-0.4	65 x 1	-0.69	-0.65	-24.41*	-0.84**	-0.40
60 x 2	-1.29	-1.42	-31.44**	-0.52*	-0.30	64 x 2	-1.02	-1.48	-12.74	-0.83**	-0.38	65 x 2	-1.02	-1.48	-12.74	-0.83**	-0.38
60 x 1	-1.29	-1.42	-20.40*	-0.44*	-0.70	66 x 2	-0.69	-0.65	-11.41	-0.51	-0.38	66 x 2	-0.69	-0.65	-11.41	-0.51	-0.38
SE	**0.93**	**1.05**	**12.66**	**0.42**	**0.30**		**0.87**	**0.82**	**12.61**	**0.35**	**0.40**		**1.71**	**1.80**	**12.56**	**0.41**	**0.29**

T/cross = testcross, AD-days to anthesis, SD- days to silking, PH - plant height, PA - plant aspect and EA - ear aspect, *, ** = significant (p≤0.05), highly significant (p≤0.01), 1=CML395/CML444 and 2=CML312/CML442

4.3.5 Heterotic orientations of lines based on specific combining ability

Below are results of the heterotic orientations of lines based on specific combining ability data for grain yield under *C. partellus* infestation. Heterotic orientation to CML312/CML442 and CML395/CML444 was determined according to the CIMMYT heterotic classification system as A and B, respectively (Table 4.5). Clustering of the lines into groups A and B depended on the direction of the SCA estimate such that lines displaying positive SCA with tester A were oriented towards the opposite heterotic group B, and vice versa, whereas lines exhibiting positive SCA to both testers were elected as AB group.

At Embu, 8 lines showed significant ($p \leq 0.05$) positive SCA effects for grain yield with CML395/CML444 therefore they were oriented towards heterotic group A. Lines 46 and 60 fitted into heterotic group AB and B, respectively (Table 4.5).

At Kakamega, 8 lines showed positive SCA estimates for grain yield with CML312/CML442, therefore they were oriented towards heterotic group B. Lines 26 and 51 were oriented towards heterotic group A and the remainder into group AB (Table 4.5).

At Kiboko, 2 lines (31 and 65) showed positive SCA estimates for grain yield with CML395/CML444, therefore they were allocated to heterotic group A, however, 9 lines displayed significant ($p \leq 0.05$) positive SCA effects with CML312/CML442 and were oriented towards heterotic group B. Lines 43 and 46 showed positive SCA effects for grain yield with both CML395/CML444 and CML312/CML442 in at least two locations, so they were consistently classified into AB group (Table 4.5).

Table 4.5. Heterotic orientation of top lines based on specific combining ability effects for grain yield under *C. partellus* infestation at Embu, Kakamega and Kiboko (averaged over 2 seasons per site)

	SCA effects for grain yield with		
Line	**CML312/CML442 (A tester)**	**CML395/CML444 (B tester)**	**Heterotic orientation**
Embu			
9	0.07**	-0.07**	A
11	0.02**	-0.02**	A
16	0.04**	-0.04**	A
26	0.04**	-0.04**	A
28	0.05**	-0.05**	A
29	0.10**	-0.10**	A
39	0.06**	-0.06**	A
40	0.10**	-0.10**	A
46	0.06**	0.06**	A/B
60	-0.11**	0.11**	B
Kakamega			
4	-0.01**	0.01**	B
11	-0.01**	0.01**	B
15	-0.01**	0.01**	B
19	-0.04	0.04	B
21	-0.03	0.03	B
26	0.01*	-0.01*	A
30	-0.04	0.04	B
43	0.02*	0.02*	A/B
46	0.03	0.03	A/B
50	-0.03	0.03	B
51	0.03*	-0.03*	A
64	-0.03**	0.03**	B
66	0.01**	0.01**	A/B
Kiboko			
3	-0.02**	0.02**	B
5	-0.02**	0.02**	B
13	-0.05**	0.05**	B
14	-0.03**	0.03**	B
31	0.05**	-0.05**	A
39	-0.05**	0.05**	B
43	-0.05**	0.05**	B
60	-0.05**	0.05**	B
61	-0.05**	0.05**	B
65	0.05**	-0.05**	A
66	-0.06*	0.06*	B

*, ** = significant ($p \leq 0.05$), highly significant ($p \leq 0.01$)

4.3.6 Heterosis of maize inbred lines relative to testers

At Embu, 10 inbred lines showed positive heterosis with both testers for grain yield and were oriented towards heterotic group AB under *C. partellus* infestation (Table 4.6). In total 15 lines were allocated to A, 18 to B and 12 to AB, while the remainder (Z) could not be classified with both testers (Figure 4.1 and Figure 4.2).

Table 4.6. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *C. partellus* infestation at Embu

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
40	116.4	103.5	AB
50	107.6	95.3	AB
47	103.3	91.2	AB
49	103.4	91.2	AB
48	95.4	83.8	AB
57	96.8	85.0	AB
1	82	71.2	AB
2	82	71.2	AB
3	82	71.2	AB
55	87	75.8	AB
CML395/CML444	**1.24**		
CML312/CML442		**1.32**	

Z= inbred lines that were unclassified, SE of heterosis for grain yield = 0.33

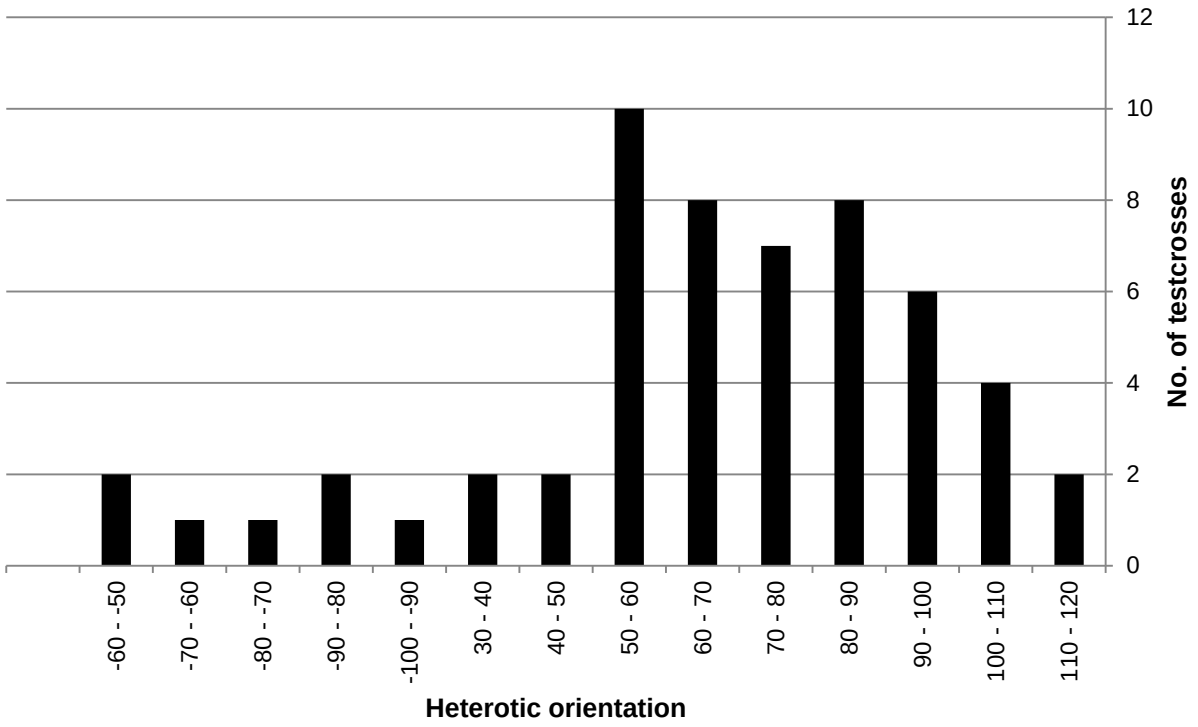

Figure 4.1. Distribution of heterosis (%) for 132 testcrosses under *C. partellus* infestation at Embu

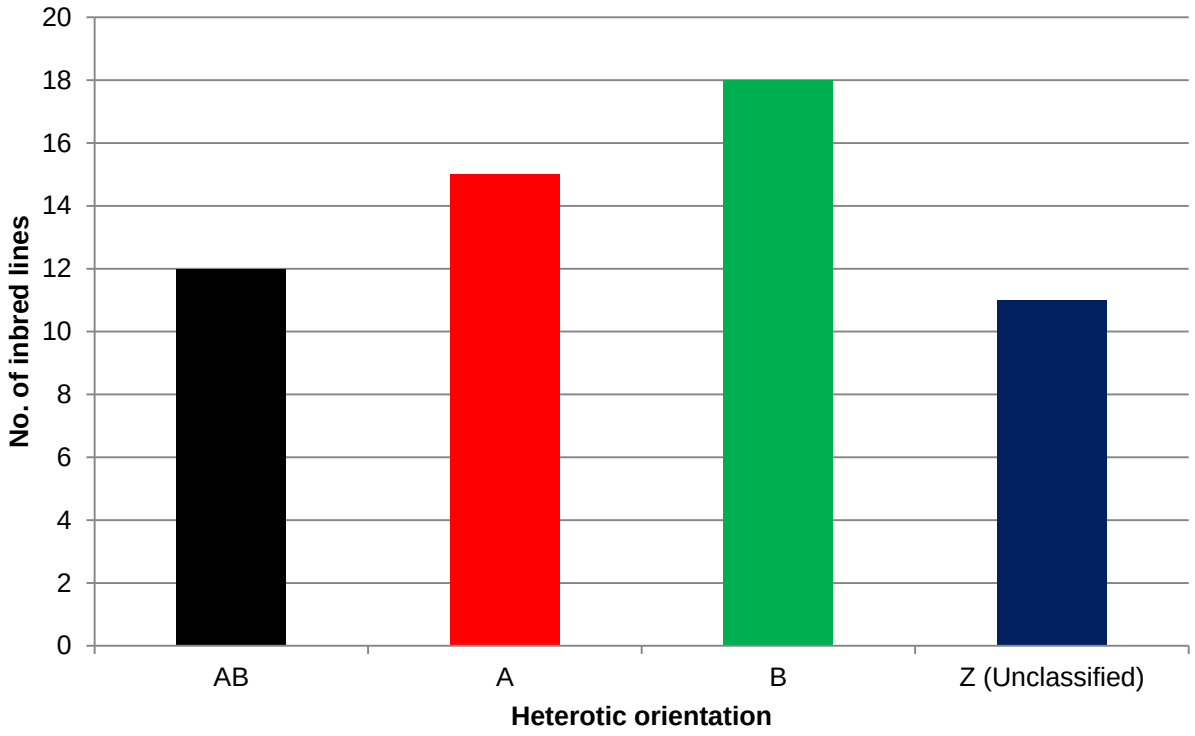

Figure 4.2. Classes of the heterotic orientations for 66 maize inbred lines under *C partellus* infestation at Embu

At Kakamega, inbred lines 29, 37, 4, 5, 6, 12, 13, 14, 20, and 27 and 28 exhibited positive heterosis with both testers for grain yield and were allocated to heterotic group AB under *C. partellus* infestation (Table 4.7). In total, 26 lines were allocated to A, 19 to B and 9 to AB, and 12 lines were not be classified with both testers (Figure 4.3 and Figure 4.4).

Table 4.7 Percent grain yield of testcrosses relative to the testers and heterotic orientation under *C. partellus* infestation at Kakamega

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
29	64.6	54.8	A
37	60.5	51.0	A
4	58.7	49.2	A
5	58.7	49.2	A
6	58.7	49.2	A
12	58.7	49.2	A
13	58.7	49.2	A
14	58.7	49.2	A
20	58.7	49.2	A
27	58.7	49.2	A
28	58.7	49.2	A
CML395/CML444	**0.59**		
CML312/CML442		**0.92**	

Z= inbred lines that were unclassified, SE of heterosis for grain yield = 0.33

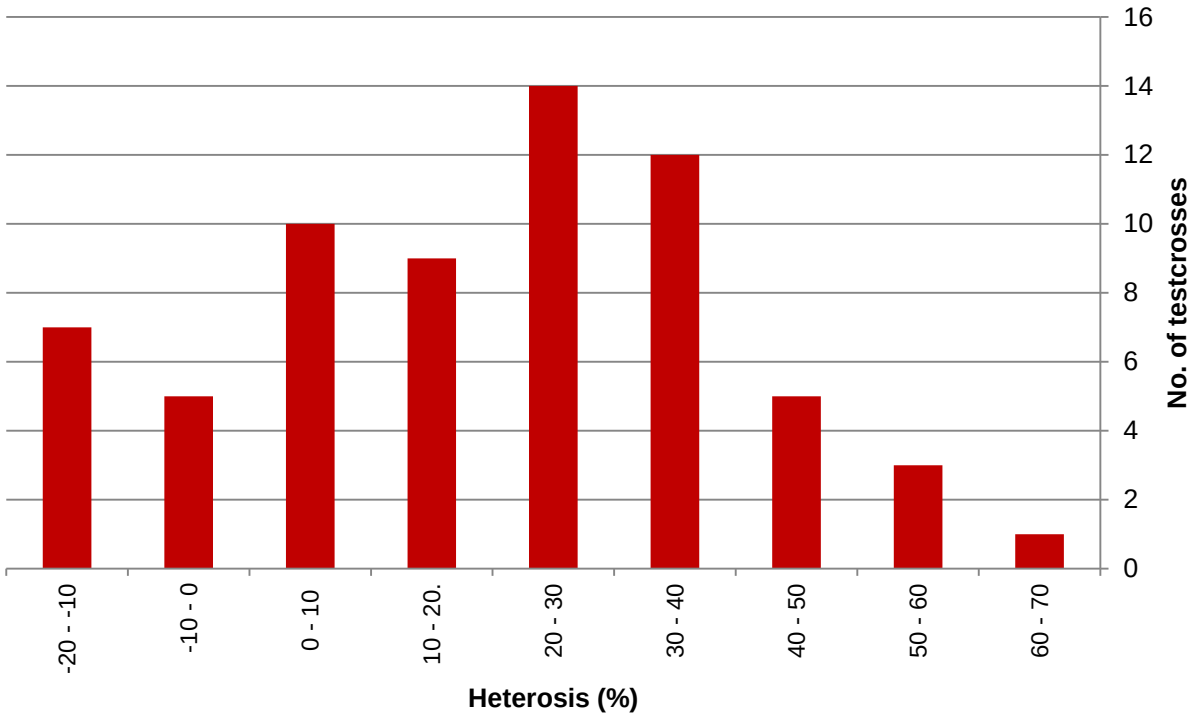

Figure 4.3. Distribution of heterosis (%) for 132 testcrosses under *C. partellus* infestation at Kakamega

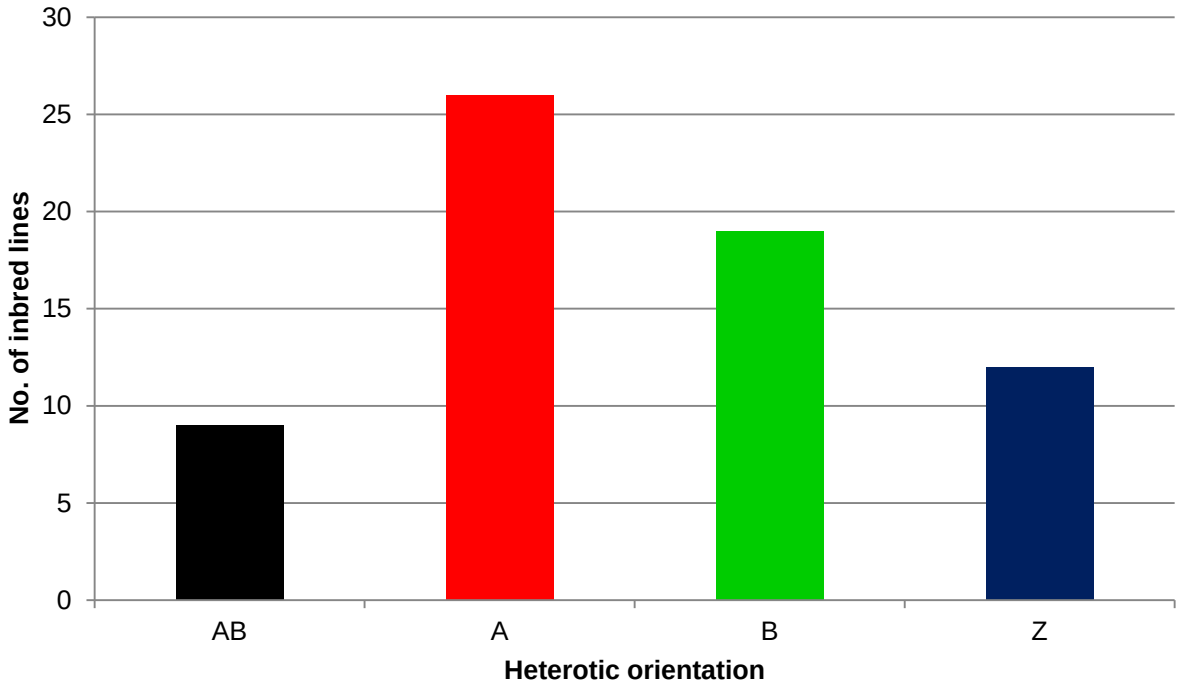

Figure 4.4. Classes of the heterotic orientations for 66 maize inbred lines under *C. partellus* infestation at Kakamega

At Kiboko, similar heterotic orientations were detected (Table 4.8), where 15 lines were allocated to A, 18 to B and 22 to AB, while the remainder could not be classified with both testers (Figure 4.5 and Figure 4.6).

Table 4.8. Percent grain yield of testcrosses relative to the testers and heterotic orientation under *C. partellus* infestation at Kiboko

Line	% yield relative to CML395/CML444	% yield relative to CML312/CML442	Heterotic orientation
25	39.6	31.3	AB
53	39.6	31.3	AB
9	36.9	28.7	AB
59	36.9	28.7	AB
10	31.5	23.7	AB
54	31.5	23.7	AB
8	28.9	21.2	AB
11	28.9	21.2	AB
CML395/CML444	**1.24**		
CML312/CML442		**1.32**	

Z= inbred lines that were unclassified, SE of heterosis for grain yield = 0.33

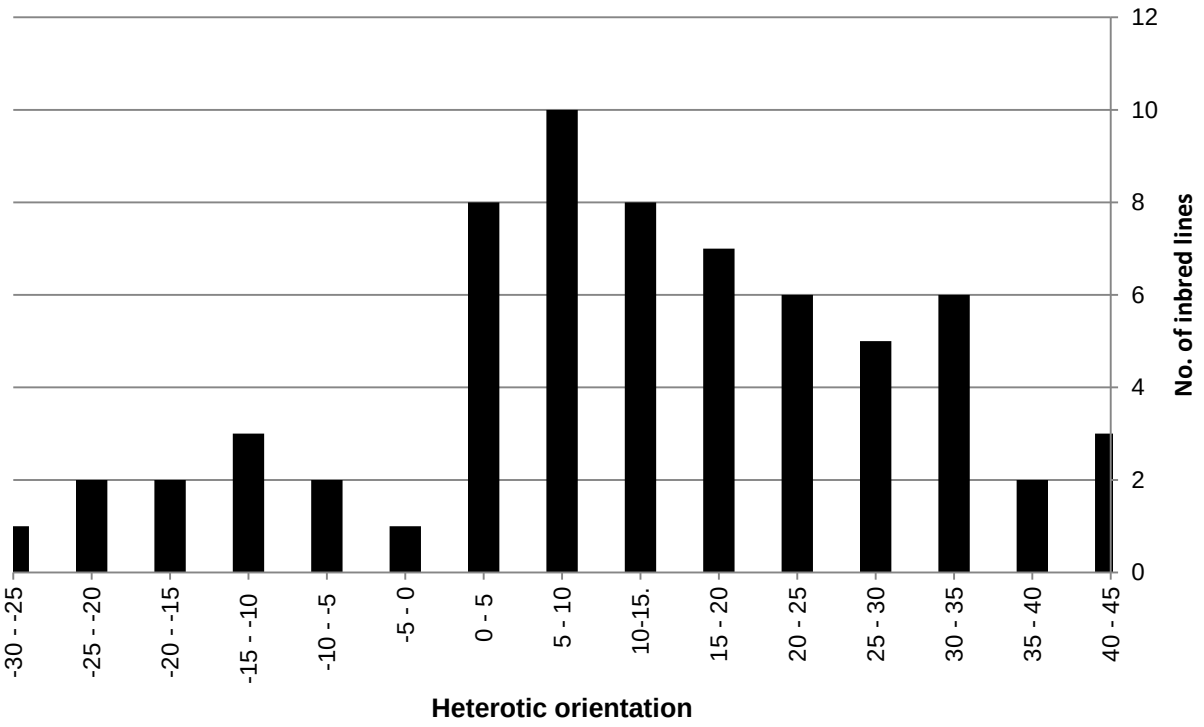

Figure 4.5. Distribution of heterosis (%) for 132 testcrosses under *C. partellus* infestation at Kiboko

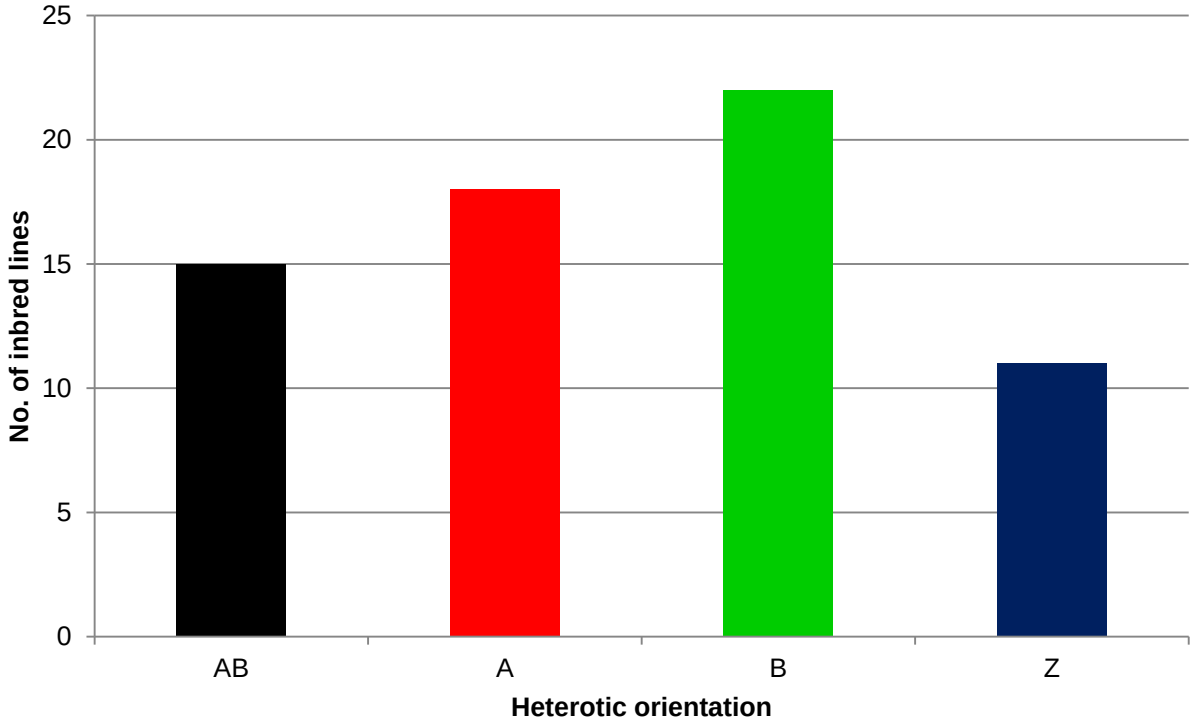

Figure 4.6. Classes of the heterotic orientations for 66 maize inbred lines under *C. partellus* infestation at Kiboko

4.4 Discussion

4.4.1 Genetic variation

The highly significant differences detected among the lines and their testcrosses for the various stem borer resistance and agronomic traits indicated the existence of considerable variation among the genotypes that allows for selection of preferred inbred lines and hybrids for *C. partellus*. There were revealed highly significant differences among lines, testers, lines x testers, and the line x tester x environment interactions for all the characters studied. The inferences that can be drawn from the findings are that additive effects were important for these characters. In addition, the results showed that the testers and the interaction lines x testers explained most of the variation in the expression of the stem borer resistance and agronomic traits. The study showed a large dissimilarity between lines and the testcrosses' for traits. The significance of the SCA effects suggested that the non-additive gene effects were crucial in influencing manifestation of stem borer resistance traits and yield. Additionally, the significance of the environment x line interactions for grain yield; environment x tester interactions implied that environmental influence is important in the expression of the characters. The separation of lines, testers and environment and their interactions into variances provided a better understanding of the different patterns among *C. partellus* and their response across locations. These findings corroborate with earlier studies on significance of genotype x environment interactions effects in maize (Fato et al., 2012; Morais et al., 2012).

For example, at Embu, Kakamega and Kiboko for all *C. partellus* treatments, the mean squares for lines, testers, and their interactions were highly significant for all the traits studied, indicating inconsistent ranking of the lines by the testers. However, the line x tester x site interactions were not significant for all traits except grain yield and leaf feeding damage. The highly significant differences among lines and testers for some traits may suggest that genotypes responded differently across locations. The significant differences for lines x testers' interaction for stem borer resistance traits and grain yield showed that specific combining ability is greatly attributed in the expression of resistance traits and shows the importance of dominance or non-additive variances. There were no significant differences detected in the site x tester, and the site x line x tester interactions for agronomic traits namely; days to anthesis, days to silking, plant and ear height. These may suggest a predominance of additive effect in the control of these traits for *C. partellus*.

4.4.2 General and specific combining ability

General and specific combining abilities in addition to gene action for different stem borer resistance and agronomic traits have been estimated by many researchers (Morais et al., 2012; Sanghera et al., 2012; Wegary et al., 2013). In the current study, the significant difference of mean squares between lines, testers, lines x testers for stem borer resistance traits and grain yield showed their suitability for combining ability studies.

Further, significant mean squares of lines, testers, lines x testers' revealed good possibility for manifestation of heterosis for all the traits studied. It is desirable that stem borer resistance traits namely; leaf feeding damage, cumulative stem tunneling, number of exit holes, and number of dead hearts to obtain negative GCA and SCA effects (Beyene et al., 2011; Morais et al., 2012). Similarly, positive GCA and SCA effects are necessary for grain yield, number of plants and ears per plant (Morais et al., 2012). The genetic variations due to lines and testers were significantly different in the Embu, Kakamega and Kiboko for *C. partellus* stem borer resistance parameters and grain yield, and other agronomic traits. These revealed a preponderance of the additive effects for these traits. Both additive and non-additive gene effects have been reported in the literature for grain yield and yield components for various crops (Sanghera et al., 2012; Schnable et al., 2013).

Specific combining ability effects were significant for *C. partellus* resistance traits and grain yield signifying that non-additive effects were also crucial for borer resistance and grain yield. Maize inbred lines with high GCA effects also revealed hybrids with high SCA values for grain yield. For example, comparison of the lines and their responses to *C. partellus* at Embu, Kakamega and Kiboko, only lines 20, 28, 47 and 53 showed positive significant GCA effects for grain yield across locations for *C. partellus*. Different lines showed negative GCA and SCA effects for cumulative tunneling, number of exit holes and the leaf feeding damage for *C. partellus* resistance. These may imply that in hybrid formation and deployment for the various mega-ecologies, there is a need to target *C. partellus* specific varieties that combine high yield and stem borer resistance. The lines that showed positive significant GCA effects for grain yield and negative GCA and SCA effects across locations and borer resistance may be subjected to further evaluations and probable exploitation as parents in hybrid pedigree breeding. The current study demonstrated that additive gene effects control cumulative stem tunneling, number of exit holes, leaf feeding damage and the related agronomic characters. These results corroborate with previous findings in studies on stem borer resistance and grain yield in maize (Beyene et al., 2011; Udaykumar et al., 2013).

4.4.3 Heterotic orientations of maize inbred lines under *C. partellus* infestation

The evaluation of testcrosses showed relative responses of the parent lines. Using two genetic testers, different probable heterotic orientations were identified for inbred lines used in the current study. Relative heterosis data and the magnitude of the SCA effects for the testcrosses were used in the clustering of heterotic orientations and the identification of response patterns for *C. partellus* in the various locations.

At Embu, 10 inbred lines (1, 2, 3, 40, 50, 47, 49, 48, 57, and 55), while at Kakamega, 8 inbred lines (4, 5, 6, 12, 13, 14, 29 and 37) showed positive heterosis for grain yield with both testers, therefore they were allocated to heterotic group AB. At Kakamega, 10 inbred lines (7, 10, 11, 20, 32, 33, 42, 43, 62 and 66) showed positive heterosis for grain yield with both testers, therefore they were allocated to heterotic group AB. A similar trend was observed at Kiboko where 8 inbred lines (8, 9, 10, 11, 25, 53, 54, and 59,) showed positive heterosis for grain yield with both testers, therefore they were oriented towards heterotic group AB.

Most testcrosses showed positive heterosis for grain yield across locations, indicating the presence of heterosis in the hybrids. Relative heterosis was highest for grain yield, which is in tandem with other reports on maize (Sanghera et al., 2012; Liberatore et al., 2013). These may imply that the various lines and groups identified may be useful in *C. partellus* borer's specific breeding programmes for the formation of hybrids. In addition, the results show that for lines that have good general combining ability, probably, all new testers with new genetic structures may be able to distinguish them for *C. partellus* resistance (Morais et al., 2012).

The high genetic variability detected for *C. partellus* resistance in the testcross hybrid's clustering with the two testers is a desirable characteristic for a good maize tester. However, some lines that did not show any heterotic orientation with both testers. Tentatively, these lines may be useful in breeding; however, in practice they would be discarded. The heterotic orientations identified based on specific combining effects suggest need for new testers with new genetic structures since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive to discriminating materials. Similar observations have been reported in previous studies (Fato et al., 2012; Guimaraes et al., 2012).

4.5 Conclusions

Genetic combining ability effects were significant for *C. partellus,* stem borers resistance. The results suggest that additive gene effects were most important in the control of resistance for both borers. It is

possible to identify specific lines that may be useful for hybrid breeding for specific ecologies where these borers occur exclusively or in league. For *C. partellus* resistance traits and grain yield the specific combining ability effects were significant demonstrating that non-additive gene effects were also essential in explaining variations in the borer resistance traits and grain yield.

The testers CML312/CML442 and CML395/CML444 were able to discriminate these materials based on the general and specific combining ability for stem borer resistance, agronomic characters and grain yield. This implies that the single cross testers from CIMMYT can be used for line evaluations in breeding programmes for the identification of heterotic orientations in a stem borer resistance hybrid breeding programme. However, in this study there was a high number of lines which could not be classified based on testers CML312/CML442 and CML395/CML444. Consequently, there is a need for new testers with new genetic structures since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive to discriminating materials.

Using specific combining ability effects various heterotic orientations identified lines that showed positive significant SCA effects for *C. partellus* resistance and grain yield. The genotypes indicating high desirable GCA and SCA effects and with heterotic orientations that are favourable for grain yield may be deployed in breeding programmes across Kenya with emphasis on stem borers where these borers occur exclusively or in league. The corollary is that these superior lines may be used in hybrid pedigree breeding programmes that focus on *C. partellus* stem borer resistance in the tropics. The products from the line x tester evaluations were three way cross hybrids that can be nominated directly into the national performance trials for further evaluation and deployment into maize growing areas where these borers occur exclusively or in league with other stem borers.

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Chapter 5

Appraisal of leaf disk bioassay method for screening for resistance to stem borers, *Busseola fusca* and *Chilo partellus* in maize inbred lines in laboratory and greenhouse trials

Abstract

Quick screening methods for borer resistance are limited as current approaches are time consuming and labour intensive. The objective of this study was to appraise a detached leaf disk bioassay method for quick screening for *B. fusca* and *C. partellus* resistance in maize in the greenhouse and the laboratory trials. One hundred and twelve inbred lines in two sets each for infestation with *B. fusca* and *C. partellus*, respectively were sown in a 28 x 4 α-lattice design with three replications. Ten larvae per plant were placed in the maize whorl using a camel brush two weeks after planting. Data was collected on leaf feeding damage, mortality (%), leaf area damaged, plant height, cumulative stem tunneling, number of larvae recovered per plant and mass of larvae recovered. Data were analyzed using PROC GLM procedures of SAS statistical package. The mean squares of the entries as well as entry x treatment interactions were highly significant ($p \leq 0.01$) for all traits studied. In the greenhouse, the genotypes were ranked based on leaf feeding damage scores and stem tunneling. In the laboratory, ranking was based on leaf area damaged and mortality (%). Among the top 20 entries for resistance to *B. fusca* and *C. partellus*, stem borers in both the greenhouse and laboratory were; 25, 54, 64, 69 and 102, while the susceptible 20 entries were entries 15, 42, 57, 83, 96, 99, 100 and 104. The results from this study demonstrate that a combination of infestation of detached leaf disks and whole plant assays in the laboratory and greenhouse is an effective and efficient means of screening maize for resistance to *B. fusca* and *C. partellus* stem borers, and contributes to the development of more efficient and effective procedures for future evaluations.

Keywords: *Chilo partellus, Busseola fusca,* leaf disk bioassays, stem borer resistance screening

5.1 Introduction

Effective breeding methods for resistance to borer damage could be designed by plant breeders using both improved and new sources of stem borer resistance. Development of effective breeding methods requires reliable screening approaches for resistance the germplasm used. However, quick screening methods for candidate maize genotypes for stem borer resistance are limited. Current methods are time consuming and labour intensive, therefore, the need to optimize a detached leaf bioassay screening method in the greenhouse and laboratory is essential. The use of isolated leaf bioassays for artificial screening of maize genotypes for stem borer resistance may be a practical alternative approach. Natural infestation may not be reliable due to lack of uniformity and seasonal variations that occur (Tefera et al., 2010). The use of artificial infestation in a controlled environment allows multiple screenings within a short time. Leaf screening bioassays has been used as a quick method for screening materials in a wide range of horticultural and agronomic crops against pests and diseases including Bt cassava, beans, maize (Mugo et al., 2001; Murenga et al., 2011; González et al., 2013). To probably predict stem borer resistance isolated leaf bioassay conditions must be favourable for optimum plant growth and for healthy neonates (Tefera et al., 2010). The objective of this study was to appraise a detached leaf disk bioassay method for screening for *B. fusca* and *C. partellus* resistance maize in the greenhouse and laboratory trials.

5.2 Materials and Methods

5.2.1 Germplasm

One hundred and twelve (112) maize inbred lines used in the study were sourced from the International Maize and Wheat Improvement Center (CIMMYT), Kenya and the Kenya Agricultural Research Institute (KARI) breeding programmes. These maize inbred lines have not been tested for resistance to *B. fusca* and *C. partellus* stem borers. Known elite but stem borer resistant and susceptible maize lines from CIMMYT and KARI were included as checks. The list pedigree information of the maize inbred lines used in the study is as described in Chapter 3, Appendix 5.1.

5.2.2 Experimental design and Treatments

In the greenhouse, the maize inbred lines were evaluated in a 28 x 4 α-lattice design with three replications at KARI, Biotechnology centre's biosafety greenhouse and laboratory (Murenga et al., 2011). The genotypes were sown in planting media composed of one part of topsoil mixed with farm yard manure, one part sand, and one part coconut peat (Murenga et al., 2011). Each

maize inbred line was sown in one pot each. Ten seeds were sown in large pots 0.30 x 0.36 m, and later thinned to six plants per pot. The pots were spaced at 0.75 m apart to minimize leaf contact between plants and migration of larvae. During the period of the experiment the mean temperatures ranged between 22 to 32°C. Soil moisture was maintained at field capacity. Appropriate fertilizers, weeding, and watering were applied as recommended for the greenhouse (Murenga et al., 2004).

A set each of 112 pots each containing six plants were infested with *B. fusca* and *C. partellus* larvae. Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and the Kenya Agricultural Research Institute at Katumani stem borer insect pests mass rearing facility. In the greenhouse, six plants were artificially infested in a controlled and uniform manner with the respective stem borer species by placing 10 larvae in the maize whorl using a camel brush at two weeks after planting.

5.2.3 Data collection

5.2.3.1 Greenhouse evaluations

Plants were evaluated for leaf damage scores 2 weeks after planting using a scale of 1 (resistant) to 9 (susceptible) (CIMMYT, 1989), while the number of dead hearts were scored 4 weeks after planting. At the mid-whorl (V8-12) stage data was collected on the plant height, number of larvae recovered per leaf sample per entry, mass of larvae recovered per leaf sample per entry, and cumulative tunneling (cm) measured by splitting along the stalk length at 8 weeks after planting.

5.2.3.2 Laboratory evaluations

In insect bioassay laboratory at KARI, Biotechnology Centre, the maize inbred lines were evaluated in a 28 x 4 α-lattice design with three replications. The experiment was performed two times. Sampling was carried out on emergent and most recently fully expanded leaves each from the same ex-plants before infestation with the respective borer at the greenhouse. A 10 x 10 mm leaf area disk per entry was used. Each leaf disk was placed individually in a sterile plastic petri dish (150 x 15 mm) on a two piece filter paper saturated with distilled water. Leaf area damaged was estimated using a transparent graphic paper superimposed over the damaged leaf and counting of the number of squares and converting to estimated area. For the two sets of maize inbred lines each, ten larvae each of *B. fusca* and *C. partellus* per 100 mm^2 leaf disk per entry were placed in each petri dish using a camel brush. Petri dishes were randomized in a controlled-environment maintained at 25°C under darkness to avoid migration

of larvae from petri dishes and leaf samples. All treatments were monitored daily for the presence of contaminating organisms, and evidence of tissue necrosis, and distilled water was added to the plastic petri dishes on a need basis to keep the filter paper moist. On the 5th day after infestation with the respective larvae, data was collected on the leaf area damaged per disk (mm^2); mortality (%) of larvae (larval mortality (%) equals to the initial number of larvae infested subtract the number of larvae recovered divided by the initial number of larvae infested); number of larvae recovered per disk per entry and mass of larvae recovered per disk (grams) per entry were estimated.

5.2.4 Data analysis

Analyses of variance (ANOVA) for all characters measured were computed using PROC GLM procedures in SAS computer package, version 9.2 (SAS Institute. Inc., 2012). The model used was as follows: Y=Treatment + Entry + Rep(Treatment) + Treatment*Entry + Error. The inbred lines and the sampling of leaves for bioassay were considered as fixed factors. The replication and interactions were considered random. Data on larval mortality (%) and larval mass were transformed into arcsine values before subjecting them to ANOVA. Using the greenhouse data on plant damage traits, a rank summation index (RSI) was constructed to determine the ranking of each line within the population for suitable response. The index was obtained by the sum of the means of each of the leaf feeding damage score, leaf area damaged by the respective borer, and cumulative stem tunnel length for each line, to get its mean performance compared with other lines within the same population. In addition, based on data from the greenhouse and laboratory cluster analysis and principal components analysis were carried out to group the genotypes into resistant and susceptible, and to show which traits were most important in explaining variations among the genotypes across the two environments.

5.3 Results

5.3.1 Trait variations in the greenhouse

The mean squares of the entries from the combined analysis of selected stem borer resistance traits for *B. fusca* and *C. partellus* were highly significant ($p \leq 0.01$) for all traits studied. The entry x treatment interaction effects were highly significant ($p \leq 0.01$) for leaf feeding damage score, plant height (cm), and number of larvae recovered per disk per entry, mass of larvae recovered and tunneling (cm) (Table 5.1). The estimates for means for *B. fusca* and *C. partellus* for the various parameters are indicated below (Table 5.1). The greenhouse experiments showed that

B. fusca showed higher means for most of the borer damage traits than *C. partellus* except for plant height, larval mortality (%) and number of larvae recovered per disk per entry (Table 5.1).

Table 5.1 Mean squares of selected stem borer damage traits in the greenhouse trials at KARI

Source	DF	Leaf feeding damage	Plant height (cm)	No. of larvae recovered	Mass of larvae recovered	Stem tunneling (cm)
Treatment	1	7.43**	30070.04**	671.91*	12.11	7.28**
Entry	111	1.11**	51.26**	3.01	24.65	0.54**
Rep(Treatment)	4	1.07**	1208.42**	55.51*	41.66	12.05**
Treatment*Entry	111	0.95*	89.65**	2.71*	24.63	0.68**
Error	581	0.49	35.19	1.11	47.5	0.69
Cp Mean		**2.75**	**4.80**	**2.39**	**1.81**	**3.65**
Bf Mean		**2.94**	**16.86**	**0.49**	**1.56**	**3.45**
Overall Mean		**2.86**	**10.8**	**1.39**	**1.77**	**3.54**
% R^2		**45.8**	**71.32**	**69.99**	**0.17**	**32.1**

*, ** = significant (p≤0.05), highly significant (p≤0.01)

5.3.2 Trait variations in the laboratory

The mean squares of the entries from the combined analysis of selected traits for *B. fusca* and *C. partellus* were highly significant (p≤0.01) for all traits studied except mass of larvae per entry (mg). The entry x treatment interaction effects were highly significant (p≤0.01) for all traits (Table 5.2). *B. fusca* showed a higher mean for most of the borer damage traits than *C. partellus* except for plant height, larval mortality (%) and number of larvae recovered per plant (Table 5.2).

Table 5.2. Mean squares of selected stem borer damage traits in the in laboratory trials at KARI

Source	DF	% Mortality	Leaf area damaged (mm^2)	Mass of larvae recovered (g)	Cumulative stem tunneling (cm)
Treatment	1	507229.37**	89595.63**	380.47**	12.55**
Rep	2	270.52**	60.87**	3.02**	24.65**
Rep(Treatment)	4	3698.30**	1272.31**	6.24	41.66**
Treatment*Entry	222	240.57**	61.38**	2.57**	24.61**
Error	581	165.22	32.16	1.14	47.49
Cp Mean		**55.68**	**0.54**	**2.40**	**0.51**
Bf Mean		**3.99**	**22.42**	**0.99**	**0.76**
Overall Mean		**30.04**	**11.50**	**1.63**	**0.72**
% R^2		**86.88**	**86.38**	**61.09**	**17.05**

*, ** = significant (p≤0.05), highly significant (p≤0.01)

5.3.3 Rank selection indices in the greenhouse and laboratory

In the greenhouse, the genotypes were ranked based on the mean estimates of leaf feeding damage scores and cumulative stem tunneling for their resistance to *B. fusca* and *C. partellus*, stem borers (see Appendix 5, Table 5.5). In the laboratory, the genotypes were ranked based on the mean estimates of leaf area damaged and mortality (%) for their resistance to both borers (see Appendix 1, Table 5.5). Both in the greenhouse and the laboratory, among the top 20 entries for resistance to *B. fusca* and *C. partellus*, stem borers were entries: 25, 54, 64, 69 and 102. The least 20 susceptible entries were entries: 15, 42, 57, 83, 96, 99, 100 and 104. The distribution of the rank selection index (RSI) based on the greenhouse and laboratory is shown below (Figure 5.1 and Figure 5.2).

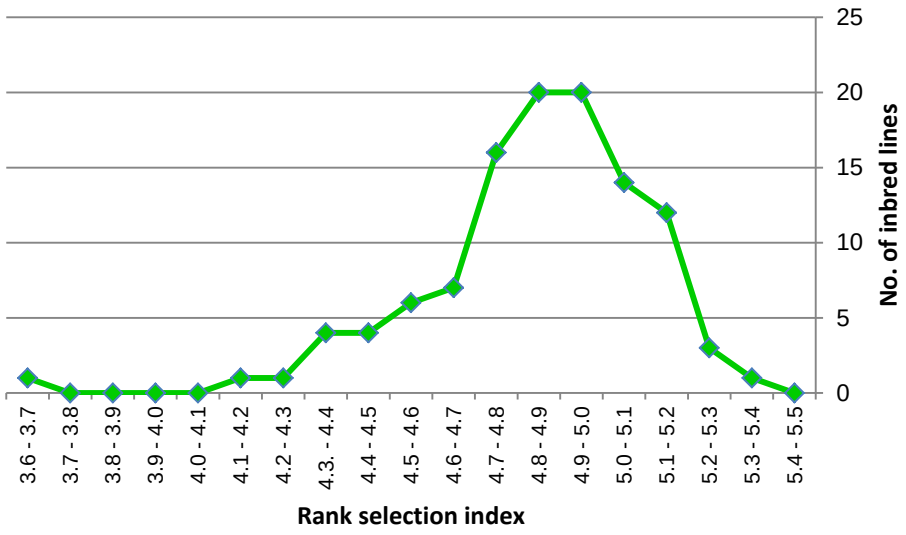


Figure 5.1. Distribution of genotypes based on the rank selection index at the greenhouse

Figure 5.2. Distribution of genotypes based on the rank selection index at the laboratory

5.3.4 Correlations among traits in the greenhouse and laboratory

There were significant (p≤0.01) correlations for some traits measured among the lines for resistance for under *B. fusca* and *C. partellus* infestation (Table 5.3). Significant (r=-0.46, p≤ 0.01) negative correlations were detected between larval mortality (%), leaf area damaged and cumulative tunneling (r=-0.82, p≤ 0.01). There were highly significant

(r=0.947, p≤ 0.01) correlations between the rank selection index in the greenhouse and the rank selection index in the laboratory. Similarly significant (r=0.458, p≤ 0.01) correlations were detected between leaf feeding damage in the greenhouse and the leaf area damaged in the laboratory (Table 5.3).

Table 5.3. Correlation coefficients for selected traits under *B. fusca* and *C. partellus* infestation in the greenhouse and laboratory

	LFD	**Mort**	**Area**	**TL**	**Larvae**	**Mass of larvae**
Leaf feeding damage	1	0.55	-0.46***	0.41***	0.28***	0.02
Mortality (%)		1	-0.86***	0.74***	0.49***	0.03
Leaf area damaged (mm^2)			1	-0.82***	-0.46***	-0.08*
Cumulative tunneling (cm)				1	0.36***	0.05
Number of larvae recovered per entry per disk					1	0.01
Mass of larvae recovered per entry per disk (g)						1
	RSIGH	**RSILab**	**LFD**	**Area**	**Mortality (%)**	
RSIGH	1	0.947***	0.043	-0.016	0.111	
RSILAB		1	0.072	0.003	0.087	
Leaf feeding damage			1	-0.274***	0.458***	
Leaf area damaged (mm^2)				1	-0.261***	
Mortality (%)					1	

LFD-leaf feeding damage, Mort-mortality (%), Area- leaf area damaged (mm^2), TL-cumulative tunneling, Larvae-number of larvae recovered per entry per disk, Mass of larvae recovered per entry per disk (g), RSIGH-rank selection index in the greenhouse, RSILab-rank selection index in the laboratory, *, ** = significant (p≤0.05), highly significant (p≤0.01)

There were significant (p≤0.01) correlations for some traits measured among the lines for resistance for under *B. fusca* and *C. partellus* infestation (Table 5.4). For example under *C. partellus*, leaf feeding damage scores and mortality (%) significant correlations were detected (r=0.21, p≤ 0.01), mortality (%) and area damaged (r=0.21, p≤ 0.01), area damaged and plant height (r=0.16, p≤ 0.01) and, larvae and larval mass (r=0.22, p≤ 0.01). The other traits showed varied trends (Table 5.4). For example, under *B. fusca* infestation significant correlations were detected for plant height and mortality (%) (r=0.59 p≤0.01), and number of larvae and plant height (r=-0.63, p≤ 0.01). Negative correlations were detected between plant height due to *B. fusca* and plant height due to *C. partellus* (r=-0.85, p≤ 0.01). However, most of the correlations between traits were (r<0.50). The other traits showed diverse trends (Table 5.4).

Table 5.4. Correlation coefficients based stem borer damage parameters in the greenhouse and laboratory trials at KARI

	Chilo partellus						*Busseola fusca*					
	LFD	**Mort.**	**AREA**	**PH**	**LARV.**	**Wt. LARV.**	**LFD**	**Mort.**	**AREA**	**PH**	**LARV.**	**Wt. LARV.**
LFD	**1**	1.00**	0.21**	-0.01	0.09	0.16*	0.07	0.08	-0.05	0.06	-0.02	0.18 **
Mortality (%)		1	0.21**	-0.02	0.09	0.16*	0.08	0.08	-0.05	0.06	-0.02	0.18**
Leaf AREA			1	0.16*	-0.11	0.07	0.09	0.01	-0.01	0.04	0.18*	-0.02*
PH (cm)				1	0.01	-0.17 *	0.05	0.01	0.07	0.02	0.02	0
LARVAE					1	0.22**	-0.05	0.06	-0.03	0.03	-0.08	-0.08
WT LARVAE						1	-0.03	-0.02	-0.01	-0.02	-0.06	0.04
LFD							1	0.14*	-0.20**	0.22**	0.20*	0.06
Mortality (%)								1	-0.55**	0.59**	0.34**	0.08
Leaf AREA									1	-0.85**	-0.63**	-0.15*
PH (cm)										1	0.69**	0.20**
LARVAE											1	0.23**
WT LARVAE												1

LFD = leaf feeding score, Mortality (%), leaf AREA- leaf area damaged (cm^2 per 10 cm^2 leaf disk), PH = plant height (cm), LARVAE = number of larvae recovered $plant^{-1}$, Wt. LARV. = mass of larvae plant-1, and *, * = significant (p≤0.05), highly significant (p≤0.01)

5.3.5 Evaluation of the maize inbred lines in the greenhouse

The two most important principal components are PC1 (leaf feeding damage scores) and PC2 (cumulative stem tunneling) as they account for most of the total variation. Leaf feeding damage scores and the cumulative stem tunneling were plotted to visualize the separation of the maize genotypes and their responses (Figure 5.3). PC1 accounted for 67.4% of the variation in resistance among the genotypes, while PC2 accounted for the rest. The genotypes showing susceptible to both *B. fusca* and *C. partellus* based on the ranks selection index in the greenhouse were 16, 107, 68, 46, 32, 80, 13 (RC), 83, 44 and 100, while, genotypes 75, 64, 89, 25, 56, 84, 102, 22 and 47 were resistant to both *B. fusca* and *C. partellus* (Figure 5.3). Evaluation of the maize inbred lines in the greenhouse is shown below (Figure 5.4).

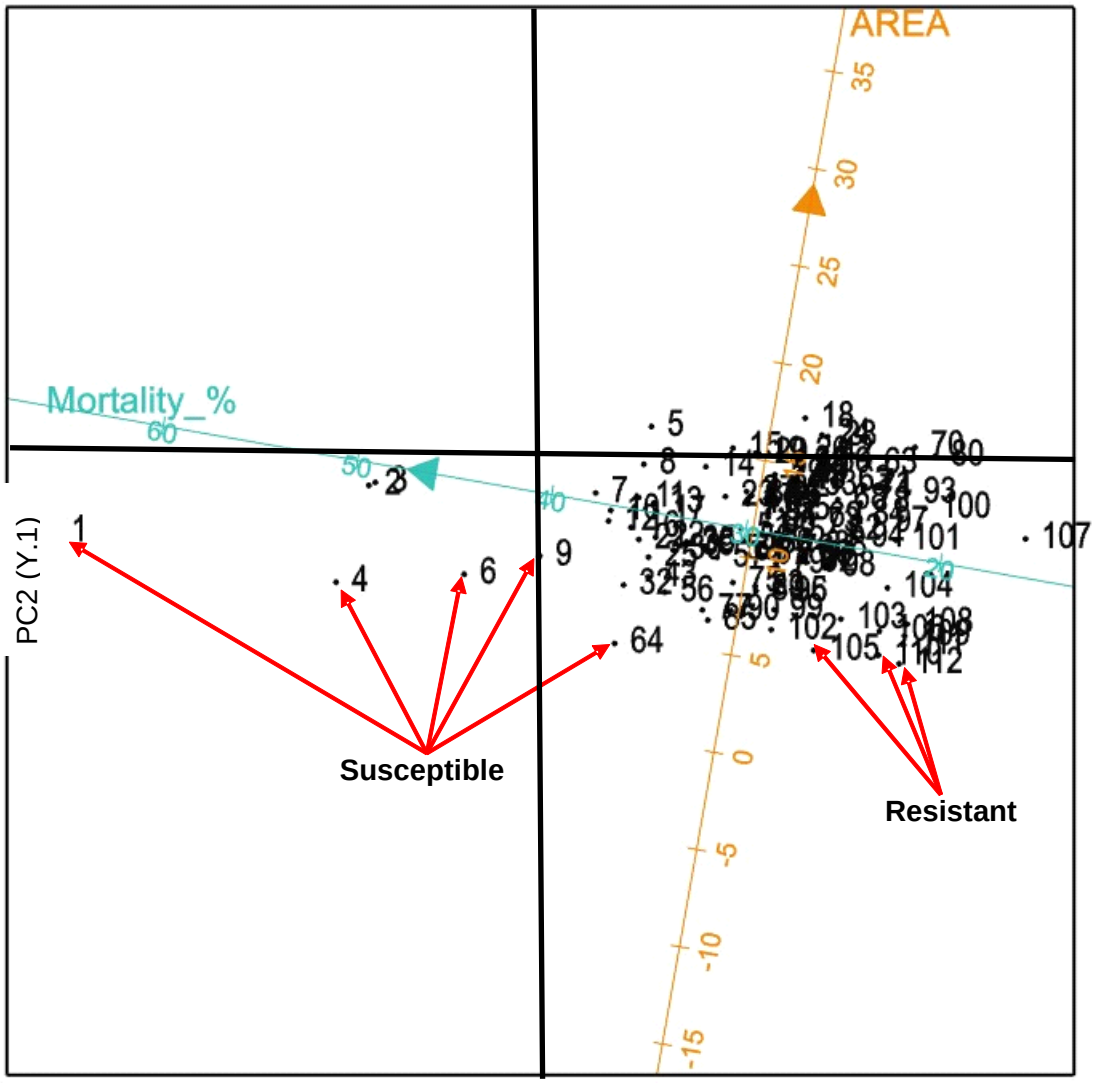


Figure 5.3. Leaf feeding damage (PC1) versus cumulative stem tunneling (PC2) score plot of 112 different genotypes at the greenhouse

Figure 5.4. Evaluation of the maize inbred lines in the greenhouse at KARI, Kabete

5.3.6 Evaluation of the maize inbred lines in the laboratory

Based on the results from the bioassays in the laboratory, the two most important principal components were leaf area damaged (PC1) and percent larval mortality as they accounted for most of the total variation. Leaf feeding damage scores and the cumulative stem tunneling were used were plotted to visualize the separation of the maize genotypes and their responses (Figure 5.5). PC1 accounted for 84.14% of the variation in resistance among the genotypes, while PC2 accounted for 15.86%. The genotypes showing resistance to both B. fusca and *C. partellus* based on the RSI from bioassays were 64, 39, 112, 84, 69 (resistant check), 82, 72, 56, 30 and 43. Genotypes 94, 36, 46, 109, 2, 16, 40, 48 (susceptible check) were susceptible to both *B. fusca* and *C. partellus* (Figure 5.5). Other susceptible genotypes in the least 20 were 102, 105 and 112 (Figure 5.5).

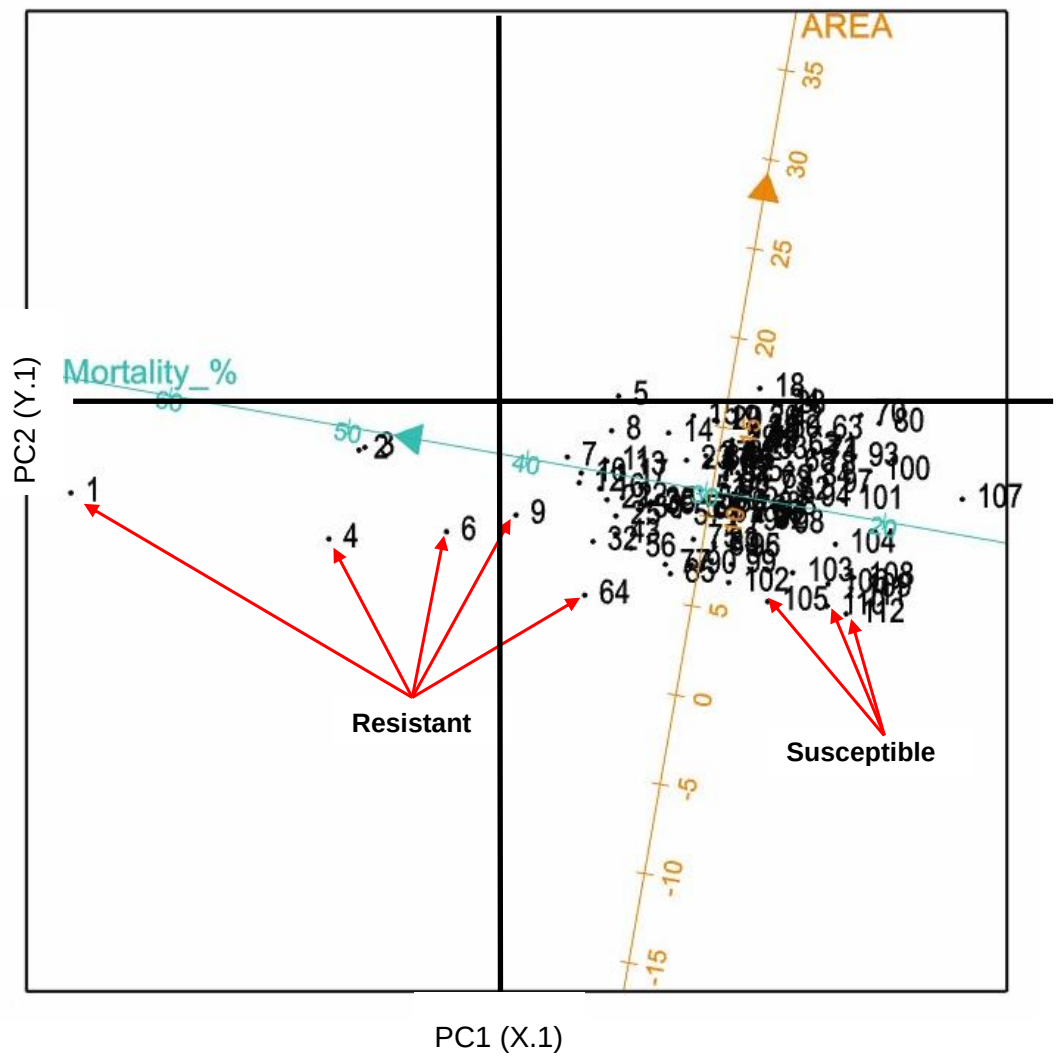
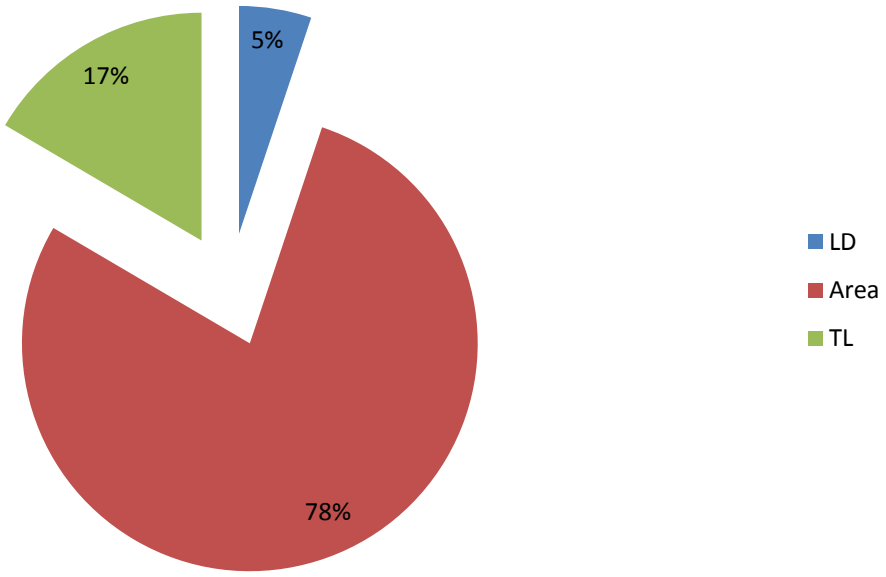


Figure 5.5. Leaf area damaged (PC1) versus larval mortality (%) (PC2) score plot of 112 different genotypes at the laboratory

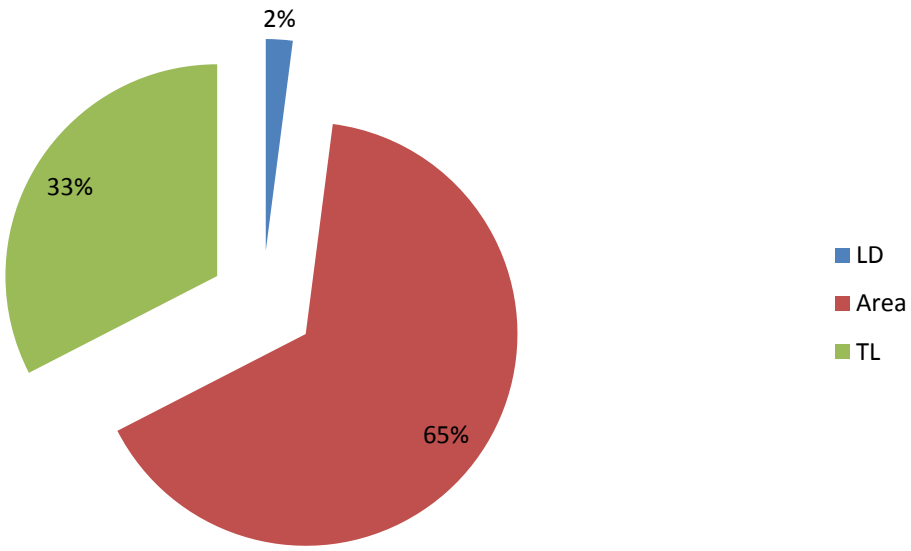
5.3.7 Partitioning of damage effects under *B. fusca* and *C. partellus* infestation

Partitioning of stem borer damage effects under *B. fusca* and *C. partellus* infestation were detected in the greenhouse and laboratory for leaf feeding damage, leaf area damaged and cumulative tunneling (Figure 5.6 and Figure 5.7). For *C. partellus* leaf damage score accounted for 5%, leaf area damaged 78% and cumulative stem tunneling 17%, while for *B. fusca* leaf damage score accounted for 2%, leaf area damaged 65% and cumulative stem tunneling 33% of the total damage. Resistant and susceptible genotypes were detected in the greenhouse (Figure 5.8).



LD –leaf feeding damage, Area-leaf area damaged and TL-cumulative stem tunneling

Figure 5.6. Leaf feeding damage, leaf area damaged and cumulative tunneling due to *Chilo partellus*



LD-leaf feeding damage, Area-leaf area damaged and TL-cumulative stem tunneling

Figure 5.7. Leaf feeding damage, leaf area damaged and cumulative tunneling under *B. fusca* infestation

Figure 5.8. Resistant (*left*) versus susceptible (*right*) plant in the greenhouse at KARI, Kabete

5.4 Discussion

The analysis of variance showed significant variation among the genotypes for all traits studied. The highly significant differences detected among the entries for the various *B. fusca* and *C. partellus* stem borer resistance traits indicated the existence of considerable variation among the genotypes that allows for selection of desired inbred lines, and that breeding progress is possible. For example, the study showed a large dissimilarity between *B. fusca* and *C. partellus* for leaf feeding scores damage, leaf area damaged, plant height, and cumulative stem tunneling, and also number of larvae recovered per plant among genotypes. *B. fusca* showed higher means for most of the borer damage traits than *C. partellus* except for plant height, mortality (%) and number of larvae recovered per plant, indicating that most genotypes were susceptible to *B. fusca* than to *C. partellus,* probably because *B. fusca* is indigenous to Africa and may have switched from wild to cultivated sorghum occurred several times from local populations of *B. fusca* and to become more adapted maize and sorghum crops unlike *C. partellus* (Mailafiya et al., 2011; Morais et al., 2012; Ong'amo et al., 2012).

The highly significant ($r=0.947$, $p \leq 0.01$) correlations between the rank selection index in the greenhouse and laboratory may suggest that the mechanisms of resistance may be comparable. Based on the rank selection index, both in the greenhouse and the laboratory, among the top 20 entries for resistance and the least 20 susceptible entries to *B. fusca* and *C. partellus*, stem borers were identified namely: 25, 54, 64, 69 and 102, and 15, 42, 57, 83, 96, 99, 100 and 104 respectively. Similar results have been reported in the literature (Tabashnik et al., 2009; Beyene et al., 2011; Murenga et al., 2011). The results showed their possible use for the improvement of resistance levels and that the lines may be screened further in the field for resistance to *B. fusca and C. partellus*. The biplots of leaf feeding scores and cumulative tunneling, and that of the larval mortality (%) and the leaf area damaged were in agreement with

the observations made on the separation of genotypes into resistant and susceptible. These evaluations may assist maize breeders in selecting genotypes for advancing in breeding.

5.5 Conclusion

The results from this study demonstrate that infestation of detached leaf assays is an effective and efficient means of screening maize for resistance to *B. fusca* and *C. partellus* stem borers. The information generated from the genotypes through artificial infestations using the detached leaf disks in the insect assay laboratory and the whole plant assays on in the greenhouse were in agreement. The experimental results appear to suggest that when selection for resistance to *B. fusca* and *C. partellus* is carried out using artificial infestations in the laboratory and greenhouse, resistant entries are shown to enhance their resistance in the greenhouse and probably in the field.

In a short time tests with leaves allows multiple assays of genotypes response to selection due to an increased probability of selecting a resistant plants in a population. A convergence in the resistance between genotypes for *B. fusca* and *C. partellus* and the consistency of results from detached leaves in the laboratory and whole plant assays in the greenhouse may facilitate in the identification of the most resistant genotypes in a population. These in turn may accelerate breeding progress and reduce the number of cycles required to attain higher resistance levels to *B. fusca* and *C. partellus* stem borers.

The results from this study demonstrate that a combination of infestation of detached leaves in the laboratory and whole plant assays in the greenhouse is an effective and efficient means of screening maize for resistance to *B. fusca* and *C. partellus* stem borers. The technique used will contribute towards the development of more efficient and effective procedures in future evaluations of maize for resistance for *B. fusca* and *C. partellus* stem borers.

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Appendix 5:

Table 5.5. Ranks based on the rank selection index of maize inbred lines at greenhouse and the laboratory under *B. fusca* and *C. partellus* infestation

Greenhouse					Laboratory					
Entry	LFD	TL	Rank selection Index	Rank	Entry	AREA	MORT (%)	MORT[¥]	Rank selection Index	Rank
75	5.2	3.5	3.6	1	58	15.9	35.6	1.6	3.1	1
69	3.6	3.9	4.2	2	39	7.5	50.0	1.7	3.4	2
41	3.1	4.2	4.3	3	64	14.1	64.5	1.8	3.5	3
56	3.6	3.8	4.3	4	81	9.7	25.6	1.4	3.7	4
89	3.9	3.5	4.3	5	109	12.9	21.2	1.3	3.7	5
102	3.5	3.9	4.3	6	21	14.2	22.3	1.3	3.8	6
25	3.8	3.4	4.4	7	11	10.0	31.1	1.5	4	7
64	3.9	3.2	4.4	8	63	8.6	27.9	1.4	4	8
71	3.2	4.0	4.4	9	69	15.2	43.7	1.6	4.2	9
84	3.6	3.6	4.4	10	16	11.1	31.5	1.5	4.2	10
17	3.0	4.0	4.5	11	25	6.5	21.1	1.3	4.3	11
22	3.4	3.6	4.5	12	55	10.6	30.0	1.5	4.3	12
39	2.9	4.0	4.5	13	2	8.5	21.2	1.3	4.4	13
47	3.4	3.7	4.5	14	74	10.2	22.4	1.4	4.4	14
54	3.2	3.7	4.5	15	102	15.3	35.6	1.6	4.5	15
61	2.8	4.1	4.5	16	86	7.0	33.5	1.5	4.5	16
92	2.9	4.0	4.5	17	78	15.3	27.9	1.4	4.6	17
4	3.0	3.8	4.6	18	110	15.1	24.5	1.4	4.6	18
5	3.0	3.8	4.6	19	40	14.3	21.1	1.3	4.7	19
59	**3.1**	**3.7**	**4.6**	**20**	**54**	**12.2**	**34.5**	**1.5**	**4.9**	**20**
73	2.8	3.9	4.6	21	82	6.3	40.1	1.6	4.9	21
76	3.2	3.6	4.6	22	31	10.3	29.0	1.5	4.9	22
79	2.8	4.1	4.6	23	13	12.7	27.8	1.4	5	23
81	2.8	3.9	4.6	24	62	10.0	27.8	1.4	5	24
21	2.9	3.7	4.7	25	8	7.0	30.2	1.5	5.1	25
26	3.1	3.6	4.7	26	103	10.3	29.0	1.5	5.1	26
31	2.9	3.8	4.7	27	59	11.8	34.6	1.5	5.4	27
37	2.8	3.8	4.7	28	4	13.8	22.4	1.4	5.5	28
40	2.5	4.1	4.7	29	61	13.1	33.5	1.5	5.6	29
48	3.1	3.6	4.7	30	37	11.2	31.2	1.5	5.6	30
49	3.1	3.4	4.7	31	7	8.4	35.3	1.5	5.7	31
53	2.8	3.7	4.7	32	24	14.4	28.2	1.5	5.8	32
60	3.1	3.5	4.7	33	60	12.1	32.9	1.5	5.8	33
63	2.6	4.1	4.7	34	38	12.9	32.9	1.5	5.8	34

65	2.8	3.7	4.7	35	111	12.9	34.4	1.5	5.9	35
72	3.1	3.5	4.7	36	53	14.0	30.6	1.5	5.9	36
74	3.1	3.6	4.7	37	107	9.7	29.0	1.5	5.9	37
78	3.0	3.6	4.7	38	89	10.3	30.2	1.5	5.9	38
95	3.0	3.6	4.7	39	22	5.2	25.7	1.4	5.9	39
106	3.1	3.6	4.7	40	26	8.1	32.9	1.5	5.9	40
2	2.6	3.9	4.8	41	41	10.3	49.2	1.7	5.9	41
8	2.7	3.7	4.8	42	14	15.7	29.1	1.5	5.9	42
9	2.6	3.8	4.8	43	85	10.5	26.7	1.4	5.9	43
10	2.6	3.8	4.8	44	87	13.3	26.9	1.4	6	44
12	2.9	3.5	4.8	45	112	13.0	48.9	1.7	6	45
14	2.8	3.6	4.8	46	12	16.7	35.8	1.6	6	46
24	3.0	3.3	4.8	47	43	15.7	26.9	1.4	6	47
28	2.9	3.5	4.8	48	105	13.4	26.9	1.4	6.1	48
35	3.3	3.1	4.8	49	84	14.4	36.9	1.6	6.1	49
43	2.7	3.6	4.8	50	56	13.6	27.9	1.4	6.1	50
52	3.1	3.3	4.8	51	52	13.3	30.0	1.5	6.1	51
55	3.0	3.3	4.8	52	75	10.8	29.0	1.5	6.1	52
58	3.1	3.4	4.8	53	10	10.3	26.7	1.4	6.3	53
66	2.6	3.8	4.8	54	65	8.2	36.9	1.6	6.3	54
67	2.6	3.9	4.8	55	30	7.2	26.3	1.4	6.4	55
82	2.8	3.7	4.8	56	92	10.6	30.2	1.5	6.4	56
85	2.9	3.5	4.8	57	98	17.5	28.5	1.5	6.5	57
98	2.8	3.5	4.8	58	106	4.6	34.6	1.5	6.5	58
101	2.7	3.8	4.8	59	27	9.2	29.0	1.5	6.6	59
103	3.0	3.4	4.8	60	91	10.1	37.8	1.6	6.7	60
109	2.6	3.8	4.8	61	71	9.8	34.8	1.5	6.7	61
112	2.8	3.6	4.8	62	72	8.6	24.5	1.4	6.7	62
1	2.4	3.8	4.9	63	33	6.6	35.6	1.6	6.8	63
3	3.0	3.2	4.9	64	20	5.2	25.7	1.4	6.8	64
6	2.9	3.3	4.9	65	76	11.2	22.4	1.4	6.8	65
11	2.6	3.5	4.9	66	80	13.7	29.0	1.5	6.8	66
20	2.6	3.6	4.9	67	73	15.7	26.8	1.4	6.9	67
23	2.8	3.4	4.9	68	88	14.6	29.0	1.5	7	68
32	2.3	4.0	4.9	69	97	6.7	26.8	1.4	7	69
33	2.4	3.8	4.9	70	23	14.3	28.4	1.5	7.1	70
51	2.6	3.6	4.9	71	95	11.9	30.1	1.5	7.1	71
62	2.6	3.6	4.9	72	5	11.9	31.3	1.5	7.1	72
68	2.2	3.9	4.9	73	35	12.3	23.4	1.4	7.2	73
70	2.6	3.6	4.9	74	34	7.5	30.1	1.5	7.2	74
77	2.8	3.3	4.9	75	29	10.8	29.1	1.5	7.2	75
87	2.6	3.6	4.9	76	45	12.1	24.6	1.4	7.3	76

88	2.8	3.5	4.9	77	48	14.2	30.0	1.5	7.3	77
90	2.8	3.4	4.9	78	93	7.6	29.0	1.5	7.3	78
97	2.5	3.8	4.9	79	44	15.3	30.3	1.5	7.4	79
105	2.8	3.3	4.9	80	51	12.2	16.0	1.2	7.4	80
107	2.2	4.1	4.9	81	50	6.0	29.1	1.5	7.5	81
110	2.8	3.4	4.9	82	101	8.3	25.7	1.4	7.6	82
18	2.9	3.0	5	83	66	14.7	24.5	1.4	7.6	83
19	2.7	3.2	5	84	28	10.5	35.7	1.6	7.7	84
27	2.9	3.0	5	85	36	10.3	21.3	1.3	7.7	85
30	2.6	3.5	5	86	3	7.6	27.3	1.4	7.8	86
34	2.7	3.3	5	87	6	10.5	29.0	1.5	7.8	87
42	2.7	3.3	5	88	77	12.6	32.6	1.5	7.8	88
50	2.9	3.1	5	89	70	10.2	28.0	1.4	7.9	89
80	2.3	3.7	5	90	19	15.0	27.9	1.4	7.9	90
86	2.7	3.3	5	91	32	12.0	29.0	1.5	7.9	91
91	2.7	3.3	5	92	49	11.4	24.5	1.4	7.9	92
94	2.5	3.5	5	93	94	13.1	22.2	1.3	8	93
96	2.9	3.1	5	94	83	14.6	25.6	1.4	8	94
108	2.7	3.3	5	95	68	12.7	24.6	1.4	8	95
111	2.9	3.2	5	96	108	16.8	27.8	1.4	8.1	96
7	2.6	3.1	5.1	97	90	12.6	27.9	1.4	8.2	97
13	2.3	3.4	5.1	98	18	11.5	28.0	1.4	8.3	98
15	2.4	3.4	5.1	99	9	17.0	28.0	1.4	8.3	99
29	2.4	3.3	5.1	100	17	15.4	30.0	1.5	8.4	100
36	2.6	3.1	5.1	101	79	13.7	30.2	1.5	8.4	101
38	2.6	3.2	5.1	102	100	7.6	31.4	1.5	8.4	102
45	2.5	3.2	5.1	103	15	8.7	26.8	1.4	8.4	103
46	2.3	3.5	5.1	104	42	16.8	35.6	1.6	8.6	104
57	2.6	3.2	5.1	105	67	10.7	28.0	1.4	8.6	105
93	2.8	3.0	5.1	106	47	11.5	24.5	1.4	8.6	106
99	2.6	3.3	5.1	107	1	10.3	26.8	1.4	8.7	107
100	2.4	3.5	5.1	108	46	14.8	21.2	1.3	9	108
16	2.1	3.5	5.2	109	104	6.0	26.7	1.4	9.1	109
83	2.4	3.3	5.2	110	96	7.8	27.0	1.4	9.1	110
104	2.4	3.2	5.2	111	99	10.2	22.3	1.3	9.2	111
44	2.4	3.0	5.3	112	57	10.3	28.0	1.4	9.5	112

LFD – leaf feeding damage, TL – cumulative stem tunneling, AREA-lea area damaged, MORT (%) - larval mortality (%), MORT¥ - transformed mortality (%) data used for the ranking from the mortality (%)

CHAPTER 6

Response to selection of S1 progeny recurrent selection to resistance to two stem borers, *Busseola fusca* and *Chilo partellus,* in two tropical maize populations

Abstract

Stem borers, *Busseola fusca* and *Chilo partellus*, are among the key devastating lepidopteran insect pests of maize causing grain yield losses. Recurrent selection studies for stem borer resistance in maize are limited. However, maize populations carrying resistance genes to these stem borers have not been exploited fully in breeding programmes. The objective of the study was to separately improve resistance to *B. fusca* and *C. partellus* stem borers for two maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc and therefore grain yield after two cycles of S1 progeny recurrent selection. Cycle 0 and the advanced generations (cycle 1-susceptible, cycle 1-resistant and cycle 2-resistant) were evaluated at three locations in Kenya using a 35 x 12 α-lattice design with 2 replications. The net reductions in cumulative tunneling, number of exit holes and leaf feeding damage scores ranged from 0% to 69% for both populations after two cycles of selection. In the two populations, each cycle of selection for borer resistance improved grain yield by 0.5 to 0.8 t ha^{-1}. Actual net gains in grain yield with reference to cycle 0 were 43% for population CML395/MBR C5 Bc under *B. fusca* infestation and 70% under *C. partellus* infestation. For population CML444/MBR/MDR C3Bc, the actual net gains in grain yield were 25% under *B. fusca* infestation and 36% under *C. partellus* infestation. The reductions in the injurious effects attributable to leaf feeding damage, cumulative stem tunneling and number of exit holes contributed towards the 43% and 70% net genetic gain in grain yield under *B. fusca* and *C. partellus* infestation respectively, for both populations. Broad sense heritability (H^2) for grain yield ranged from 2% to 98% in both maize populations. The study showed that two cycles of S1 progeny recurrent selection was effective in accumulating favourable alleles for *B. fusca* and *C. partellus* stem borer resistance.

Keywords: *Busseola fusca*, *Chilo partellus*, genetic gain, heritability, maize populations, S1 progeny recurrent selection

6.1 Introduction

There are limited studies on recurrent selection for stem borer resistance in tropical maize. Although many maize varieties with high yield potential are available on the market, some limitations have precluded some farmers in Kenya from access to these modern varieties because of the high cost of hybrid seed. In addition, hybrid seed may impose other limitations namely fertilization, pesticides, mechanized equipment and efficient management required to exploit the full genetic potential of these high yielding maize varieties in many parts of Kenya and in the tropics (Acquaah, 2009; Ana Paula et al., 2013).

Plant breeding has led to the development of new maize varieties with better resistance and agronomic traits to biotic and abiotic stresses. Among these biotic stresses, the African stem borer, *Busseola fusca* and the spotted stem borer *Chilo partellus* are serious insect pests of maize in tropical environments. Breeding for stem borer resistance in maize is challenging because the trait is quantitative and involves polygenes with low heritability (Sharma et al., 2007; Sandoya et al., 2010; Oloyede-Kamiyo et al., 2011). Cartea et al. (1999) suggested that recurrent selection approaches would be the most suitable for the improvement of stem borer resistance. This breeding scheme is effective in increasing favourable alleles of agronomic and economic traits of importance in maize populations. The S1 progeny recurrent selection scheme is characterized by the additive genetic effects that are more important than the non-additive gene effects in stem borer resistance in maize populations (Sandoya et al., 2008; Schnable et al., 2013) .

The S1 progeny recurrent selection scheme is widely used in maize breeding. Various successful examples of its application in various crops against pests and diseases were reported (Ordas et al., 2009). A greater amount of breeding efforts have been dedicated towards improvement of resistance to maize stem borers in Africa and Asia (Mugo et al., 2005; Butrón et al., 2009; Sandoya et al., 2010; Barros et al., 2011). Several cycles of recurrent selection have been used to improve maize for resistance against various stem borers species (Ana Paula et al., 2013; Dhillon et al., 2013; Oloyede-Kamiyo et al., 2013). However, limited work has been carried out on maize populations through recurrent selection for resistance to *B. fusca* and *C. partellus* stem borers in Kenya.

The objectives this study were to separately improve resistance to two stem borers *B. fusca* and *C. partellus* in two tropical maize populations through S1 progeny recurrent selection. The test hypothesis was that both resistance improvement to two stem borers, *B. fusca* and *C. partellus* and grain yield could be achieved through cycles of S1 progeny recurrent selection.

6.2 Materials and methods

6.2.1 Germplasm

Two maize breeding populations used in this study were CML395/MBR C5 Bc F114-1-2-3-B-4-2-B-B (hereafter referred to as CML395/MBR C5 Bc) and CML444/MBR/MDR C3Bc F1-1-1-1-B-3-2-B-B (hereafter referred to as CML444/MBR/MDR C3Bc). These populations from CIMMYT-Nairobi are unrelated and originate from various nurseries. They have not been improved for resistance to *B. fusca* or *C. partellus* resistance through a recurrent selection scheme. The two populations were chosen for improvement because they are popularly grown as open pollinated varieties by farmers. Prior to this study the means of various agronomic and borer resistance traits were recorded from previous studies on these maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc.

6.2.2 Experimental sites

The experimental sites used in this study are as described in Chapter 2 (Figure 2.1).

6.2.3 Formation of S1 progenies

The recurrent selection scheme was applied separately to each of the two maize populations. One thousand and five hundred plants were established for each population at each site between 2011B and 2012B and evaluated in 2013A season. The design was an un-replicated nursery with lines sown into 75 rows of 5 m lengths, with inter-row spacing of 0.75 m and inter-hills spacing of 0.25 m within the rows.

Plants were artificially infested in a controlled and uniform manner with *B. fusca* or *C. partellus* larvae respectively by placing 10 larvae in the maize whorl using a camel brush at two weeks after planting. Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and the Kenya Agricultural Research Institute at Katumani stem borer insect pests mass rearing facility (Tefera et al., 2010; Tefera et al., 2011). Plant evaluations on stem borer resistance as well as agronomic traits were measured as described in Chapter 3. After harvesting, a rank summation index (RSI) was constructed to determine the ranking of each line within each population for appropriate reaction. The index was obtained by the sum of the means of each of the leaf feeding damage score; number of dead-hearts; number of exit holes; and cumulative stem tunnel length for each line, to get its mean performance compared with other lines within the same population. An entry with the least value was ranked higher for the resistance traits (Mulamba et al., 1978; Mutinda et al., 2013). The timelines

and activities for the S1 progeny recurrent selection for the two maize populations in three locations for resistance under *B. fusca* and *C. partellus* stem borers infestation are described below (Table 6.1).

Table 6.1. Recurrent selection scheme for two maize populations under *B. fusca* and *C. partellus* infestation at three locations

Season	Activity
2010B	Two cycle 0 (C_0) populations of CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc were established in rows in fields at Kiboko. Two seeds were planted per hill and later thinned to one. Recommended agronomic practices such as were implemented. About 1000 plants per population were selfed based on general performance to generate S_1 population. Only S_1 ears with sufficient seed were advanced.
2011A	Field evaluation of S_1 progenies under *B. fusca* and *C. partellus* infestation at Embu, Kakamega with 2 replications laid out in single rows in a 25 x 12 α-lattice design. The evaluations at Kiboko were exclusively under *C. partellus* infestation because the borer occurs in the region. Planting were ear-to-row to maintain genetic purity of the S_1 progenies. Two local (one resistant and the other susceptible) varieties were included as checks¥. Remnant seed were stored. Data were collected on stem borer resistance traits on about 300 plants per population and grain yield to form the basis for selection. Fifty extra plants were sown to ensure a minimum of 300 healthy plants for advancement during selection. Divergent selection was carried out for *B. fusca* and *C. partellus* resistance and susceptibility at each site.
2011B	Remnant seed of about 300 S_1 progeny rows showing resistance and susceptibility to stem borers were selected for recombination per population. Seed per progeny were reserved. Susceptible progeny were used as checks Recombination involved ear-to-row planting of the S_1 seed and hand-pollination using bulk pollen was carried out with one half pollinating the other to ensure random mating. Cycle 1 (C1S and C1R) seed was formed from this recombination per population (300 lines of C_1 seed expected/population).
2012A	Field evaluation of S_1 progenies under *B. fusca* and *C. partellus* infestation at Embu, Kakamega with 2 replications laid out in single rows in a 25 x 12 α-lattice design. The evaluations at Kiboko were exclusively under *C. partellus* infestation.

	Evaluation of C_1 (C1S and C1R) seed was carried out in replication trials for resistance and susceptibility to stem borers, keeping remnant seed per ear. Two local (one resistant and the other susceptible) varieties were included as checks. The field design was laid out with two replicates in a 10 x 5 α lattice design. Twenty extra plants for each cycle were sown to ensure a minimum of 35 healthy plants for advancement during selection. Divergent selection was carried out for *B. fusca* and *C. partellus* resistance and susceptibility at each site.
2012B	Remnant C_1 (C1S and C1R) seed from about 35 progenies showing similar characteristics of resistance and susceptibility to stem borers were selected for recombination per population. Recombination involved ear-to-row planting of the S_1 seed and hand-pollination using bulk pollen was carried out with one half pollinating the other to ensure random mating. Cycle 2 (C2R) seed were formed from this recombination (35 lines of C_1 seed expected/population).
2013A	Field evaluation of all cycles for each population, C_0, C_1 (C1S and C1R) and C_2 (C2R) of S_1 progenies under *B. fusca* and *C. partellus* infestation at Embu, Kakamega with 2 replications laid out in single rows in a 35 x 4 α-lattice design. The evaluations at Kiboko were exclusively under *C. partellus* infestation. Seed from each location for each cycle for each population, C_0, C_1 (C1S and C1R) and C_2 (C2R) of S_1 progenies under *B. fusca* and *C. partellus* infestation was bulked separately. In addition to the cycles of the susceptible progeny per population, two local (one resistant and the other susceptible) varieties were included as checks.

¥commercial varieties and a resistant check hybrid CKIR6009

6.2.4 Multi-site evaluation of the cycle 0 and the advanced cycles

The population cycles C_0, C_1S, C_1R and C_2R were evaluated in a 35 x 4 α-lattice design with two replications in each location. Each 6.75 m plot was divided into three parts namely, *B. fusca* and *C. partellus* infested on either side of the plot at Embu and Kakamega, while the middle part was protected using insecticide Bulldock® (active ingredient, *beta cyfluthrin 25g/L*). At Kiboko, 5 m row plots were used, and were infested with *C. partellus* on half the plot while the remaining part was protected. Two seeds were sown per hill and later thinned to one. Inter-row spacing of 0.75 m and inter-hills spacing of 0.25 m within the rows was used. Recommended fertilizer application of nitrogen (60 kg N ha^{-1}) and phosphate (60 kg P_2O_5 ha^{-1}) and irrigation were applied as recommended for each location to ensure

healthy and vigorous plants. Nitrogen was applied in two splits, while supplementary irrigation was applied when needed. The fields were kept free of weeds by hand weeding throughout the growth period.

6.2.4 Artificial infestation with insects

Insect larvae were obtained from the International Centre for Insect Physiology and Ecology (ICIPE) and KARI Katumani stem borer mass rearing facilities. Plants were artificially infested in a controlled and uniform manner with the respective stem borer species by placing 10 larvae in the maize whorl using a camel brush at two weeks after planting.

6.2.5 Data collection

Plants in each population cycles C_0, C_1S, C_1R and C_2R were evaluated for leaf damage using a scale of 1 (resistant) to 9 (susceptible) (CIMMYT, 1989). The number of dead-hearts was assessed as proportion of plants in the plot indicating death of the growing points. Other plant damage parameters were measured at harvest namely; cumulative tunnel length, number of exit holes, stalk strength, and number of larvae recovered per plant. Stalk strength was measured using a rind penetrometer 8 weeks after planting. Agronomic traits were measured following standard protocols used at CIMMYT (CIMMYT, 1989). Grain yield (kg $plot^{-1}$) was obtained as grain weight adjusted for moisture content at 13%, and converted to t ha^{-1}.

6.2.6 Data analysis

Analyses of variance (ANOVA) for all characters measured were computed using PROC GLM procedures in SAS computer package, version 9.2 (SAS Institute. Inc., 2012) combined over locations and separately for each treatment with *B. fusca* and *C. partellus* using the following model:

$Y = \mu + r + t + rt + c + ct + cr(t) + l + lr + lt + rtl + cl + tcl + lcr(t)$

Where;

Y = detected value

μ = overall mean

r = replication effect with 2 levels

t = treatment effect with 2 levels (*B. fusca* and *C. partellus*)

c = cycle effect with 4 levels (C_0, C_1S, C_1R and C_2R)

l = location effect with 3 levels for *C. partellus* (Embu, Kakamega and Kiboko), and 2 levels for *B. fusca* (Embu and Kakamega)

There were 5 error terms specifically;

a) replication interaction with treatment effect - for testing significance of treatments
b) cycle interaction with replication effects nested in treatment - for testing significance of cycles, and treatment x cycles interaction;
c) location interaction with replication effects - for testing significance of locations;
d) replication x treatment x location interaction effects - for testing significance of locations x treatment interaction;
e) location x cycle x replication nested in treatment interaction - for testing significance of locations x cycle interaction, and locations x cycles x treatment interaction.

Population cycles C_0, C_1S, C_1R and C_2R were considered as fixed factors. The locations, replication and interactions were considered random. Data on number of dead-hearts and cumulative stem tunnel length were transformed into arcsine values before subjecting them to analysis of variance (ANOVA). The C_1 susceptible progeny was used as the check. The response to selection was carried out by comparison of each population cycles C_0, C_1S, C_1R and C_2R.

The net genetic gain to selection was carried out by comparison of each population cycles C_0, C_0, C_1S, C_1R and C_2R. The net gain (%) was calculated using the formulae;

The percent net genetic gain to selection was calculated as:

$$\left[\frac{\mu C_n - \mu C_0}{\mu C_0}\right] \text{ x } 100$$

Where, μC_0 and μC_n are means of the stem borer damage traits evaluated at cycles 0 and the n^{th} cycle. The mean of cycle C_0 was used as the reference population.

PROC VARCOMP procedures in SAS computer package, version 9.2 (SAS Institute. Inc., 2012) were used for the estimation of the variance components. Each population was analyzed for the grain yield, stem borer resistance and agronomic traits to establish the genetic variance under *B. fusca* and *C. partellus* infestation. Broad sense heritability (H^2) was obtained using the formula σ^2g/σ^2p (Dabholkar, 1992; Falconer et al., 1996), where ; σ^2g - genotypic variance and σ^2p - phenotypic variance. The standard error of broad sense heritability was calculated as;

SE (H^2) = 2SE {σ^2g} / {$\sigma^2g + \sigma^2p + \sigma^2we$} and,

2SE{ σ^2g} – square root of the genotypic variance and σ^2we - is the within plot variance (Dabholkar, 1992; Falconer et al., 1996).

Selection differential (S) was calculated by subtracting the populations mean for all S_1 progeny from the mean of the selected S_1s to be advanced;

a) $S = \mu_{sel}2 - \mu_o$ for C2

b) $S = \mu_{sel}1 - \mu_o$ for C1

where; $\mu_{sel}2$ is mean of the best 50 selected lines to advance to C_2R,

; $\mu_{sel}1$ is mean of the best 100 selected lines to advance to C_1R,

; μ_o is the mean of the original reference population prior to selection of the best stem borer resistant lines.

PROC CORR procedures in SAS computer package, version 9.2 (SAS, Institute, 2012) were used for the estimation of the genetic correlations between borer resistance and agronomic characters.

6.3 Results

6.3.1 Trait variations in cycles under *C. partellus* infestation

The mean squares of the populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc from analysis of variance for grain yield, stem borer resistance and agronomic traits for were significant (p≤0.01 to p≤0.05) for most traits under *C. partellus* infestation (Table 6.2 and Table 6.3). In population CML395/MBR C5 Bc, the cycle main effects were significant (p≤0.05) for grain yield, plant height, plant and ear aspects, number of exit holes, and cumulative stem tunneling, and leaf feeding damage scores (p≤0.05). The location main effects were significant for all traits measured (p≤0.01). The location x cycle interaction effects were significant (p≤0.05) for grain yield, plant height, plant aspect, number of exit holes, and cumulative stem tunneling, and leaf feeding damage scores (Table 6.2). In population CML444/MBR/MDR C3Bc, the cycle main effects were significant (p≤0.05) for grain yield, plant height, plant aspect, number of exit holes, cumulative stem tunneling, and leaf feeding damage scores (p≤0.05). The location main effects were significant for all traits measured (p≤0.01). The location x cycle interaction effects were significant (p≤0.05) for all traits measured (Table 6.3).

Table 6.2. Mean squares of combined analysis for selected traits in cycles of CML395/MBR C5 Bc under *C. partellus* infestation at Embu, Kakamega and Kiboko

Source	DF	GY	AD	SD	PH	PA	EA	EXH	TL	LD
Rep	3	3.75***	11.02	18.94	17898.97***	1.36	0.35	6.15	0.05	0.06
Cycle	3	9.18***	2.13	9.42	7494.75***	25.04***	4.83***	121.19**	940.99***	17.62***
Location	2	1.11***	25364.82***	21449.33***	1111930.56***	87.19***	255.50***	14023.41***	8464.40***	30.32***
Rep*Location	2	0.35***	40.46***	36.46	2918.23**	0.23	2.93**	23.15	4.03	2.05***
Location*Cycle	6	5.81***	8.53	14.84	5647.59***	16.88***	12.55	388.72***	983.19***	15.79***
Rep*Location*Cycle	6	0.64***	13.12	5.90	18597.11***	8.34***	2.50**	69.02	64.44	1.25***
Error	816	0.03	6.08	7.56	1099.15	0.59	0.89	41.35	63.53	0.30
%R^2		**73.30**	**91.13**	**87.49**	**72.99**	**45.93**	**47.38**	**48.14**	**33.61**	**48.09**

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

Table 6.3. Mean squares of combined analysis for selected traits in cycles of CML444/MBR/MDR C3Bc under *C. partellus* infestation at Embu, Kakamega and Kiboko

Source	DF	GY	AD	SD	PH	PA	EA	EXH	TL	LD
Rep	3	0.19	0.75	11.60	9244.11***	0.19	2.90	35.52	29.46	4.34**
Cycle	3	6.77***	48.50***	2.23***	2634.99***	12.12***	0.56	91.76***	1423.07***	54.14***
Location	2	38.06***	350.53***	748.40***	1234077.27***	87.63***	187.10***	1562.26***	3942.17***	88.25***
Rep*Location	2	0.14	1.74	30.40	16935.14***	0.44	7.60**	3.16	115.64	1.89**
Location*Cycle	6	8.67***	34.12***	35.32***	5292.81***	8.53***	8.83***	134.25***	863.04***	6.96***
Rep*Location*Cycle	6	0.10	6.26***	17.48***	10045.54***	1.56**	4.37**	24.66**	382.37**	2.76***
Error	816	0.13	2.75	4.86	738.98	0.69	1.22	9.93	174.09	0.55
%R^2		**81.34**	**61.17**	**94.58**	**81.34**	**34.50**	**32.53**	**35.97**	**12.66**	**50.10**

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

6.3.2 Trait variations in cycles under *B. fusca* infestation

The mean squares of the populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc from analysis of variance for grain yield, stem borer resistance and agronomic traits were significant (p≤0.01 to p≤0.05) for most traits under *B. fusca* infestation (Table 6.4 and Table 6.5). In population CML395/MBR C5 Bc, the cycle main effects were highly significant (p≤0.01) for grain yield, plant height, plant aspect, number of exit holes, and cumulative stem tunneling, and leaf feeding damage scores except days to anthesis under *B. fusca* infestation (Table 6.4). The location main effects were significant for all traits measured (p≤0.01). The location x cycle interaction effects were highly significant (p≤0.01) grain yield, plant height, plant and ear aspects and cumulative stem tunneling, and leaf feeding damage scores (Table 6.4). In population CML444/MBR/MDR C3Bc, the cycle main effects were highly significant (p≤0.01) for all traits except ear aspect. The location main effects were significant for all traits measured (p≤0.01). The location x cycle interaction effects were highly significant (p≤0.01) for all traits measured (Table 6.5).

Table 6.4. Mean squares of combined analysis for selected traits in cycles of CML395/MBR C5 Bc under *B. fusca* infestation at Embu and Kakamega

Source	DF	GY	AD	SD	PH	PA	EA	EXH	TL	LD
Rep	1	2.38***	3.50	25.20	18490.22*	2.08*	7.80*	73.04***	60.92***	10.68***
Cycle	3	13.02***	2.90	3.54	1603.26	19.95***	4.39***	160.49***	235.92***	3.31***
Location	2	12.80***	19163.53***	16107.52***	1400720.46***	6.14***	44.31***	14608.68***	2087.94***	199.15***
Rep*Location	2	1.31***	71.59***	69.76***	3518.99**	1.79	0.75	4.09	1.22	0.88
Location*Cycle	6	1.75***	2.53	4.17	15057.08***	20.37***	2.42***	57.71***	184.05***	12.16***
Rep*Location*Cycle	6	1.09***	21.71	15.95	472.71	3.52***	0.30	46.89	3.01	5.87***
Error	816	0.15	6.94	7.82	605.57	0.82	0.51	3.33	2.69	0.28
%R^2		**46.34**	**83.68**	**79.28**	**81.62**	**28.45**	**19.44**	**89.75**	**69.75**	**64.92**

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

Table 6.5. Mean squares of combined analysis for selected traits in cycles of CML444/MBR/MDR C3Bc under *B. fusca* infestation at Embu and Kakamega

Source	DF	GY	AD	SD	PH	PA	EA	EXH	TL	LD
Rep	1	0.61***	5.66	17.18	3568.75	1.05	12.70**	13.99	2.81	4.16**
Cycle	3	20.40**	115.79	27.67**	18295.31***	1.30**	4.93***	1152.32***	105.60***	10.78***
Location	2	43.06**	46973.17**	47456.02***	1453219.00***	149.00***	195.27***	401.97***	7910.75***	90.40***
Rep*Location	2	0.10	14.67	29.09***	884.45	0.13	5.67**	0.39	3.18	0.48
Location*Cycle	6	0.58**	64.17	0.75	5619.72**	4.59***	4.94*	809.02***	80.75***	7.69***
Rep*Location*Cycle	6	0.12	133.63	113.12***	3430.43	5.03***	4.01*	16.47	20.82*	1.08
Error	816	0.08	67.24	8.85	1574.88	0.34	1.01	6.50	5.25	0.44
%R^2		**70.93**	**56.96**	**90.93**	**64.90**	**49.87**	**32.14**	**64.98**	**75.36**	**41.98**

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

6.3.3 Mean performance of cycles of two maize populations

Results from the evaluation of the reference cycle (C_0) and the advanced cycles (C_1R and C_2R) of two maize populations across locations under *B. fusca* and *C. partellus* infestation are shown (Table 6.6). In population CML395/MBR C5 Bc, the overall mean grain yield was significantly ($p \leq 0.05$) higher in C_1R and C_2R compared to C_1S and C_0. Grain yield was higher under *B. fusca* infestation compared to *C. partellus* in C_1R and C_2R compared to C_0 and C_1S. No significant differences were detected for days to anthesis and silking. Cumulative stem tunneling was significantly ($p \leq 0.05$) lower in C_1R and C_2R compared to C_0 and C_1S. *B. fusca* caused more tunneling in the cycles than and *C. partellus*. The number of exit holes was significantly ($p \leq 0.05$) higher in C_0 and C_1S than in C_2R except under *C. partellus* infestation. Leaf feeding damage scores were significantly ($p \leq 0.05$) higher in C_0 and C_1S. Cycles C_1R and C_2R had similar mean leaf feeding damage scores under *B. fusca* infestation. Plant and ear aspects were significantly higher in C_1R and C_2R compared to C_0 and C_1S under both *B. fusca* and *C. partellus* infestation. However, plant height for all cycles was not significant under *B. fusca* and *C. partellus* infestation (Table 6.6).

In population CML444/MBR/MDR C3Bc, the overall mean grain yield was significantly ($p \leq 0.05$) higher in C_1R and C_2R compared to C_1S and C_0. No significant differences were identified for days to anthesis and silking. Cumulative stem tunneling was significantly ($p \leq 0.05$) lower in C_1R and C_2R compared to C_0 and C_1S. Although there was a reduction in the level of cumulative tunneling from C_0 to C_2R, *B. fusca* caused more tunneling in the cycles compared to *C. partellus*. The number of exit holes was significantly ($p \leq 0.05$) higher in C_0 and C_1S than in C_2R except under *C. partellus* infestation. Leaf feeding damage scores were significantly ($p \leq 0.05$) higher in C_0 and C_1S in comparison with C_1R and C_2R. However, no significant differences were detected for mean plant aspect and ear aspect, and plant height for all cycles under *B. fusca* and *C. partellus* infestation (Table 6.6).

6.3.4 Genetic gains from selection in cycles

There were net genetic gains from selection in cycles under *B. fusca* and *C. partellus* infestation (Table 6.6 and Table 6.7). Under *B. fusca* infestation, in population CML395/MBR C5 Bc, the net genetic gain in grain yield was 43%, cumulative stem tunneling -41%, number of exit holes -35%, and leaf feeding damage score 0%. In population CML444/MBR/MDR C3Bc, the net genetic gain in grain yield was 25%, cumulative stem tunneling -57%, number of exit holes -69% and leaf feeding damage score 10%. For both populations, the other agronomic traits showed varied trends in the net genetic gain under *B. fusca* infestation (Table 6.6 and Table 6.7).

Under *C. partellus* infestation, in population CML395/MBR C5 Bc, the net genetic gain in grain yield was 70%, cumulative stem tunneling -35%, number of exit holes -35%, and leaf feeding damage score 9%. In population CML444/MBR/MDR C3Bc, the net genetic gain in grain yield was 36%, cumulative stem tunneling -24%, number of exit holes -15% and leaf feeding damage score -29%. For both populations, the other agronomic traits showed wide-ranging inclinations in the net genetic gain under *C. partellus* infestation (Table 6.6 and Table 6.7).

Table 6.6. Means for selected traits in two maize populations under *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

Cycle	**GY**		**LD**		**TL**		**EXH**		**AD**		**SD**		**PA**		**EA**		**PH**	
CML395/MBR C5 Bc																		
Treatment	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**
C0(Reference)	0.81	0.64	2.68	2.78	12.91	6.67	11.24	8.12	72.19	67.95	72.69	68.87	3.16	2.37	3.19	2.37	154.60	176.80
C1Susceptible	0.44	0.66	2.89	2.99	10.97	4.24	8.93	11.50	72.53	68.07	72.92	68.88	2.75	2.97	3.19	2.64	153.50	174.90
C1Resistant	0.99	0.86	2.99	2.32	12.56	4.11	9.76	9.92	72.29	67.94	72.73	68.58	3.54	3.10	3.38	2.68	147.40	183.00
C2Resistant	1.16	1.09	2.69	2.53	8.37	3.91	7.25	10.18	72.39	68.11	73.02	69.05	2.77	2.54	3.56	2.69	154.10	168.90
LSD $_{(0.05)}$	**0.02**	**0.03**	**0.08**	**0.12**	**0.41**	**0.62**	**0.82**	**0.78**	**0.81**	**0.91**	**0.76**	**0.86**	**0.11**	**0.17**	**0.12**	**0.18**	**6.47**	**9.71**
Net gain (%)	**43**	**70**	**0**	**-9**	**-35**	**-41**	**-35**	**25**	**0**	**0**	**0**	**0**	**-12**	**7**	**12**	**14**	**0**	**-4**
CML444/MBR/MDR C3Bc																		
Treatment	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**
C0(Reference)	0.58	0.73	2.98	3.83	15.36	8.11	8.75	8.87	74.71	70.18	75.79	70.73	2.61	3.16	2.40	2.85	158.70	166.80
C1Susceptible	0.61	0.84	3.12	3.43	14.57	6.77	7.73	8.37	75.31	69.06	76.81	70.52	2.79	3.28	2.78	2.94	182.20	175.10
C1Resistant	1.23	0.86	3.62	2.90	16.51	7.66	4.19	9.04	76.85	69.20	76.62	70.18	2.62	3.46	2.56	2.83	159.10	172.60
C2Resistant	1.28	1.15	3.28	2.71	10.49	6.18	2.69	7.55	75.34	70.42	76.56	70.97	2.77	2.88	2.38	2.89	173.00	171.40
LSD $_{(0.05)}$	**0.03**	**0.05**	**0.12**	**0.18**	**0.57**	**0.86**	**1.14**	**1.02**	**1.11**	**1.67**	**1.05**	**1.58**	**0.15**	**0.20**	**0.16**	**0.24**	**8.96**	**10.12**
Net gain (%)	**25**	**36**	**10**	**-29**	**57**	**-24**	**-69**	**-15**	**1**	**0**	**1**	**0**	**6**	**-9**	**-1**	**2**	**9**	**3**

Bf - *B. fusca*, Cp - *C. partellus*, GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage

Table 6.7. Genetic gains[§] for selected traits in two maize populations under *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

CML395/MBR C5 Bc																		
Cycle	**GY**		**AD**		**SD**		**TL**		**EXH**		**LD**		**PA**		**EA**		**PH**	
	Bf	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**
C_1R-C_0	0.18	0.22	0.1	-0.01	0.04	-0.29	-0.35	-2.56	-1.48	1.8	0.31	-0.46	0.38	0.73	0.19	0.31	-7.2	6.2
C_1R-C_1S	0.55	0.2	-0.24	-0.13	-0.19	-0.3	1.59	-0.13	0.83	-1.58	0.1	-0.67	0.79	0.13	0.19	0.04	-6.1	8.1
C_2R-C_1R	0.17	0.23	0.1	0.17	0.29	0.47	-4.19	-0.2	-2.51	0.26	-0.3	0.21	-0.77	-0.56	0.18	0.01	6.7	-14.1
Net Gain (C_2R-C_1S)	**0.72**	**0.43**	**-0.14**	**0.04**	**0.10**	**0.17**	**-2.60**	**-0.33**	**-1.68**	**-1.32**	**-0.20**	**-0.46**	**0.02**	**-0.43**	**0.37**	**0.05**	**0.60**	**-6.00**
Net Gain (C_2R-C_0)	**0.35**	**0.45**	**0.20**	**0.16**	**0.33**	**0.18**	**-4.54**	**-2.76**	**-3.99**	**2.06**	**0.01**	**-0.25**	**-0.39**	**0.17**	**0.37**	**0.32**	**-0.50**	**-7.90**
CML444/MBR/MDR C3Bc																		
	Bf	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**	**Bf**	**Cp**
C_1R-C_0	0.65	0.13	2.14	-0.98	0.83	-0.55	1.15	-0.45	-4.56	0.17	0.64	-0.93	0.01	0.3	0.16	-0.02	0.4	5.8
C_1R-C_1S	0.62	0.02	1.54	0.14	-0.19	-0.34	1.94	0.89	-3.54	0.67	0.5	-0.53	-0.17	0.18	-0.22	-0.11	-23.1	-2.5
C_2R-C_1R	0.05	0.29	-1.51	1.22	-0.06	0.79	-6.02	-1.48	-1.5	-1.49	-0.34	-0.19	0.15	-0.58	-0.18	0.06	13.9	-1.2
Net Gain (C_2R-C_1S)	**0.67**	**0.31**	**0.03**	**1.36**	**-0.25**	**0.45**	**-4.08**	**-0.59**	**-5.04**	**-0.82**	**0.16**	**-0.72**	**-0.02**	**-0.40**	**-0.40**	**-0.05**	**-9.20**	**-3.70**
Net Gain (C_2R-C_0)	**0.70**	**0.42**	**0.63**	**0.24**	**0.77**	**0.24**	**-4.87**	**-1.93**	**-6.06**	**-1.32**	**0.30**	**-1.12**	**0.16**	**-0.28**	**-0.02**	**0.04**	**14.3**	**4.60**

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, Bf - *B. fusca*, Cp - *C. partellus*, § - refers to the true means and their unit of measurement used to calculate the net genetic gain per cycle

6.3.5 Broad sense heritability estimates

The broad sense heritability estimates of cycle 0 and the advanced cycles (C_1R and C_2R) of two maize populations across locations under *B. fusca* and *C. partellus* infestation are presented below (Table 8). Broad sense heritability estimates were high for all traits measured in both maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc (Table 6.8).

Under *B. fusca* infestation, in population CML395/MBR C5 Bc, the broad sense heritability estimates for grain yield were 95.7%, 93.5% and 98.4% for cycles 0, C1R and C2R, separately. In population CML444/MBR/MDR C3Bc under *B. fusca* infestation, the broad sense heritability estimates for grain yield were 99.1%, 99.6% and 99.1% for cycles 0, C1R and C2R, respectively (Table 6.8). Similar trends were detected for leaf feeding damage, cumulative stem tunneling and number of exit holes. Other agronomic characters showed diverse trends for broad send sense heritability estimates (Table 6.8).

Under *C. partellus* infestation, in population CML395/MBR C5 Bc, the broad sense heritability estimates for grain yield were 79.5%, 77.7% and 77.0% for cycles 0, C1R and C2R, in that order. In population CML444/MBR/MDR C3Bc under *C. partellus* infestation, the broad sense heritability estimates for grain yield were 79.1%, 79.9% and 78.8% for cycles 0, C1R and C2R, individually (Table 6.8). Comparable tendencies were identified for leaf feeding damage, cumulative stem tunneling and number of exit holes. Other agronomic traits displayed varied inclinations for broad send sense heritability estimates (Table 6.8).

For both maize populations the genetic variances showed less variation from cycles 0, C1R and C2R for most of the traits.

Table 6.8. Estimates of genetic variances and broad sense heritability for grain yield, stem borer resistance and agronomic traits under *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

CML395/MBR C5 Bc

	GY			**LD**			**TL**			**EXH**		
	C_0	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**
σ^2g	409.9	678.59	660.65	6136.46	5325.9	4381.9	549864.9	1007121.1	254567.5	137550.1	375.38	121048.5
H^2 Cp	**0.795**	**0.797**	**0.770**	**0.765**	**0.799**	**0.787**	**0.799**	**0.800**	**0.799**	**0.799**	**0.566**	**0.799**
H^2 Bf	**0.957**	**0.935**	**0.984**	**0.974**	**0.975**	**0.951**	**0.996**	**0.997**	**0.994**	**0.998**	**0.998**	**0.999**
SE												
	AD			**SD**			**PH**					
	C_0	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**			
σ^2g	253756.90	267619.30	247718.50	205825.30	242765.10	212634.90	6921878.70	17941363.00	2893750.50			
H^2 Cp	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**	**0.799**			
H^2 Bf	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.994**	**0.995**	**0.993**			

CML444/MBR/MDR C3Bc

	GY			**LD**			**TL**			**EXH**		
σ^2g	3315.31	2075.7	3236.03	10137.2	7269.3	4611.2	741840	1105263.3	255637.1	34226.33	332634.3	325475.3
HCP	**0.791**	**0.799**	**0.788**	**0.800**	**0.800**	**0.800**	**0.800**	**0.800**	**0.799**	**0.647**	**0.800**	**0.800**
HBF	**0.991**	**0.996**	**0.991**	**0.981**	**0.978**	**0.965**	**0.999**	**0.998**	**0.998**	**0.885**	**0.871**	**0.877**
	AD			**SD**			**PH**					
	C_0	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**	**C_0**	**C_1R**	**C_2R**			
σ^2g	289104.10	846278.00	333503.70	361250.00	571555.00	429627.00	929757.00	2568774.00	35907.40			
H^2 Cp	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**0.997**	**0.999**	**0.931**			
H^2 Bf	**0.999**	**0.999**	**0.999**	**1.000**	**1.000**	**1.000**	**0.996**	**0.996**	**0.996**			

GY-grain yield (t ha^{-1}), AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, Bf - *B. fusca*, Cp - *C. partellus*

6.3.6 Correlations of selected traits and grain yield

In population CML395/MBR C5Bc, there were significant correlations between grain yield and days to anthesis (r=0.28 p≤0.01) and days to silking (r=0.28 p≤0.01) for *B. fusca* and no significant correlations with *C. partellus*. There were significant negative correlations between grain yield and plant height (r=-0.08 p≤0.01), plant aspect (r=-0.08 p≤0.01), ear aspect (r=-0.09 p≤0.01), number of exit holes, (r=-0.04 p≤0.01), cumulative stem tunneling (r=-0.06 p≤0.01), leaf feeding damage (r=-0.09 p≤0.01) for *C. partellus*. A similar trend was found for *B. fusca* where there were significant negative correlations between grain yield and plant height (r=-0.23 p≤0.01), plant aspect (r=-0.11 p≤0.01), ear aspect (r=-0.09 p≤0.01), number of exit holes, (r=-0.34 p≤0.01), cumulative stem tunneling (r=-0.18 p≤0.01) and leaf feeding damage (r=-0.25 p≤0.01). The correlations between damage traits were stronger for *B. fusca* compared to *C. partellus*. Other agronomic traits displayed varied inclinations for both borers (Table 6.9).

Table 6.9. Pearson correlation coefficients between selected traits for CML395/MBR C5Bc under *B. fusca* and *C. partellus* infestation at Embu, Kakamega and Kiboko

		Chilo partellus								
		GY	**AD**	**SD**	**PH**	**PA**	**EA**	**EXH**	**TL**	**LD**
Busseola fusca	**GY**	1	0.01	0.01	-0.08**	-0.08**	-0.09**	-0.04**	-0.06	-0.09*
	AD	0.28***	1	0.99***	-0.69***	0.35***	0.42***	-0.63***	0.15***	-0.07**
	SD	0.28***	0.99***	1	-0.68***	0.34***	0.41***	-0.62***	0.14***	-0.07**
	PH	-0.23***	-0.79***	-0.77***	1	-0.12***	-0.14***	0.50***	-0.31***	0.16***
	PA	-0.11*	0.09**	0.09**	-0.09**	1	0.31***	-0.29***	-0.08**	-0.07**
	EA	-0.07	-0.33***	-0.31***	0.33***	-0.10**	1	-0.30***	-0.14***	0.05
	EXH	-0.34***	-0.82***	-0.81***	0.81***	-0.07	0.29	1	-0.07**	0.22***
	TL	-0.18***	0.60***	0.59***	-0.54***	0.02	-0.26***	-0.60***	1	-0.08*
	LD	-0.25***	-0.61***	-0.60***	0.62***	-0.01	0.18***	0.87***	-0.45***	1

GY-grain yield, AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

In population CML444/MBR/MDR C3Bc, there were significant correlations between grain yield and days to anthesis (r=0.38 p≤0.01) and days to silking (r=0.51 p≤0.01) under *B. fusca* infestation and significant correlations between grain yield and days to anthesis (r=0.33 p≤0.01) and days to silking (r=0.42 p≤0.01) under *C. partellus* infestation. There were significant positive correlations between grain yield and plant aspect (r=-0.08 p≤0.01) and ear aspect (r=-0.09 p≤0.01). There were significant negative correlations between grain yield and plant height (r=-0.46 p≤0.01), number of exit holes,

(r=-0.11 p≤0.01), cumulative stem tunneling (r=-0.06 p≤0.01), leaf feeding damage (r=-0.37 p≤0.01) under *C. partellus* infestation. Comparable significant correlations were detected under *B. fusca* infestation where there were significant negative correlations between grain yield and plant height (r=-0.44 p≤0.01), number of exit holes (r=-0.27 p≤0.01), cumulative stem tunneling (r=-0.42 p≤0.01) and leaf feeding damage (r=-0.10 p≤0.01). However, positive significant correlations were found between grain yield and days to anthesis (r=0.38 p≤0.01), days to silking (r=-0.51 p≤0.01), plant aspect (r=-0.31 p≤0.01) and ear aspect (r=-0.32 p≤0.01) for under *B. fusca* infestation*. B. fusca* appeared to have a stronger correlation coefficient for damage traits than *C. partellus*. There were diverse trends for the other agronomic traits under *B. fusca* and *C. partellus* infestation (Table 6.10).

Table 6.10. Pearson correlation coefficients between selected traits for CML444/MBR/MDR C3Bc under *B. fusca at* Embu, Kakamega and *C. partellus* infestation at Embu, Kakamega and Kiboko

		Chilo partellus								
		GY	**AD**	**SD**	**PH**	**PA**	**EA**	**EXH**	**TL**	**LD**
Busseola fusca	**GY**	1	0.33***	0.42***	-0.46***	0.12***	0.12***	-0.11***	-0.06*	-0.37***
	AD	0.38***	1	0.79***	-0.62***	-0.10***	-0.10***	0.09***	-0.18***	-0.20***
	SD	0.51***	0.77***	1	-0.81***	-0.09**	-0.08*	0.11***	-0.18***	-0.24***
	PH	-0.44***	-0.55***	-0.70***	1	-0.07*	-0.10**	-0.16**	0.11**	0.32***
	PA	0.31***	0.47***	0.60***	-0.47***	1	0.25***	0.25**	0.11**	-0.14**
	EA	0.32***	0.36***	0.47***	-0.37***	0.31***	1	0.23**	0.11**	-0.22**
	EXH	-0.27***	0.19***	0.16***	-0.20***	0.22****	0.12***	1	0.07**	-0.12
	TL	-0.42***	0.61***	0.76***	-0.70***	0.57***	0.44***	0.56***	1	0.00
	LD	-0.10**	-0.40***	-0.42***	0.33***	-0.37***	-0.18***	-0.33***	-0.43***	1

GY-grain yield, AD-days to anthesis, SD-days to silking, PH-plant height, PA-plant aspect, EA-ear aspect, EXH-number of exit holes, TL-cumulative stem tunneling (cm), LD-leaf feeding damage, *, ** = significant (p≤0.05), highly significant (p≤0.01)

6.4 Discussion

There were highly significant differences detected among the different cycles of the two maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc for the various stem borer resistance and agronomic traits showed the existence of significant variation among the populations that allows for selection of preferred resistant genotypes. The population studied exhibited wide genotypic variability and heritability estimates showing possible projections of selection gain for the subsequent cycles.

For the two maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc, the partition of treatments, cycles and locations and their interactions into variances provided a better insight of the dissimilar patterns among treatments and cycles, and their reaction across locations. In both populations, grain yield was significantly higher in cycles C2R than in C0 and C1S, indicating positive response to selection. Even though incessant genetic gains in successive cycles of recurrent selection has been argued among researchers, in population CML395/MBR C5 Bc, each cycle improved grain yield by 0.5 t ha^{-1}, while in population CML444/MBR/MDR C3Bc, each cycle improved grain yield by 0.8 t ha^{-1}. Similar findings have been reported in literature on maize (Ana Paula et al., 2013). These genetic gains may imply the losses incurred by farmers for not controlling the stem borers in the maize agro-ecologies where they exist. The reduction in the cumulative stem tunneling, number of exit holes per plant, leaf feeding damage scores, in both maize populations' for all cycles and for both *B. fusca* and *C. partellus* stem borers was an improvement in the mitigation of damaging effects of borers in maize plants. The days to anthesis and silking and plant height, plant and ear aspect marginally maintained the same values with the advancing cycles of selection. Similar findings have been reported from previous studies on maize (Ordas et al., 2012; Ordas et al., 2013).

Under *B. fusca* infestation the net genetic gain in cumulative stem tunneling of -41%, number of exit holes -35%, and leaf feeding damage score 0% in population CML395/MBR C5 Bc contributed towards the 43% net genetic gain in grain yield. In this population the reduced stem tunneling and number of exit holes were crucial in the gains in the grain yield. However, in population CML444/MBR/MDR C3Bc, the net genetic gain in cumulative stem tunneling of 57%, number of exit holes -69%, and leaf feeding damage 10% was important in the 25% net genetic gain in grain yield. Similarly, reductions in cumulative stem tunneling, number of exit holes, and leaf feeding damage were of considerable importance towards grain yield gain in population CML444/MBR/MDR C3Bc (Ana Paula et al., 2013; Liberatore et al., 2013). Under *C. partellus* infestation, in population CML395/MBR C5 Bc, the net genetic gain in cumulative stem tunneling of -35%, number of exit holes -35%, and leaf feeding damage score 9% were attributable to the 70% genetic gain grain yield. In population CML444/MBR/MDR C3Bc, the net genetic gain in cumulative stem tunneling of -24%, number of exit holes -15% and leaf feeding

damage score -29% contributed towards the 36% genetic gain in grain yield. For both populations, the other agronomic traits showed wide-ranging inclinations in the net genetic gain under *C. partellus* infestation (Table 6 and Table 7). Comparable outcomes on estimates of gains in selection for yield have been reported in the literature (Ana Paula et al., 2013; Liberatore et al., 2013). Although, results with similar heritability values have been reported, the value of heritability for grain yield detected in this study is an estimate of high magnitude, considering the quantitative and polygenic nature of this trait (Hallauer et al., 2010).

Although broad sense heritability estimates were high (>0.5) for most traits in both populations, they are not reliable (Falconer et al., 1996). The heritability estimates for the stem borer resistant parameters are specific to the populations and the mega environments under study, therefore predictions based on these estimates should be carried out with caution. The characters with low heritability estimates may require more cycles of selection. The variations detected among cycles for heritability estimates may be due to experimental error, genotype x environment interaction effects and possibly due to linkage disequilibrium leading to over estimation of genetic variances. Similar results were reported among cycles of maize populations (Sandoya et al., 2008; Ana Paula et al., 2013). The improvement of grain yield and a reduction in the number of exit holes, cumulative stem tunneling, and leaf feeding damage scores is possible through the S1 progeny recurrent selection. The scheme may be effective for the accumulation of favourable alleles for breeding progress in maize for resistance to *B. fusca* and *C. partellus* attack. In the two populations studied, it is possible to conclude that the success of new selection cycles, which provides a continuous concentration of favorable alleles and the production of hybrids, is likely.

Understanding the level and pattern of genetic correlations between borer resistance and agronomic characters is essential in constructing selection standards (Sujiprihati et al., 2003). Numerous correlations for traits were examined to understand their relationships. For example, in both maize populations, grain yield had positive correlations with days to anthesis and silking, plant and ear aspect implying that improvement in grain yield may be achieved if the magnitude of these traits is reduced. Negative correlations were detected between grain yield and the number of exit holes, plant height and leaf feeding damage scores. The results suggest that a reduction in the degree of the damaging effects due to exit holes, leaf feeding may contribute to an increase in grain yield. Similar findings have been reported from other studies on maize stem borers (Morais et al., 2012; Dhillon et al., 2013). From this study, there were positive correlations between the days to anthesis and silking and number of exit holes in population CML395/MBR C5 Bc, while there were negative correlations between the days to anthesis and silking and number of exit holes in population CML444/MBR/MDR C3Bc. These

corroborate with findings of Ordás et al. (2013) and may imply that selection for reduced number of days to anthesis and silking may reduce the number of exit holes exhibited in this population CML444/MBR/MDR C3Bc due to early maturity. Early maturity increases the probability of a crop to escape the peak periods of pest invasion.

6.5 Conclusion

The study showed that the S1 progeny recurrent selection scheme is effective for the accumulation of favourable alleles for stem borer resistance and indirectly contribute towards genetic gain in grain yield. Through this scheme there was a reduction in the injurious effects of *B. fusca* and *C. partellus* stem borers attributable to number of exit holes, cumulative stem tunneling, and leaf feeding damage scores in the maize populations. This was evident with the improvement of grain yield in the advancing cycles of maize through the S1 progeny recurrent selection scheme. These results suggest that further S1 progeny recurrent selection cycles may further improve the stem borer resistance. The S1 progeny recurrent selection scheme is useful in the development of improved populations and to borer resistance. The method is appropriate in making elite germplasm available for breeding. The advanced cycles of maize populations CML395/MBR C5 Bc and CML444/MBR/MDR C3Bc from the current study will be evaluated further for *B. fusca* and *C. partellus* stem borer resistance and grain yield to confirm their stability. The advanced cycles of these maize populations will be used in breeding with emphasis on borer resistance breeding programmes in the tropics.

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Chapter 7

General Discussion, Conclusion and Recommendations

7.1 Introduction

The purpose of this chapter is to provide a summary of the research findings through a review of the completed investigations, and to draw their inferences for maize breeding. The main objective of the study was to carry out a genetic analysis and establish the responses to selection for resistance to two stem borers, *Busseola fusca* and *Chilo partellus,* in tropical maize (*Zea mays* L.) germplasm across 3 locations in Kenya. To understand the genetics of stem borer resistance in maize the following specific objectives were addressed through studies to:

a) evaluate tropical maize inbred lines for resistance to two stem borers, *Busseola fusca* and *Chilo partellus*,
b) determine combining ability for resistance and heterotic orientation of maize inbred lines under *Busseola fusca* infestation,
c) determine combining ability and heterotic orientation of maize inbred lines under *Chilo partellus* infestation,
d) appraise a detached leaf disk bioassay method for screening for *Busseola fusca* and *Chilo partellus* resistance maize in the greenhouse and laboratory trials, and
e) separately improve resistance to two stem borers *Busseola fusca* and *Chilo partellus* in two tropical maize populations through S1 progeny recurrent selection.

7.2 Summary of key research findings and implications for breeding

7.2.1 Genetic variation for stem borer resistance

It was established that,

a) Twenty one maize inbred lines are resistant to both *Busseola fusca* and *Chilo partellus* in at least two locations, and only four inbred lines showed resistance to both species across the locations, indicating that genotype x environment interactions is involved for conditioning stem borer resistance in maize. These inbred lines will be crucial for breeding programs that breed for resistance to both pests, and for deployment in areas where both pests would occur in the field.

b) Among all the test inbred lines, 26 exhibited resistance to *Chilo partellus* only, while five displayed resistance to *Busseola fusca* only. These lines will be deployed in areas where only one of the pests occurs.

c) Furthermore, 84 and 28 inbred lines showed susceptibility to *Busseola fusca* and *Chilo partellus* respectively. These lines do not have any breeding utility in areas where the pest occurs. Unless they have other desirable attributes they will be discarded from the program.

d) The remaining genotypes were categorized as either moderately resistant or moderately susceptible to either or *Busseola fusca* and *Chilo partellus*. This group of inbred lines will be subjected to further improvement provided they have other desirable attributes such as high grain yield potential.

e) The results showed that most of the test genotypes were susceptible to *Busseola fusca* and less so to *Chilo partellus,* indicating that breeding for *Busseola fusca* would be more challenging. Therefore more resources would be required to improve maize germplasm for resistance to *Busseola fusca* to broaden the base from which breeders will select suitable lines for breeding.

f) Cumulative stem tunnel length and number of exit holes were the most effective parameters for discriminating the test genotypes for resistance to the two borers. These two parameters will be adopted and recommended to breeding programs that emphasise stem borer resistance to enable rapid screening of germplasm with reliable measurements. The other methods will only be included at the advanced stage when genotypes are few to confirm stem borer resistance mechanisms before varieties are deployed.

7.2.2 Combining ability and heterotic orientation under *Busseola fusca* infestation

Allocation of lines into heterotic Group A (CML312/CML442) and Group B (CML395/CML444) was done on the basis of SCA and heterosis data for yield potential under *Busseola fusca* infestation. The Group AB was constituted by inbred lines that showed both good heterosis and good specific combining ability with both testers. Because genotype x environment interactions were large and the sites represented different mega environments results are reported for individual environments.

a) There was significant variation among testcrosses for heterosis for grain yield relative to both testers, which ranged from -91.9% to 98.9%, at Embu; and from -87.3% to 80.2% at Kakamega under *Busseola fusca* infestation. This provided opportunities for selection of testcrosses for advancement to product development, and allocation of the lines into distinct heterotic groups to enhance effectiveness and efficiency of the hybrid program that emphasise stem borer resistance in Kenya.

b) Based on heterosis for grain yield data,

i. At Embu, 9 lines (1, 2, 3, 4, 5, 52, 53, 54 and 55), and at Kakamega 10 lines (62, 11, 33, 20, 32, 42, 66, 10, 7, and 43) showed positive heterosis for grain yield relative to both testers, respectively, indicating that productive three-way cross hybrids can be developed from these germplasm lines. The lines will be advanced in the program.

ii. At Embu, 22 lines were allocated to Group A, 18 to Group B and 8 to Group AB, while at Kakamega, 24 lines were oriented towards Group A, 13 to Group B and 9 to Group AB, whilst the remainder could not be classified.

c) Based on the SCA data,

i. At Embu, 12 lines were assigned to Group A, 9 lines to B and the line 30 was classified as AB.

ii. At Kakamega, 11 lines were allocated to Group A; 9 lines to Group B; and three lines 10, 30, and 32 were oriented towards Group AB.

- Across environments, the lines 16, 18, 38, and 40 displayed positive SCA effects with both testers CML312/CML442 and CML395/CML444 qualifying them as group AB members. In addition, they combined high level of resistance to *Busseola fusca* with high grain yield potential. These lines will be advanced the stem borer resistance program in Kenya, and their three-way cross hybrids will be advanced into the national variety trials.

7.2.3 Combining ability and heterotic orientations under *Chilo partellus* infestation

The maize inbred lines were also classified into heterotic groups under *Chilo partellus* infestation based on heterosis and SCA data as described above.

a) Based on heterosis for grain yield data;

i. At Embu, 15 lines were allocated to group A, 18 to group B and 12 to group AB;

ii. At Kakamega, 26 lines were oriented towards group A, 19 to group B and 9 to group AB.

iii. At Kiboko, 15 lines were inclined towards group A, 18 to group B and 11 to group AB, whilst the remainder could not be classified.

b) Based on the SCA data,

i. At Embu, 8 lines were oriented towards heterotic group A. The lines 46 and 60 fitted into heterotic group AB and B, respectively

ii. At Kakamega, 8 lines inclined to heterotic group B. The lines 26 and 51 were oriented towards heterotic group A and the remainder into group AB.

iii. At Kiboko, 2 lines (31 and 65) were allocated to heterotic group A, however, 9 lines were oriented towards heterotic Group A.

iv. In at least two locations, the lines 43 and 46 showed positive SCA effects for grain yield with both CML395/CML444 and CML312/CML442, so they were steadily classified into AB heterotic group

- In at least two locations, there were lines that displayed combined high level of resistance to *Chilo partellus* with high grain yield potential. However, due to the genotype x environment interactions the lines were oriented differently in the different locations. For example, lines 11, 39 and 66 exhibited dissimilar heterotic orientations in at least two locations except lines 39 and 60. These lines will be advanced the stem borer resistance breeding program in Kenya, and their three-way cross hybrids will be nominated for the national performance trials.

7.2.4 Appraisal of leaf disk bioassay method

The leaf disk bioassay method was appraised for its efficacy for screening maize genotypes for resistance to stem borers *Busseola fusca* and *Chilo partellus* in tropical maize inbred lines in the greenhouse and laboratory. The study can reveal that,

a) A combination of the detached leaf disk bioassay method in the laboratory and infestation with whole plants in the greenhouse is an effective and efficient means of screening maize for resistance to *Busseola fusca* and *Chilo partellus* stem borers.
b) In the greenhouse evaluations, leaf feeding damage scores and cumulative stem tunnelling were the best resistance measurements; while in the laboratory bioassay, leaf area damaged and percent larvae mortality were key parameters for discrimination of genotypes according to stem borer resistance or susceptible.
c) Based on the rank selection index using leaf feeding damage scores and cumulative stem tunnelling in the greenhouse; and using leaf area damaged and percent larvae mortality in the laboratory, 112 test genotypes were evaluated for resistance. The results indicated that 5 genotypes (25, 54, 64, 69 and 102) were among the top 20 for resistance under *Busseola fusca* and *Chilo partellus* infestation. Eight genotypes (15, 42, 57, 83, 96, 99, 100 and 104) were among those considered susceptible in both the laboratory and greenhouse. The remaining 72 genotypes were categorized as moderately resistant and moderately susceptible in both the laboratory and greenhouse under *Busseola fusca* and *Chilo partellus* infestation.

- Therefore the Leaf Disk Bioassay method was considered to be effective and efficient for discriminating genotypes according to resistance to both pests. For this reason, it will be recommended for use in future studies. This is the first time that the method has been appraised for its application in classical breeding for stem borer resistance; therefore the results have implications for breeding programs that emphasise stem borer resistance in maize and similar crops.

7.2.5 Response of maize populations to S1 progeny recurrent selection

Studies carried out on response to two cycles of S1 progeny recurrent selection for resistance to two stem borers *B. fusca and Chilo partellus* in two tropical maize populations CML395/MBR C5 Bc F114-1-2-3-B-4-2-B-B and CML444/MBR/MDR C3Bc F1-1-1-1-B-3-2-B-B revealed that:

a) The net reductions in cumulative tunneling, number of exit holes and leaf feeding damage scores ranged from 0% to 69% for both populations after only two cycles of selection, indicating tremendous genetic gains were realised.
b) In the two maize populations, each cycle of selection for borer resistance improved grain yield by 0.5 to 0.8 t ha^{-1}, indicating that selection for stem borer resistance would not compromise yield. There was no yield penalty which is feared to happen when breeders emphasise resistance parameters during selection.
c) Actual net gains in grain yield with reference to cycle 0 were 43% and 70% for population CML395/MBR C5 Bc F114-1-2-3-B-4-2-B-B, and 25% and 36% for population CML444/MBR/MDR C3Bc F1-1-1-1-B-3-2-B-B under *Busseola fusca* and *Chilo partellus* infestation, respectively after two cycles of selection. Results indicated that response to selection would depend on the population.
d) Negative correlations were recorded between grain yield and the number of exit holes, plant height and leaf feeding damage scores for both borers and populations, indicating that these are the primary traits that must be emphasized during breeding for stem borer resistance.

7.3 General observations on stem borer resistance

The study also indicated that there will be less chances of confounding when test genotypes are infested with both pests in the field, because symptoms which are caused by the pests can be easily differentiated. This is crucial because under field situation both pests might occur which can affect rating if the damage symptoms are confounded.

The following section describes observations on the two stem borers (Figure 7.1 and Figure 7.2). For *Busseola fusca* the first instar larvae feed and produce a characteristic pattern of small holes where leaf tissues have been consumed. The observation may be due to the fact that *B. fusca* bores tissue in a more straight fashion unlike *Chilo partellus* that probably bores unevenly. The emerging leaves appear to have the typical patterns for *Busseola fusca* feeding. The larvae may feed into the growing points causing dead hearts and it produces visible frass. However, *Chilo partellus* feeding damage occurs as a series of small pin holes on juvenile plant leaves and as transparent leaf epidermis 'windowing' symptoms in older leaves. Equally, the larvae may feed into the growing points of maize causing dead hearts (Figure 7.1).

Figure 7.1. Differences in patterns of leaf feeding damage by *B. fusca* (*left*) and *C. partellus* (*right*) in maize field

Figure 7.2. *Busseola fusca* and *Chilo partellus* larvae recovered from susceptible plants in the greenhouse.

7.4 General Discussion and Recommendations

The findings from the completed study indicate high variability of germplasm for resistance to *Busseola fusca* and *Chilo partellus* stem borers exists. Further genetic improvement may be explored through selection for a reduction in the number of exit holes, cumulative stem tunneling, and leaf feeding damage, which are the most effective resistance measurements. This can be done alongside emphasis of the key agronomic traits such as yield potential. The evidence generated suggests that breeders should probably focus more on using cumulative stem tunnel length and number of exit holes in discriminating the test genotypes for resistance to the two borers. Genotypes identified from the current study showed their possible use in hybrid breeding programmes in tropical maize programs that emphasize stem-borer resistance especially in eastern and southern Africa.

The results from the combining ability effects for *Busseola fusca* and *Chilo partellus* resistance suggest that additive gene effects were more important for the control of resistance for both borers. The level of resistance detected in the lines is mainly attributable to the general combining ability effects, therefore should be relatively easy to use. This implies that selection would be effective to improve the levels of resistance, which has been demonstrated. The study identified hybrids with high yield advantage over commercial hybrids indicating significant progress in breeding for resistance to *Busseola fusca* and *Chilo partellus*. In maize agroecologies where these *Busseola fusca* and *Chilo partellus* stem borers

occur exclusively or in league, different genotypes indicating high desirable GCA and SCA effects, and with favourable heterotic orientations for grain yield will be deployed in breeding programmes in Kenya with emphasis on *Busseola fusca* and *Chilo partellus* resistance. The inference is that these superior maize inbred lines have high utility in hybrid pedigree breeding programmes that emphasise stem borer resistance.

The study showed that using testers CML395/CML444 and CML312/CML442, SCA effects based classification was more reliable because of its predictive value for F1 grain yield than heterosis based classification. The heterosis based grouping is subject to environmental effects which might mask expression of heterosis or heterosis for grain yield may change from one site to another due to genotype x environment interactions. Through the SCA effects classification, hybrids with high yield advantage over commercial hybrids were identified demonstrating significant breeding progress for resistance to *Busseola fusca* and *Chilo partellus* stem borers.

The concluded investigations indicated that under *Busseola fusca* infestation using testers CML395/CML444 and CML312/CML442, in Embu and Kakamega, 21 and 20 lines were unclassified into heterotic groups, respectively. Furthermore, under and *Chilo partellus* infestation using testers CML395/CML444 and CML312/CML442, in Embu, Kakamega and Kiboko 21, 12 and 11 lines, in that order were not classified. Overall, greater than 20% of the lines were unclassified into heterotic groups using the two testers. The implication for plant breeding is that there is a need to source new testers to improve the efficiency of discrimination of genotypes for breeding of stem borer resistant hybrids since continuous introduction of new and diverse germplasm into breeding programs may render some testers insensitive.

The completed breeding investigations have sufficiently demonstrated that a combination of infestation of detached leaf disks in the laboratory and whole plant assays in the greenhouse is an effective and efficient for screening maize genotypes for resistance to *Busseola fusca* and *Chilo partellus* stem borers. In a short time, tests with leaves allow multiple assays of genotypes' response to selection due to an increased probability of selecting a resistant plant in a population. This will in turn accelerate breeding progress and reduce the number of cycles required to attain higher resistance levels to *Busseola fusca* and *Chilo partellus* stem borers. The technique will contribute to rapid development of stem borer resistant varieties.

The study demonstrated that S1 progeny recurrent selection scheme is effective for accumulation of favourable alleles for grain yield and stem borer resistance in maize populations. Clearly there was a significant reduction in the injurious effects of both *Busseola fusca* and *Chilo partellus* stem which manifest in reduced number of exit holes, cumulative stem tunneling, and leaf feeding damage scores in the maize populations. These results suggest that supplementary S1 progeny recurrent selection cycles may further improve the stem borer resistance to desired levels. The scheme can be useful in the development of improved populations and later derive lines with good general and specific combining ability with other complementary heterotic groups. The method is appropriate in making elite germplasm available for breeding. The advanced cycles of maize populations CML395/MBR C5 Bc F114-1-2-3-B-4-2-B-B and CML444/MBR/MDR C3Bc F1-1-1-1-B-3-2-B-B from the current study will be advanced to further breeding cycles in the breeding programme in Kenya. The populations will also be recommended to other programmes with emphasis on the *Busseola fusca* and *Chilo partellus*, stem borer resistance in tropical environments.

7.5 Conclusion

Findings from the foregoing breeding investigations will positively impact on both food security and plant breeding capacity. The completed study was successful in identifying new maize inbred lines with resistance to stem borers, *Busseola fusca* and *Chilo partellus.* These lines have high utility to maize breeding programmes that emphasise stem borer resistance in tropical environments. For the hybrid oriented programmes, combining ability and heterotic orientation data for the maize inbred lines will be crucial. In this regard the study was very successful in classifying the lines in three heterotic groups according to single cross testers (CML395/CML444, and CML312/CML442) that are widely used at CIMMYT, and by public breeding programs throughout sub-Saharan Africa. Importantly, this was done based on grain yield potential under *Busseola fusca* and *C. partellus* infestation in three mega environments. The detached leaf disk bioassay method was proven to be effective for screening maize genotypes for *Busseola fusca* and *Chilo partellus* resistance under greenhouse and laboratory conditions. Above all the study demonstrates that S1 progeny recurrent selection is effective for improving stem borer resistance. In sum, this represents significant contribution to plant breeding capacity, especially maize breeding for stem borer resistance.