MAKERERE UNIVERSITY

RESISTANCE TO THE SPOTTED STEM BORER AND AFRICAN MAIZE STEM BORER IN TROPICAL MAIZE

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DECLARATION

This is my original work and has not been presented in this or any other University for the award of a degree.

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Dedication

With immeasurable love, I dedicate this Thesis to my daughter Ciku; thank you for your unwavering love, for courageously bearing with my absence and remaining steadfast and focused in your growth in all aspects of your life at a tender age. Sweetheart, you inspire me.

To my prayerful and hard working mother Muchiru, my sister Wangechi, my brothers Macharia and Gichuru and your families; thank you for being there for Ciku and I, for your support and prayers.

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Preamble

The results sections of this thesis are based on the following paper chapters, some of which have been published while others are to be published:

Chapter 3

Munyiri S., Mugo S. N., Otim M., Tefera T., Beyene Y., Mwololo J. K and Okori P. 2013. Responses of tropical maize landraces to damage by *Chilo partellus* stem borer. 2013. *African Journal of Biotechnology* 12 (11):1229-1235.

Chapter 4

Resistance to the spotted stem borer and African stem borer in commercial maize hybrids, open pollinated varieties and inbred lines.

Paper submitted for Young Women in Africa Science Competition 2013.

Chapter 5

Munyiri S. W., Mugo S. N. Okori P., Otim M. and Mwololo J. K. 2013. Mechanisms of resistance in tropical maize inbred lines to *Chilo partellus* (Lepidoptera: Crambidae). *Journal of Agricultural Science* 5 (7): 51-60.

Chapter 6

Quantitative trait loci for resistance to spotted stem borer and African maize stem borer in a tropical maize population. Manuscript to be submitted for publication.

Chapter 7

General discussions, conclusions and future perspectives.

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RESISTANCE TO SPOTTED STEM BORER AND AFRICAN STEM BORER IN TROPICAL MAIZE

ABSTRACT

In sub-Saharan Africa (SSA), maize (*Zea mays* L.) is the staple food for about 50% of the population. However, lepidoptera stem borer poses a major threat to sustained food sufficiency in SSA causing annual yield losses of 15% and particularly in Kenya where they cause losses estimated at 13.5%. The potential to manage insect pests using host-plant resistance exists, but has not been adequately exploited. The goal of this study was to determine the resistance levels in tropical maize to the African (*Busseola fusca* Fuller) and the spotted stem borer (*Chilo partellus* Swinhoe) to support breeding efforts for improved maize productivity. Specific objectives were; i) Determine the variability in resistance to spotted and African stem borers in tropical maize; ii) Investigate the mechanisms of resistance in CIMMYT tropical maize inbred lines and; iii) Map the quantitative trait loci (QTL) associated with resistance to *C. partellus* and *B. fusca* stem borers using a tropical maize population. Two hundred and ninety five (295) germplasm which included 120 inbred lines, 75 landraces, 100 hybrids and open pollinated varieties (OPVs) were evaluated for two seasons at the Kenya Agricultural Research Institute (KARI)-Kiboko and KARI-Embu field stations. Data were recorded on leaf damage on a 1-9 scale, number of stem borer exit holes, stem tunnel length (cm) and grain yield. A selection index was computed using the damage traits leaf damage, number of stem borer exit holes and cumulative tunnel length to categorize genotypes into resistant and susceptible categories. Germplasm with selection index of below 0.8 were regarded as highly resistant, 0.8-1.00 as moderately resistant, 1.0-1.2 as moderately susceptible and above 1.2 as highly susceptible. Evaluations for mechanisms of resistance were carried out for two seasons at KARI-Kiboko on 120 inbred lines. Data were recorded on leaf toughness, stem penetrometer resistance, trichome density and pith sugar content. To map the QTL for stem borer resistance, a population of 203 F2:3 individuals were developed between 2009 and 2011. Field evaluations were carried out at six sites, three for each stem borer species. Data were recorded on leaf damage, number of stem borer exit holes and cumulative tunnel length as putative stem borer resistance traits. The mapping population was genotyped with 152 single nucleotide polymorphism (SNP) molecular markers. Phenotypic data were subjected to ANOVA using PROC GLM of SAS 2007 and means

separated using Fisher's protected LSD ($P<0.05$). Variability for resistance to maize stem borers was identified in the genotypes evaluated. Top ten highly resistant landraces against *C. partellus* were GUAT 1050, GUAT 280, GUAT 1093, GUAT 1082, GUAT 1014, CHIS 114, GUAT 1034, GUAT 1038, CAQU 321 and GUAN 34. Topmost highly resistant commercial hybrids and OPVs to *C. partellus* were DH01, PH1, ECA-STRIGOFF-VL-102-#-, KDV1-1-#, KDV1-2-#, and PH3253 while, KDV1-3-#, EEQPM-8-EA-#, DH02, KDV1-2-#, DKC8053, POOL15QC, KDV1-1-#, WH403, EEQPM-9-EA-#, and PH4 were highly resistant to *B. fusca* among others. Open pollinated varieties KDV1-1-#, KDV1-2-#, KDV1-3-#, EEQPM-8-EA-#, POOL15QC and EEQPM-9-EA exhibited high resistance to both stem borer species. Most CIMMYT MBR lines exhibited high resistance levels, with CKSBL10008, CKSBL10005, CKSBL10025, CKSPL10273 and CKSBL10027 being the top five highly resistant lines to *B. fusca* and CKSBL10039, CKSBL10025, CKSBL10026, CKSBL10014 and CKSBL10004 the top five highly resistant to *C. partellus*. Dual and high resistance to both stem borer species was found in CIMMYT MBR lines CKSBL10025, CKSBL10026, CKSBL10027, CKSBL10034, CKSBL10014 and CKSBL10039 among other inbred lines. Trichome density was the best mechanism in discriminating genotypes into resistant and susceptible categories, followed by leaf toughness and stem sugar content in that order. Number of stem borer exit holes and cumulative tunnel length were the most consistent traits in assessing resistance. A linkage map spanning 1248.01 cM on 10 chromosomes with an average 8.21cM was constructed. Several QTL for putative resistance traits were detected on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9 based on data from both individual sites and different species. In the combined *B. fusca* sites analysis, one QTL for stem tunnelling was revealed on chromosome 4 (LOD 2.86) while in the *C. partellus* combined sites, one QTL for reduced stem tunnelling on chromosome 4 (LOD 2.81), and another QTL for reduced number of borer exit holes was revealed on chromosome 5 (LOD 2.53). Individual sites analyses revealed five QTL for reduced stem tunnelling, three for stem exit holes and two for leaf damage. Phenotypic variances explained by each QTL ranged from 6 to 10% suggesting a need to validate these QTL using a larger population and in different environments. Variability for resistance against maize stem borers was identified and germplasm identified as highly resistant are recommended as novel sources of resistance for stem borer resistance breeding in SSA. Information on resistant commercial hybrids and OPVs should to be

disseminated to farmers in the relevant ecologies for adoption to curb grain yield losses. Trichome density, leaf toughness and stem sugar content could be adopted as satisfactory indicators of resistance mechanisms and used for pyramiding of resistance genes for high and durable resistance. Quantitative trait loci for the three putative resistance traits were detected in the CIMMYT tropical population studied. Overall, this study identified new sources of resistance to spotted and African stem borers in tropical maize germplasm that could be used as new varieties and/or used as sources of resistance in breeding for resistance to stem borers.

CHAPTER 1

INTRODUCTION

1.1 Importance of maize

In many parts of sub-Saharan Africa (SSA), food insecurity still remains a major challenge with it being escalated by high population growth. Thus the pressure on agriculture to supply staple cereals including maize, rice, and wheat remains high. Indeed, the demand for maize in the SSA outstrips production levels with about 2-3 million tons imported annually to bridge the production gap (FAOSTAT, 2010; Ogunniyi, 2011). About 13% (17.4 million ha) of the global world area under maize (*Zea mays* L.) is planted in Africa, majority of which is in the SSA, where maize is the staple food for approximately 50% of the population (FAOSTAT, 2010; M'mboyi *et a*l., 2010). Maize yields, however, are low due to various biotic and abiotic stresses including pests, diseases, drought, salinity and low soil fertility among others. The average per capita maize consumption in the region is high; for example in Kenya it is about 100 kg per year (Pingali, 2001). In Kenya, annual maize production is 2.3 million tons produced on 1.5 million hectares at an average grain yield of 1.5 t/ha (Pingali, 2001). The average maize yields remain low and annual losses have been estimated to be equivalent to $90M (Mugo and Hoisington, 2001; De Groote, 2002). Songa *et al.* (2001) reported that stem borer damage greatly reduced maize yield, with tunnel lengths greater than 20 cm causing a 40% reduction of potential yield and estimated 33% yield loss found in plants with more than one stem borer exit hole. Farmers viewed stem borer damage as the major insect pest problem reducing maize yields in their farms (Mugo *et al.,* 2001). There is, therefore, need to reduce the yield gap through intensified research. This thesis was an effort to improve management of insect pests, a key challenge to maize production that is needed to partly bridge the yield gap.

1.2 The stem borer challenge to maize production

In spite of the existence of high yielding varieties, farmers in SSA continue to realize low maize yields due to several biotic and abiotic stresses. Kenya is no exception where maize production has historically been constrained by a number of biotic and abiotic factors which include drought, low soil fertility, insects, diseases and weeds (Sallah *et al.,* 2002). Lepidopteran pests especially stem borers pose a major threat to increased production with estimated losses

averaging 13.5% reported in Kenya (De Groote, 2002). For self-reliance in maize, productivity must be increased in SSA countries. This effort is partly constrained by lack of resistant varieties to pests including the maize stem borer.

The major maize stem borer species in eastern Africa include the spotted stem borers (*Chilo partellus* Swinhoe), African stem borers (*Busseola fusca* Fuller), pink stem borers (*Sesamia calamistis* Hampson) and African sugarcane borer (*Eldana saccharina* Walker) (Mugo *et al.*, 2002). In a stem borer distribution study conducted in Kenya, about 54% of the recovered larvae were *C. partellus*, 40% *B. fusca*, 4% *S. calamistis* and 1% *C. orichalcociliellus*, the rest were minor stem borer species (Ong'amo *et al*., 2006). Stem borers may directly or indirectly attack and reduce the optimum development of the maize plant between seedling and grain filling stages, specifically affecting ear development which greatly impacts negatively on grain yield (Afzal, *et al.*, 2009).

Feeding and stem tunneling by stem borer larvae on plants results in crop losses as a consequence of destruction of the growing point, early leaf senescence, interference with translocation of metabolites, and nutrients that result in malformation of the grain, stem breakage, plant stunting, lodging, and direct damage to ears. Infestations by stem borers increase the incidence and severity of stalk rots (Kfir *et al.*, 2002).

The adult moth prefers smooth surfaces of the plant for oviposition, and lays its eggs on the lower surfaces of the leaves and upper part of mid ribs (Kfir, 1997; Hutchingson *et al.*, 2008). The whorl stages (V2-V5 of the maize growth stages) are the most preferred for oviposition (Ofomata *et al.*, 2000; Belfield and Brown, 2008). Incubation of eggs takes about 5−7 days, depending on prevailing environmental conditions. Upon hatching, the neonate larvae migrate from the oviposition site into the leaf whorls, where they establish and feed on tender young leaves. The third larval instar migrates from the leaf whorls and bore into the stems. On older plants, the young larvae may feed on leaf collar tissue before boring into the stem. Fully grown larvae feeding in the stem prepare for emergence as moths by cutting a circular exit hole in the

stem rind before pupation. Pupation takes place inside the stem within a period of 7−10 days depending on temperature (Kfir, 1997; Ofomata *et al.*, 2000; Hutchinson *et al*., 2008).

Chilo partellus is highly invasive and has become the most important stem borer in Kenya and in many SSA countries since its introduction in 1932 (Mbapila *et al*., 2002). In Kenya *C. partellus* and *B. fusca* dominate the stem borer populations though they respectively vary in proportions between agroecological zones (Onga'mo *et al*., 2006). *Sesamia calamistis*, a native species showed spatial overlap and occur in all areas of Kenya although in low densities. The search for durable resistant to both *B. fusca* and *C. partellus* is nascent in most of the SSA countries, but the existing breeding pool is narrow and needs to be improved with novel sources of resistance (Mugo *et al*., 2012).

1.3 Host plant resistance to stem borers

Control of maize stem borers using agrochemicals has not been effective because of the fact that during the destructive stage, the larvae is hidden within the stem and is unreachable by regular chemical control measures. Pesticide control methods are also, environmentally unsafe and are costly for most resource-poor small scale farmers (Kfir *et al.,* 2002; Mugo *et al.,* 2002; Mugo *et al.,* 2005). The nature of stem borer larvae development within the host plant hinders effective control using standard control measures such as regular contact pesticides and predators. Host plant resistance, one of the approaches to manage pests, is important because it is packaged in the seed, causes no hazard to the environment and is compatible with all other control methods (Mugo *et al*., 2005). Host plant resistance to stem borers can be due to antibiosis, antixenosis or tolerance. Antibiosis results in reduced longevity and reproduction and increased mortality of insects. Antixenosis refers to reduced plant attractiveness to feeding and oviposition, whilst tolerance refers to the plant's ability to withstand and recover from damage (Sarwar, 2012). Antibiosis decreases larval development as well as the number of larvae per plant, thereby decreasing the damage levels of stem borers (Pimentel, 2002). In maize, antibiosis is a response to insect damage and is conditioned in part by the toxin hydroxamic acid, 2,4-dihydroxy- 7-methoxy- (2H)-1,4-benzoxazin-3- (4H)-one (DIMBOA) that decreases as the plant matures (Santiago *et al.,* 2003; Smith, 2005). It should, however, be noted that in some maize resistant

genotypes, DIMBOA are lower than levels found in susceptible genotypes, suggesting the presence of other resistance mechanisms (Klun and Brindley, 1966). Antixenosis affects the insect's behaviour towards the plant while tolerance increases a plants ability to survive or recover from pest damage. Resistance may, therefore, involve different mechanisms that interact to determine the level of resistance for each genotype (Santiago *et al*., 2003). Classifying the mechanisms of resistance in existing breeding maize genotypes will improve efficiency of selection through pyramiding of different mechanisms into the preferred breeding materials.

1.4 Exploiting variability for resistance

While germplasm variation within temperate maize has been studied extensively, the levels and patterns of diversity in tropical maize for resistance to biotic and abiotic stresses are still not well understood (Larboda *et al.,* 2005). Knowledge about germplasm diversity and relationships among breeding materials are important in crop improvement strategies (Mohammadi and Prasanna, 2003). Variation in germplasm allows farmers and plant breeders to adapt a crop to heterogeneous and changing environments, for instance providing resistance to insect pests and foliar diseases (Bellon, 2002). New knowledge on tropical maize germplasm's variability to stem borer resistance will promote the search for high and durable resistance by providing breeders with novel sources of maize stem borer resistance (Mugo *et al.,* 2012). Previous studies have recommended that the search for new sources of resistance to stem borers should be continuous and focus on broad groups of germplasm to capture distinct resistance profiles (Malvar *et al*., 2004). The new sources of resistance identified in this study will be incorporated in the ongoing search for borer resistant maize in SSA.

CIMMYT has developed stem-borer resistant germplasm in the SSA (Mugo *et al*., 2005; Beyene *et al*., 2011). However, the diverse variability for borer resistance in these lines needs to be studied and documented. Mapping of the quantitative trait loci (QTL) for resistance will increase utility of these germplasm in breeding programmes. The mechanisms of resistance prevalent in stem borer resistant germplasm are not well known and there is need to characterize them. This study will generate knowledge on the variation to stem borer resistance in tropical maize germplasm that will be useful resources for resistance breeding, variety deployment and

germplasm conservation. Some of the germplasm evaluated in this study are CIMMYT experimental materials previously screened for drought tolerance, low soil nitrogen tolerance, striga (*Striga* spp) resistance and post-harvest storage insects. These, therefore, will build on initiatives to improve maize to more than one production constraint.

1.5 Statement of the problem

Maize yield losses associated with stem borers in SSA region can be as high as 50%, but averages between 13-40% (FAOSTAT, 2010; Abate, 2012). Maize productivity in SSA is, therefore, under threat due to major grain yield losses attributed to stem borer damages. Controlling the pest through the recommended control methods have been challenging, inefficient and so far not successful. Many high yielding maize varieties are susceptible calling for introgression of resistance which is challenging as the trait is quantitatively inherited. Improved varieties have been developed but most of them are from a narrow germplasm base, hence, new sources of resistance are needed to support the breeding programs by increasing the germplasm breeding pool. The breeding programs are also nascent and need to be supported with knowledge on the genetics of inheritance of the stem borer pest for efficiency in the breeding process. Available tropical germplasm have not been evaluated for mechanisms of resistance that would offer opportunity to pyramid resistance genes in elite materials.

1.6 Justification of the study

One of the recommended methods of stem borer control is the use of chemical pesticides. However, control using pesticides has not been efficient partly because during the destructive stage, the larvae is hidden within the stem and is unreachable by regular chemical control measures. Pesticide control methods are also, environmentally unsafe and are costly for most resource-poor small scale farmers in SSA. Studies on the effectiveness of indigenous predators and introduced parasitoids on several stem borer species in the past have concluded that they are not able to keep stem borer populations below economic injury levels (Songa *et al*., 2002). Cultural control method, the oldest traditional practice used for stem borer control cannot be relied upon as a tactful and successful means of control and needs to be combined with other control methods (Kfir *et al.*, 2002). Further, cultural control practices tend to be complicated and

are unpopular in SSA due to the need for more labour and technical knowledge for successful implementation. Accordingly, host plant resistance is one of the important approaches that can easily be used in an integrated manner at a relatively low cost. A resistant crop variety inherently controls the pest without any environmental problems and is compatible with other control methods. Moreover, many high yielding maize varieties are susceptible to stem borers calling for introgression of resistance into these materials. The breeding process, is slow because of the nature of resistance being quantitative and, therefore, difficult to introgress.

Breeding programmes require a supply of well characterized material whose underlying mechanisms to resistance have been elucidated and that are diverse enough to enhance hybrid vigour. Many of the breeding lines in SSA are not well characterized for resistance to the stem borers, neither are the mechanisms of resistance well understood. Thus, to enhance resistance breeding, it is important to identify different mechanisms of resistance exhibited in the breeding lines. Such information allows for pyramiding of resistance genes associated with the different mechanisms into already well adapted high yielding genotypes. In order to exploit natural variations for resistance to stem borers, it is imperative that the mechanisms of variation be elucidated. This is one focus of this thesis study.

In principle, variation for insect resistance exists in maize due to the fact that both the plant and its pest have co-evolved over the years and as such developed mechanisms that regulate the pest-host interactions. Thus, evaluating the resistance levels of diverse collection of maize germplasm may reveal some novel sources of resistance to the stem borers. Studies on variability for resistance in the available tropical maize germplasm coupled with QTL mapping studies of resistance loci can be used to characterize resistance and support crop improvement. A common mapping tool that can be used to enhance breeding activities is the quantitative trait loci (QTL). Mapping of QTL associated with the major stem borer damage for tropical maize germplasm will advance breeding for durable resistance in maize. The goal of this study was, therefore, to identify new sources of resistance to *C. partellus* and *B. fusca* stem borers, characterize resistance mechanisms to these stem borers and map the QTL associated with stem borer resistance to support breeding efforts for improved maize productivity in SSA.

1.7 Specific objectives

1. Determine resistance to *C. partellus* and *B. fusca* maize stem borers among tropical maize germplasm.
2. Identify the mechanisms associated with resistance to *C. partellus* and *B. fusca* stem borers in tropical maize inbred lines.
3. Map the quantitative trait loci associated with resistance to *C. partellus* and *B. fusca* stem borers in the tropical population derived from CML442 x CKSBL10026 cross.

1.8 Hypotheses (Ha)

1. There exists resistance to *C. partellus* and *B. fusca* stem borers in tropical maize germplasm.
2. Resistance in maize to *C. partellus* and *B. fusca* stem borers is conditioned by antibiosis, antixenosis and tolerance mechanisms.
3. There are quantitative trait loci associated with resistance to *C. partellus* and *B. fusca* in the tropical maize population.

1.9 References

Abate, T. 2012. Maize stalk borers of Ethiopia. M. Worku, *et al.*, (eds) Meeting the challenges of global climate change and food security through innovative maize research.). Proceedings of the Third National Maize Workshop of Ethiopia 19-20 April 2011. International Maize and Wheat Improvement Centre (CIMMYT), Addis Ababa, Ethiopia.

Afzal, M., Nazir, Z., Bashir, M. and Khan, B. 2009. Analysis of host plant resistance in some genotypes of maize against *Chilo partellus* (Swinhoe) (Pyralidae: lepidoptera). Pakistan Journal of Botany 41:421-428.

Belfield, S. and Brown, C. 2008. Field crops manual: Maize, A guide to upland production in Cambodia. NSW Department of primary industries. The state of New South Wales.

Bellon, M.R. 2002. Conceptualizing interventions to support on farm genetic resource conservation. World Development 32:159-172.

Beyene, Y., Mugo, S., Gakunga, J., Karaya, H., Mutinda, C., Tefera, T., Njoka, S., Chepkesis, C., Shuma, J.M. and Tende R. 2011. Combining ability of maize (*Zea mays* L.) inbred lines resistant to stem borers. African Journal of Biotechnology 10:4759-4766.

De Groote, H. 2002. Maize yield losses from stem borers in Kenya. Insect Science Applicata 22:89-96.

FAOSTAT. 2010. FAOSTAT Statistics Division: Agricultural Data Retrieved from http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Food and Agriculture Organization of the United Nations, Rome, Italy.

Hutchison, W.D., Venette, R.C., Bergvinson, D. and van den Berg, J. 2008. Pest distribution profile: (*Chilo partellus* Swinhoe) (Lepidoptera: Crambidae). Harvest choice and CIMMYT workshop. Retrieved on 2/11/ 2010 from http://harvestchoice.org/sites/default/files/Chilo%20profile-HC001_v05.pdf.

Kfir, R. 1997. Competitive replacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). Annals of the Entomological Society of America 90:619-624.

Kfir, R., Overholt, W.A,. Khan, Z.R. and Polaszek, A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. Annual Review of Entomology 47:701-731.

Klun, J.A. and Brindley, T.A.. 1966. Role of 6-methoxybenzoxazolinone in inbred resistance of host plant (maize) to first brood larvae of European corn borer. Journal of Economic Entomology 59:711-718.

Larboda, P.R., Garcia, A.A., Paterniani, M.E. and de Souza, A.P. 2005. Tropical maize germplasm: what can we say about its genetic diversity in the light of molecular markers? Theoretical and Applied Genetics 111:1288-1299.

M'mboyi, F., Mugo, S., Mwimali, M. and Ambani, L. 2010. Maize production and improvement in sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF), Nairobi, Kenya.

Malvar, R.A., Butron, A,. Alvarez, A., Ordas, B., Soengas P., Revilla, P. and Ordas, A. 2004. Evaluation of the European union maize landrace core collection for resistance to *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology 97:628-634.

Mbapila, J.C., Overholt, W.A. and Kayumbo H.Y. 2002. Comparative development and population growth of an exotic stem borer, *Chilo partellus* (Swinhoe), and an ecologically similar congener, *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae). Insect Science Application 22:21-27.

Mohammadi, S.A., and Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants-salient statistical tools. Crop Science 43:1235-1248.

Mugo, S. and Hoisington, D. 2001. Biotechnology for the improvement of maize for resource Poor farmers: the CIMMYT approach. Second National Maize Workshop of Ethiopia. 12-16 November, 2001.

Mugo, S., Songa J., DeGroote, H. and Hoisington D. 2002. Insect Resistant Maize for Africa (IRMA) Project: An overview. CIMMYT, Washington DC.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa,. M., Songa, J. and Gichuki, S. 2005. Developing Bt maize for resource-poor farmers - Recent advances in the IRMA project. African Journal of Biotechnology 4:1490-1504.

Mugo, S., Tefera, T. and Beyene Y. Stem Borer Pest Resistant Maize: An Update from Africa. 2012. Presented to the SI 4 Stress tolerant maize for the poorest (Session M1 – Group 1) During the Global Launch of the MAIZE and WHEAT CGIAR Research Programs, Mexico City, 16-20 Jan 2012.

Ofomata, V.C., Overholt, W.A., Lux, S.A. and Van Huis, A. 2000. Comparative studies on the fecundity, egg survival, larval feeding and development of *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae) on five grasses. Annals Entomological Society of America 93:492-499.

Ogunniyi, L.T. 2011. Profit efficiency among maize producers in Oyo state, Nigeria. ARPN Journal of Agricultural and Biological Science 6:11-17.

Ong'amo, G.O., Le Ru, B.P., Dupas, S., Moyal, P., Calatayud, P.A. and Silvain, J.F. 2006. Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. Annales de la Societe Entomologique de France 42:171-177.

Pimentel, D. 2002. Encyclopedia of pest management. Marcel Dekker Inc, New York.

Pingali, P.L., (ed.) 2001. CIMMYT 1999-2000 World maize facts and trends. Meeting world maize needs: technological opportunities and priorities for the public sector. CIMMYT, Mexico, D.F.

Sallah, P.Y.K., Obeng Antwi, K. and Ewool, M.B. 2002. Potential of elite maize composites for drought tolerance in stress and non drought environments. African Crop Science Journal 10:9-19.

Santiago, R., Souto, X.C., Sotelo, J., Butrón, A. and Malvar, R.A. 2003. Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: Noctuidae) attack. Journal of Economic Entomology 96:1563-1570.

Sarwar, M. 2012. Management of rice stem borers (Lepidoptera: Pyralidae) through host plant resistance in early, medium and late plantings of rice (*Oryza sativa* L.). Journal of Cereals and Oil seeds 3:10-14.

Songa, J.M., Zhou, G. and Overholt W.A. 2001. Relationship of stem borer damage and plant physical conditions to yield in a semi-arid zone of eastern Kenya. *Insect Science and its Application* 21:243-9.

Songa, J.M., Mugo, S., Mulaa, M., Taracha, C., Bergvinson, D., Hoisington, D. and De Groote, H. 2002. Towards development of environmentally safe, insect resistant maize varieties for food security in Kenya. Paper Presented to the Syngenta Symposium, June 25, 2002, Washington, USA.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Increased maize productivity is critical for food security in SSA. Stem borers are some of the major pests that account for the low production, with lepidopteran stem borers, including the African stem borer (*B. fusca*), the spotted stem borer (*C. partellus*) and the pink stem borer (*S. calamistis)* being the most damaging pests in eastern and southern Africa, where they cause 13-40% yield losses (De Groote, 2002; Songa *et al*., 2002; Mailafiya *et al.,* 2011). In Kenya, the major maize stem borer species are *C. partellus, B. fusca* and *S. calamistis. Chilo partellus* is found in the warmer and lower areas, *B. fusca* is predominant in the cooler and higher altitudes areas while *S. calamistis* is found in low densities in all ecologies in Kenya (Onga'amo *et al.,* 2006).

Control of maize stem borers using pesticides has not been fully effective due to the pests' mode of crop destruction through feeding and development within the stem of the plant. Pesticide control methods are also, environmentally unsafe and are costly for most resource-poor small scale farmers (Kfir *et al.,* 2002; Mugo *et al.,* 2002; Mugo *et al.,* 2005). Studies on the effectiveness of indigenous predators on several stem borer species in the past have concluded that indigenous predators are not able to keep stem borer populations below economic injury levels (Songa *et al*., 2002). Cultural control method, one of the oldest traditional practices used for borer control cannot be relied upon as a fully successful means of control (Kfir *et al.,* 2002). Cultural control practices tend to be complicated and unpopular in SSA due to the need for more labour and technical knowledge for their successful implementation. Host plant resistance is, therefore, an economically viable control option that is also compatible with all other control methods (Kfir *et al.,* 2002; Mugo *et al.,* 2005; Murenga *et al.,* 2011). Host plant resistance has not been fully utilized in maize breeding, and the focus of this study was to explore opportunities to use host plant resistance through breeding for the management of stem borers' pest in maize.

2.2 Maize stem borers and crop damage

Stem borers are among the most damaging insect pests of maize in Africa (Songa *et al*, 2002). Stem borers destroy maize leaves, stem, ear and the cob reducing grain quantity and quality. Cereal stem borers occur as a complex of species with overlapping spatial and temporal distributions (Overholt *et al*., 2002). Maize stem borers display a high level of significant geographic differentiation in ecological preferences. For example the pest status of *B. fusca* on cereal crops varies between regions in many SSA countries. The population density varies across years, seasons and ecological zones. These variations have been attributed to geographical and genetic differences (Sezonlin *et al*., 2006). These factors make the search for an effective control even more complex. Climatic factors, human activity, availability of wild and cultivated plants and natural enemies, in general influence population fluctuations from bb year to year (Ong'amo *et al.,* 2006).

Amongst stem borer insect pest species, *C. partellus* is the most widely distributed and most damaging maize field pest in Kenya. It has been found in all areas below 1500 m above sea level and occasionally between 1500-2300 m above sea level. The highest stem borer density was found in the semi-arid ecologies. *Busseola fusca* is predominant in the highland areas above 600 m above sea level (Guofa, *et al.*, 2001). Though *B. fusca* is an indigenous species, *C. partellus* an exotic stem borer has continued to expand its area of coverage from the warm low level to the mid-altitude and the moist transitional agroecological zones of Kenya since first reported in Africa in 1932 (Mbapila *et al*., 2002).

Besides maize, the pests' alternative hosts include, many grass species and cereals crops such as sugarcane and sorghum (Ong'amo *et al*., 2006). For example, *C. partellus* stem borer attacks maize, sorghum, millet, rice, sugarcane and many grass species. After the hatching of eggs which are laid on young maize plant seedlings, larvae crawl up the plant and into the maize funnel where they start feeding (Ofomata *et al*., 2000; Hutchison *et al.,* 2008). The early stages of the caterpillars feed on the leaves in the funnel of the plant resulting in characteristic lines of holes and "windows" (Tefera *et al*., 2010). Older larvae enter into the stem and start feeding inside of stems and in the last instar, the mature caterpillar cuts a hole in the stem through which the adult

moth emerges after pupation (Ofomata *et al*., 2000; Hutchison *et al.,* 2008). The larvae feeding on the leaves and stem are the destructive stages but damage to the growing point may results in dead hearts in highly susceptible varieties (Kfir, 1997; Ofomata *et al.,* 2000). Under favourable weather conditions and with ample host crops to maintain larval populations, stem borer pests can continuously reproduce (cycle) throughout the year. However, the stem borer pests initiate diapause in the larval stage to escape unfavourable seasons complicating their control (Kfir *et al.,* 2002; Hutchison *et al.,* 2008). The reported 13.5% yield loss attributed to stem borer damages is equivalent to 400,000 metric tons of maize annually in Kenya (De Groote, 2002; Beyene *et al.,* 2011).

2.3 Progress in management of stem borers losses

In spite of the heavy losses caused by maize stem borers and storage pests in Africa, only CIMMYT's Global Maize Program (GMP) and the International Center for Insect Physiology and Ecology (ICIPE) carry out research on stem borer management in the region (Mugo *et al.* 2008). This has been attributed to the genetic and logistical challenges posed by screening and selecting for insect resistance. Stem borer resistance breeding has also been elusive due to the limited genetic variation, difficulty in maintaining a quantitative trait, and the intricacies of dealing with two organisms i.e. the pests and hosts (Mugo *et al.,* 2002). Efforts have, however, been made to incorporate stem borer resistance into good agronomic background and many genotypes are still undergoing trials (Kfir *et al.,* 2002; Mugo *et al*., 2012). At CIMMYT, insect resistant germplasm has been developed (populations, open pollinated varieties, inbred lines, and hybrids) and several elite inbred line donors for stem borer resistance and CIMMYT inbred lines (CMLs) for use in hybrids combinations have been produced (Mugo *et al*., 2012). Old sources of resistance against stem borer damage comprise of local landraces, collections from exotic germplasm and open pollinated varieties developed within the country by either ICIPE or CIMMYT (Mugo *et al.,* 2012). In 2007, the CIMMYT insect resistant maize project released the first conventionally bred stem borer resistant maize three maize OPVs and three maize hybrids mainly for the low to mid transitional zones (Mugo *et al.,* 2008). More of such conventionally bred varieties are still needed for all maize growing ecologies.

At the ICIPE, an integrated pest management (IPM) project for stem borer control was initiated in the coastal region of Kenya in 1991 and had as part of its objective, the assessment of the relative contribution of different IPM components to maize stem borer yield loss reduction (Ajala *et al.,* 2010). The project saw the introduction of several resistant maize varieties such as ICZ5, IC92M2 and IC92M5 among other varietal crosses and open pollinated populations against *C. partellus* in early 1990s. However, the need for superior genotypes in terms of yield and higher resistance levels is still present (Ajala *et al.*, 2010; Mugo *et al.,* 2012). In 1993 a stem borer larval parasite *Cotesia flavipes* was also released in coastal Kenya, however, only about 30% decrease in the stem borer density has been reported in the region (Kfir *et al.,* 2002; Mailafiya *et al.,* 2011). Parasitism has, therefore, remained low with low recoveries of parasitized stem borer larvae since its release in 1993. Resistant varieties are still needed to supplement these efforts as part of an IPM control strategy. Major challenges that still constraints development of resistant maize include limited sources of resistance to stem borers, and lack of adequate stem borer breeding programs, which slows down improvement strategies (Mugo *et al.,* 2012). Further, stem borer species are distributed by maize growing ecologies, increasing costs and complicating the screening procedures (Ong'amo *et al.,* 2006; Mugo *et al.,* 2012).

2.4 Common management strategies to stem borer pests

Insect pests are more damaging in the tropics than in temperate environments because the climatic conditions are more conducive for accelerated insect development with multiple and overlapping generations leading to high infestation levels and losses (M'mboyi *et al*., 2010). Therefore, effective stem borer insect pest control requires a combination of relevant and affordable tactics for the subsistence farmer (Mugo *et al.,* 2005; Murenga *et al.,* 2011). Damage by insect pests in the field, however, remains a challenging problem for resource poor farmers in Kenya (Mugo *et al.,* 2002). Currently, the management of maize stem borers involves deployment of pesticides, parasitoids as well as cultural control methods as management strategies (Kfir *et al,* 2002; Mugo *et al.,* 2005). These approaches vary in their efficacy and are the focus of this section of the review.

2.4.1 Cultural control methods

Cultural methods that have been tried by farmers in SSA include destruction of crop residues, intercropping, crop rotation, manipulation of planting dates, and tillage methods among others (Kfir *et al.,* 2002). Cultural methods would be the most relevant for maize stem borer control as they are economical and appropriate for resource-poor farmers in Africa (Mugo *et al.,* 2005). These methods are mostly considered the first line of defense against pests, and have little adverse effects on the environment; however, they require the farmers' understanding of stem borers' behavior and the relationships with the crop and the environment (Kfir *et al.,* 2002). There is, therefore, low success of these methods in controlling stem borers mainly due to the techniques unique complexities (Kfir *et al.,* 2002; Mugo *et al,.* 2005; Murenga *et al.,* 2011). Most of these methods come with specific challenges and limitations such as the universal need for intensive labour, knowledge of the pest and the habitat and their interactions for effective use. For instance, the use of the push and pull strategy, timely destruction of residues may require some technical skills for their efficient use (Kfir *et al.,* 2002; Murenga *et al.,* 2011). These methods are, therefore, constrained by lack of enough technical knowledge, are not amenable for large scale farming, and this has reduced them to minimal use in SSA (Kfir *et al*., 2002; Mugo *et al*., 2005; Murenga *et al.,* 2011). These methods would be best used in combination with other control methods such as host plant resistance.

2.4.2 Chemicals use to control stem borer pests

Chemical control methods are the most effective but are expensive to small scale farmers and pose risks to humans, livestock, and the environment (M'mboyi *et al*., 2010; Murenga *et al,* 2011). Stem borers cause damage by tunnelling within the maize plant stem. This nature of damage complicates chemical control because the developing larvae are protected within the host plant and the most effective pesticides against such pests would be systemic in nature (Mugo *et al.,* 2002). The cost of pesticides is prohibitive to resource poor farmers, and some farmer's result to using no control at all (Kfir, *et al.,* 2002; Mugo *et al.,* 2005). Coupled with the low return to investment for cereals such as maize, the use of pesticides is generally low. Accordingly, host plant resistance has continued to gain importance since chemicals are also not accessible to farmers due to poor agro-dealer distribution networks in developing countries, and

concerns over human and environmental health, as well as possibilities of resistance build-up (Mugo *et al.,* 2005; M'mboyi *et al*., 2010). Breeding for host plant resistance thus remains the a very viable and economic method for maize stem borer control in Kenya and most SSA countries (Mugo *et al.,* 2005; Beyene *et al.,* 2011). Where pesticides are readily available and correctly used, they ensure optimum performance of varieties. Chemical pesticides do not increase yields per se, but ensures the yield potential of any genotype is realized by keeping off insect pests' that may interfere with the plant's physiological processes leading to a decrease in production.

2.4.3 Host plant resistance

Host plant resistance (HPR) can be defined as the collective heritable characteristics by which a plant species can reduce the possibility of successful use of the plant as a host by an insect pest (Beck, 1965). The effects of resistant plants on insects can be manifested as antibiosis, in which the biology of the pest is adversely affected; or as antixenosis, in which the plant acts as a poor host and the pest searches for an alternate host plant. When the inherent genetic qualities of the plant provide the ability to endure or recover from insect damage, it is said to express tolerance to the pest (Painter, 1951; Smith, 2005).

Many plant characters are responsible for host plant resistance and these may be divided into morphological and biochemical, with morphological characters being the most important in host plant resistance (Afzal *et al.,* 2009). In maize these are the characters that are responsible for suitability of a variety for feeding, oviposition and development. Trichome densities and surface waxes are considered to comprise negative effects on the oviposition and development of stem borers (Kumar, 1997). Host plant resistance is an important method of stem borer control as it is technically and economically feasible, ecologically sound, and socially acceptable and is compatible with other control methods (Kfir *et al.,* 2002; Mugo *et al.,* 2005).

In cereals, resistance to the stem borers, includes non-preference for oviposition, reduced feeding by on young leaves, low dead heart formation, reduced tunneling, and tolerance to leaf damage and stem tunneling (Sharma *et al.,* 2006). The use of host plant resistance as a part of IPM is, therefore, an acceptable concept that could reduce grain yield losses. Unlike chemical control

methods, resistant varieties can suppress even low pest densities, whereas pesticide usage is justifiable only when the density reaches the economic injury level (Kfir, 1997). To sustain resistance in a crop variety, multiple genes are required to counter the threat for continued pest adaptation (Sadasivam and Thayumanavan, 2003). All the three components of resistance (antibiosis, antixenosis and tolerance) have been identified in stem borer resistant cereals (Singh *et al.*, 2011). Pyramiding these different mechanisms controlled by multiples of diverse genes into suitable varieties could improve durability of resistance and contribute to improving productivity of maize. Understanding the possible mechanisms of resistance to stem borers in different maize germplasm will, therefore, enhance the search for durable resistance to maize stem borer pest.

2.5 Mechanisms of host plant resistance to stem borers

Resistance may involve the different mechanisms which may interact to determine the final level of resistance for a genotype. For instance, morphological characters accounted for 60% of resistance to stem borer damage in maize, with trichome density contributing 57% of the morphological resistance (Kumar, 1997). Ovipositional non-preference (antixenosis) by *C. partellus* has been reported on maize genotypes due to the presence of trichomes and surface waxes (Kumar and Saxena, 1992). Most plants produce morphological and secondary compounds to protect them against herbivores. Resistance studies have earlier focused on the isolation and identification of biologically active compounds in plants (Santiago *et al*., 2003; Kvedaras and Keeping, 2009). When DIMBOA was found in maize plants at high levels during the early stages of plant development, it was found to inhibit stem feeding in several borer species. However, as the maize plant grows, DIMBOA protection against insect herbivores fail, and other mechanisms therefore underlie host plant resistance identified in such plants (Santiago *et al*., 2003).

Trichome density, leaf and stem hardness are important forms of physical resistance against maize stem borers. Cultivars of maize with numerous trichomes are less preferred by stem borers for oviposition (Smith, 1997). Leaf hardness affects borer feeding and is an important factor in conferring resistance to stem borers. In leaves, presence of high quantities of p-coumaric and

ferulic acid provide resistance to borer feeding through cell wall fortification and lignifications (Ukwungwu and Odebiyi, 1985). Trichomes or pubescence (plant hairs) deter feeding, oviposition or both, resulting in non-preference type of resistance (Eyal *et al.,* 2005). The concentration of pith sugars in maize influences resistance to stem borers (Kumar *et al.,* 2006; Sarwar, 2006). High stem sugar content promotes borer feeding and occured in higher levels in susceptible maize genotypes (Kabre and Ghorpade, 1999). Indeed, susceptible maize varieties have been reported to contain significantly higher sugars contents than resistant ones (Arabjafari and Jalali, 2007).

Stem hardness through physical resistance reduced tissue digestibility and increased hardness of plant tissues thereby reducing susceptibility (Arabjafari and Jalali, 2007). Stem hardness also prevented larvae entry through the stem and hindered feeding on the pith, a major cause of weakened stems and yield losses through lodging (Santiago *et al*., 2003). Stem hardness, therefore, forms a logical mechanism that reduces stem tunnelling. Deployment of different mechanisms of resistance enhances resistance against evolution of the aggressive insect populations (Kvedaras and Keeping, 2009). The mechanisms of resistance in tropical inbred lines have not been elucidated. Generating knowledge on the different types of mechanisms operative in the current tropical inbred lines breeding pool will enable researchers to deploy the different mechanisms through pyramiding of resistance genes for increased resistance in future. This information will also guide breeders on the best inbred lines combinations to use for improved resistance in hybrids.

2.6 Deployment of resistance variability for breeding

The need for documentation of maize genetic variability within the available germplasm has increased as breeders search for new sources of resistance to biotic and abiotic stresses reducing maize productivity in SSA. To improve diversity for resistance in tropical maize germplasm, it is important to assess the extent of existing variability within the available tropical germplasm. Such information will support the identification of superior materials that can be exploited in maize improvement (Ahmad and Sharma, 2011; Ahmad *et al.,* 2011).

Maize improvement has been accompanied by a narrowing germplasm base, as newer lines and varieties are derived from crosses of existing elite materials. To widen the genetic base of breeding material, exogenous materials such as landraces introduced from other regions is needed (Li *et al*., 2004). Tropical inbred lines have been shown to hold the greatest diversity capturing over 80% of the allelic diversity in landrace accessions (Liu *et al*., 2003). Tropical inbred lines, therefore, represent an important source of diversity for broadening the genetic base for breeding for resistance to the stem borers. CIMMYT has over the years developed maize inbred lines with superior traits including resistance to field and post-harvest insect pests, drought tolerance, and low N-tolerant. Many tropical landrace accessions which are important crop genetic-resource conservation have also been collected and preserved and hold many genes with potential for maize improvement (Pressoir and Berthaud, 2004). The CIMMYT gene bank for example holds many tropical accessions that could be reservoirs for resistance genes against the stem borer pests. The levels of resistance to maize stem borers in commercial maize hybrids and open pollinated varieties have not been adequately documented. Information, therefore, on variability for stem borer resistance in the available breeding materials is needed for the diverse maize growing ecologies in Kenya as well as for other SSA countries.

2.7 Mapping of resistance loci to support breeding for stem borer resistance

Quantitative trait loci (QTL) are the regions of genome that are associated with quantitative phenotypic trait. Quantitative traits are phenotypes that appear in varying degrees attributed to polygenic effects mainly due to two or more genes and the environment (Doerge, 2002). Quantitative trait loci are identified through mapping, a process that locates genes conditioning the trait's resistance (Semagn *et al.,* 2006a). Quantitative mapping is done using both phenotypic/morphological and molecular markers. Of these markers, single nucleotide polymorphism provides one of the best options for studying metabolism, crop improvement, linkage map construction, marker-trait association and marker-assisted selection (Semagn *et al.,* 2006a). Today the single nucleotide polymorphism provides one of the most ideal genetic markers for studying metabolism, crop improvement, linkage map construction, marker-trait association and marker-assisted selection (Semagn *et al.,* 2006b). When mapping QTL, a segregating population is generated from a cross involving two divergent parents that show clear

genetic differences for the trait of interest. In maize, a cross pollinated plant, backcross and F2 progeny are the simplest types of mapping populations because they are easy to construct and require shorter production durations, however, they have the disadvantage of being too heterozygous (Semagn *et al*., 2006b). The International Maize and Wheat Improvement Centre (CIMMYT) has embarked on the development of insect resistant germplasm using molecular methods including use of QTL to select for improved stem borer resistance (Semagn *et al.*, 2006a; Mugo *et al.*, 2012). Consensus molecular marker maps are available from which possible markers can be identified (Mugo and Hoisington, 2001). The simplest approach for detecting linkage between a DNA marker and a QTL is by using single marker analysis (Holland, 2007). This involves testing a DNA marker in a mapping population and determining if significant differences exist between trait means based on the genotype of a marker using t-tests, analysis of variance (ANOVA) or simple linear regression (Doerge, 2002). Another approach includes utilization of flanking markers called interval mapping (simple interval mapping). Advanced methods such as composite interval mapping utilize flanking markers and require additional markers (Semagn *et al.,* 2006b). Such methods are based on maximum likelihood regression and calculate the most likely position of a QTL within a given interval between two markers. Once tightly-linked markers that reliably predict a trait phenotype have been identified, they may be used for marker-assisted breeding (Collard and Mackill, 2008).

Association mapping, also called linkage disequilibrium mapping, is a new and promising method for mapping complex traits. Unlike the methods mentioned earlier which deal with a fixed population, association mapping is based on a random larger population and is more efficient and effective in confirming candidate genes or in identifying new genes (Yan *et al.*, 2011). Association mapping has higher mapping resolution through the exploitation of historical recombination events at the population level that enable gene level mapping on non-model organisms where linkage-based approaches are not possible (Ersoz *et al.,* 2009). Association mapping can help in crop improvement because genes with significant associations to target traits can be re-sequenced to identify causal mutations and the most favourable alleles for trait improvement, and to develop simple PCR-based markers for marker assisted breeding (MAB) (Yan *et al*., 2011). By determining the allele of a DNA marker, plants that possess particular

genes or QTLs may be identified thus bringing efficiency and effectiveness in breeding (Semagn *et al.,* 2006b). Stem borer resistance is quantitatively inherited, a challenge that has compounded breeding of borer resistant genotypes (Scott *et al*., 1966; Mugo *et al.*, 2005). The availability of genetic maps for markers linked to resistance traits will, therefore, advance breeding for resistant maize. Such genetic maps are currently not available for some of the local stem borer species.

In the European corn borer (ECB), several QTL have been identified in different chromosomes. Although some of the differences in QTL detected may be due to methods used in measuring the insect damage, the results suggest that different QTL for ECB resistance are present among resistant inbred lines (Jampatong *et al*., 2002). Two QTL for stem tunnelling at bins 1.06 and 9.04 have been detected in maize conditioning resistance to Mediterranean corn borer. The two QTL detected for resistance are close to other QTL consistently found for ECB resistance, indicating that mechanisms of resistance are common to either pests or gene clusters controlling the resistance. In the case of ECB, nine QTL for stem tunnelling have been associated with 59% of genetic variation (Jampatong *et al*., 2002). From the above findings it is evident that resistance loci tend to cluster together in several borer species. Several reports have suggested the presence of genes involved in cell wall biosynthesis or fortification with effects on resistance to different corn borers (Ordas *et al.*, 2010). Five candidate genes related to cell wall characteristics which could explain the QTL for stem tunnelling have been suggested for Mediterranean corn borer but the small proportion of genotypic variance explained by the QTLs, however, suggest that there were also many other genes with small effects conditioning resistance (Ordas *et al*., 2010). These studies allow for speculation that QTL for damage parameters associated with major maize stem borers can be detected and such information used to improve breeding for durable resistance. The genetic maps of QTL linked to stem borer resistance traits will assist in the development of maize varieties that are resistant to stem borers through molecular marker assisted breeding in the future in the SSA region. Quantitative trait loci mapping for stem borers resistance has not been carried out using tropical maize populations.

2.8 Sectional conclusion

It is evident that maize productivity as the staple food crop in SSA is under threat due to the maize stem borer pest through grain yield losses. New sources of resistance need to be identified and documented from among the available tropical germplasm for use in advancing resistance breeding for stem borer damage as they are reservoirs for different resistance genes. The challenge in successfully controlling insect pests through the various recommended cultural control methods and pesticides have been limited thus far. Moreover, available tropical germplasm have not been evaluated for mechanisms of resistance that would offer the opportunity to pyramid resistance genes towards reducing losses. The discovery of different mechanisms of resistance in the tropical inbred lines will pave way for pyramiding of these genes. Marker assisted breeding, based on characterized QTL will reduce breeding evaluation cycles currently in use. In the temperate regions, several QTL for maize stem borer resistance have been documented but none from any tropic populations. This study, therefore, intended to bridge these research gaps by identifying new sources of resistance, different mechanisms of resistance, and map the QTL associated with spotted and African stem borer resistance to underpin prospects for marker assisted breeding in maize against these pest species.

2.6 References

Afzal, M., Nazir, Z., Bashir, M. and Khan, B. 2009. Analysis of host plant resistance in some genotypes of maize against *Chilo partellus* (Swinhoe) (Pyralidae: lepidoptera). Pakistan Journal of Botany 41:421-428.

Ahmad, R. and Sharma, J.P. 2011. Genetic variability studies of yield and quality traits in tomato (Solanum lycopersicum L.). International Journal of Plant Breeding and Genetics 5:168-174.

Ahmad, S.Q., Khan, S., Ghaffar, M. andAhmad, F. 2011. Genetic diversity analysis for yield and other parameters in maize (Zea mays L.) genotypes. African Journal of Agricultural Sciences 3:385-388.

Ajala, S.O., Nour, A.M., Ampong-Nyarko, K. and Odindo, M.O. 2010. Evaluation of maize (*Zea mays* L.) genotypes as a component of integrated stem borer (*Chilo partellus* Swinhoe) management in coastal region of Kenya. African Journal of Agricultural Research 5:758-763.

Arabjafari, K.H. and Jalali, S.K. 2007. Identification and analysis of host plant resistance in leading maize genotypes against spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Pakistan Journal of Biological Sciences 10:1885-1895.

Beck, S. D. 1965. Resistance of plants to insects. *Annual Review of Entomology* 10, 207−232.

Beyene, Y., Mugo, S., Gakunga, J., Karaya, H., Mutinda, C., Tefera, T., Njoka, S., Chepkesis, C., Shuma, J.M. and Tende R. 2011. Combining ability of maize (*Zea mays* L.) inbred lines resistant to stem borers. African Journal of Biotechnology 10:4759-4766.

Collard, C.Y.B. and Mackill, D.J. 2008. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Biological Science 363:557-572.

De Groote, H. 2002. Maize yield losses from stem borers in Kenya. Insect Science Applicata 22:89-96.

Doerge, R.M. 2002. Mapping and analysis of Quantitative trait loci in Experimental populations. Nature Reviews: Genetics 3:43-52.

Ersoz, E.S., Yu, J. and Buckler, E.S. 2009. Molecular Genetic Approaches to Maize Improvement. Biotechnology in Agriculture and Forestry 63:173-195

Guofa, Z., Overholt, W.A. and Mochiaha, M.B. 2001. Changes in the distribution of lepidopteran maize stem borers in Kenya from the 1950s to 1990s. International Journal of Tropical Insect Science 21:395-402.

Holland, J.B. 2007. Genetic architecture of complex traits in plants. Current Option in Plant Biology 10:156-161.

Hutchison, W.D., Venette, R.C., Bergvinson, D. and van den Berg, J. 2008. Pest distribution profile: (*Chilo partellus* Swinhoe) (Lepidoptera: Crambidae). Harvest choice and CIMMYT workshop. Retrieved on 2/11/ 2010 from http://harvestchoice.org/sites/default/files/Chilo%20profile-HC001_v05.pdf.

Jampatong, C., McMullen, M.D., Barry, B.D., Darrah, L.L., Byrne, P.F. and Kross, H. 2002. Quantitative trait loci for first- and second-generation European corn borer resistance derived from the maize inbred Mo47. Crop Science 42:584–593.

Kabre, G.B. and Ghorpade, S.A.. 1999. Susceptibility to maize stem borer, *Chilo partellus* (Swinhoe) in relation to sugars, protein and 3 amino acid contents of maize germplasm and FI hybrids. Journal of Insect Science 12:37-40.

Kfir, R. 1997. Competitive replacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). Annals of the Entomological Society of America 90:619-624.

Kfir, R., Overholt, W.A,. Khan, Z.R. and Polaszek, A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. Annual Review of Entomology 47:701-731.

Kumar, H. 1997. Resistance in maize in *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): an overview. Crop Protection 16:243-250.

Kumar, H. and Saxena, K.N. 1992. Resistance in certain maize cultivars to first and third-instar *Chilo partellus* larvae. Entomologia Experimentalis et Applicata 65:75-80.

Kumar, V.K., Sharma, H.C. and Reddy, K. 2006. Antibiosis mechanism of resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. Crop Protection 25: 66–72.

Kvedaras, O.L. and Keeping, M.G. 2009. Silicon impedes stalk penetration by the borer *Eldana saccharina* in sugarcane. Journal of Applied Entomology 125:103-110.

Li, Y., Shi, Y., Song, Y., Du, Y.J., Tuberosa, R. and Wang T.W. 2004. Analysis of genetic diversity in maize inbred lines based on AFLP markers. Maydica 49:89-95.

Liu, K., Goodman, M., Muse, S., Smith, J., Buckler, E. and Doebley, J. 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165:2117-2128.

M'mboyi, F., Mugo, S., Mwimali, M. and Ambani, L. 2010. Maize production and improvement in sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF), Nairobi, Kenya.

Mailafiya, D.M., Le Ru, B.P., Kairu, E.W., Stéphane, D. and Paul-André C. 2011. Parasitism of lepidopterous stem borers in cultivated and natural habitats. Journal of Insect Science:11:15-25.

Mbapila, J.C., Overholt, W.A. and Kayumbo H.Y. 2002. Comparative development and population growth of an exotic stem borer, *Chilo partellus* (Swinhoe), and an ecologically similar congener, *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae). Insect Science Application 22:21-27.

Mugo S. and Hoisington D. 2001. Biotechnology for the Improvement of Maize for Resource Poor Farmers: The CIMMYT Approach. Second National Maize Workshop of Ethiopia. 12-16 November, 2001. CIMMYT, Mexico, D.F.

Mugo, S., Songa J., DeGroote, H. and Hoisington, D. 2002. Insect Resistant Maize for Africa (IRMA) Project: An overview. CIMMYT, Washington DC.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa,. M., Songa, J. and Gichuki, S. 2005. Developing Bt maize for resource-poor farmers - Recent advances in the IRMA project. African Journal of Biotechnology 4:1490-1504.

Mugo, S., Gethi, J., Ouma, J.O., Ajanga, S., Beyene, J., Murenga, G.M., Mulaa, M., Likhayo, P., Gichuki, S., Kega, V., De Groote, H. and Chavangi, A. (Eds). 2008. Book of abstract of the "Consolidating Experiences from IRMA I and II: Achievements, Lessons and Prospects", IRMA Project End-of-Phase II Conference, 28-30 Oct 2008, Nairobi, Kenya: IRMA Document No. 35: KARI and CIMMYT**.**

Mugo, S., Tefera, T. and Beyene Y. Stem Borer Pest Resistant Maize: An Update from Africa. 2012. Presented to the SI 4 Stress tolerant maize for the poorest (Session M1 – Group 1) During the Global Launch of the MAIZE and WHEAT CGIAR Research Programs, Mexico City, 16-20 Jan 2012.

Murenga, G.M., Githiri, S.M., Mugo, S.N. and Olubayo, F.M. 2011. Levels of control of *Chilo partellus* stem borer in segregating tropical Bt maize populations in Kenya. African Journal of Biotechnology 10: 4725-4731.

Ofomata, V.C., Overholt, W.A., Lux, S.A. and Van Huis, A. 2000. Comparative studies on the fecundity, egg survival, larval feeding and development of *Chilo partellus* (Swinhoe) and *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae) on five grasses. Annals Entomological Society of America 93:492-499.

Ong'amo, G.O., Le Ru, B.P., Dupas, S., Moyal, P., Calatayud, P.A. and Silvain, J.F. 2006. Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. Annales de la Societe Entomologique de France 42:171-177.

Ordas, B., Malvar, R., Santiago, R. and Butron, A. 2010. QTL mapping for Mediterranean corn borer resistance in European flint germplasm using recombinant inbred lines. BMC Genomics 11:1-10.

Overholt, W.A., Ngi-Song, A.J., Omwega, C.O., Kimani-Njogu, S.W., Mbapila, J., Sallam, M.N. and Ofomata, V. 2002. An Ecological Approach to Biological Control of Gramineous Stem borers in Africa: The Introduction and Establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae). Biological Control of Gramineous Stemborers in Africa. University of Minnesota.

Painter, R. H. 1951. Insect resistance in crop plants. New York, USA. Macmillan.

Pressoir, G. and Berthaud, J. 2004. Population structure and strong divergent selection shape phenotypic diversification in maize landraces. Heredity 92:95-101.

Sadasivam, S. and Thayumanavan, B. 2003. Molecular host plant resistance to pests. Marcel Dekker Inc, New York.

Santiago, R., Souto, X.C., Sotelo, J., Butrón, A. and Malvar, R.A. 2003. Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: Noctuidae) attack. Journal of Economic Entomology 96:1563-1570.

Scott, G.E., Dicke, F.F. and Pesho, G.R. 1966. Location of genes conditioning resistance in corn to leaf feeding of the European corn borer. Crop Science 6:444-446.

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M.N. 2006a. Principles, requirements and prospects of genetic mapping in plants. African Journal of Biotechnology 5:2569-2587.

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M.N. 2006b. An overview of molecular marker methods for plants. African Journal of Biotechnology 5:2540-2568.

Sezonlin, M., Dupas, S., Le rü, B., Le Gall, P., Moyal, P. and Calatayud, P.A. 2006. Phylogeography and population genetics of the maize stalk borer *Busseola fusca* (Lepidoptera, Noctuidae) in sub-Saharan Africa. Molecular Ecology 15:407-420.

Singh, B.U., Sharma, H.C. and Rao, K.V. 2011. Mechanisms and genetic diversity for host plant resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. Journal of Applied Entomology 135:333–392.

Smith, C.N. 1997. An overview of bases and mechanisms of insect resistance in maize., pp. 316, *In* J. A. Mihn, (ed.) Proceedings Of An International Symposium Held At The International Maize And Wheat Improvement Centre (CIMMYT), 27 November-3 December, 1994. CIMMYT, Mexico D.F.

Smith, M. C. 2005. Plant Resistance to Arthropods Molecular and Conventional Approaches: Molecular and conventional approaches. Springer, Dordrecht, Netherlands.

Songa, J.M., Mugo, S., Mulaa, M., Taracha, C., Bergvinson, D., Hoisington, D. and De Groote, H. 2002. Towards development of environmentally safe, insect resistant maize varieties for food security in Kenya. Paper Presented to the Syngenta Symposium, June 25, 2002, Washington, USA.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2010. Mass rearing of stem borers, maize weevil, and larger grain borer insect pests of maize. CIMMYT: Nairobi, Kenya.

Ukwungwu, M.N. and Odebiyi, J.A. 1985. Resistance of some rice varieties to the African striped borer, *Chilo zacconius* Bleszynski. Insect Science and its Application 6:163-166.

Yan, J., Warburton, M. and Crouch, J. 2011. Association Mapping for Enhancing Maize (Zea mays L.) Genetic Improvement. Crop Science 51:433-449.

CHAPTER 3

REACTION OF TROPICAL MAIZE LANDRACES TO *Chilo partellus* INFESTATION

3.1 Introduction

Maize serves as a source of carbohydrate, protein and minerals in sub-Saharan Africa. Stem borers negatively affects maize growth leading to a reduction in grain quantity and quality. Though the African stem borer *Busseola fusca* Fuller (Lepidoptera: Noctuidae) is the indigenous species in Africa, *C. partellus* Swinhoe (Lepidoptera: Crambidae) has continued to expand its distribution in Kenya since its appearance in Africa in 1932 (Mbapila *et al*., 2002).

Host plant resistance is a practical way to overcome the stem borer constraint in maize production among smallholder resource-poor farmers (Afzal *et al*., 2009). Host plant resistance in most plants is manifested as non-preference or antixenosis, which negatively affects the feeding or oviposition behaviour of the pest. Antibiosis denotes adverse effects on the pest due to plant chemical composition, while tolerance is where the plant grows and reproduces in spite of infestation and/or damage (Painter, 1951). These factors contribute to impaired feeding or oviposition, or contribute to the action of other mortality factors that hinder increase in insect population (Kumar, 1997).

Morphological traits are important in conferring host plant resistance and are responsible for the suitability of a cultivar for feeding by the insect, oviposition and development. In many crop species, the degree of a genotype's resistance to insect pests is associated with the plant's morphological characteristics (Afzal *et al*., 2009). Antixenosis and/or antibiosis for leaf feeding, stem tunnelling, and number of stem exit holes results in resistance to *C. partellus* damage (Singh *et al.,* 2011). Stem borers directly or indirectly attacks and affects the development of the maize plant and thus affect grain yield. Stem borer damage has been reported to affect plant growth and specifically ear development, and this impact negatively on grain yield (Afzal *et al*., 2009). The number of stem exit holes is an indicator of the number of borers that have successfully completed the life cycle within a stem, while the stem tunnels indicate the extent of plant damage.

Old sources of resistance to stem borers in Kenya include local landraces, collections from exotic germplasm and open pollinated varieties used for constituting resistant inbred lines for hybrid combinations. Landraces are especially important for crop genetic-resource conservation and for sustainable agricultural development. They are a reservoir of genes with potential for maize improvement that needs to be exploited (Pressoir and Berthaud, 2004). An understanding of the effects of *C. partellus* damage on the ear morphological characteristics, plant height, stem lodging, stem diameter and grain yield could add information on the selection process in maize landraces. The objective of this study was to identify new sources of resistance to *C. partellus* from among CIMMYT gene bank maize-landrace accessions.

3.2 Materials and Methods

3.2.1 Site description

The experiments were conducted at the Kenya Agricultural Research Institute (KARI) Kiboko Farm in the mid-altitude, dry agro-ecological zone of Kenya. Kiboko lies at 950 m above sea level, 37.75E, and 2.15S and receives about 530 mm of rainfall per annum that falls in two short rainy seasons. Kiboko's maximum daily temperature is 35.1°C with a minimum of 14.3°C. The soils are sandy clays.

3.2.2 Germplasm and field experimental set up

Sixty-nine (69) white endosperm CIMMYT gene-bank accessions previously evaluated for drought tolerance and six insect-resistant CIMMYT hybrid checks were grown in a 15x5 α-lattice experimental design, on two-row plots replicated three times, for two seasons in 2009 and 2010. These landraces represent Caribbean gene bank accessions collected from *P. truncatus* endemic areas of Latin America stored in CIMMYT maize germplasm bank (Kumar, 2002). Each entry was planted in two rows of five meters spaced at 75 cm x 25 cm, respectively. Planting, weeding, harvesting and shelling operations were performed manually. Each plot was divided into two parts; one section for 10 stem borer-infested plants, while the remaining portion consisted of 28 plants protected from borer damage by applying insecticide (Bulldock® 25 EC = 25 g/l Beta-Cyfluthrin – AI). Ten plants per plot (5 plants per row) were infested with five *C.*

partellus first-instar neonates three weeks after planting. The crop was grown under rain-fed conditions but supplemental irrigation was applied as needed. Fertilizers were applied at the rate of 60kg/ha N and 102 kg/ha P2O5 at planting. The crop was top-dressed at the rate of 48kg N/ha 30 days after planting (Ministry of Agriculture, 1987).

3.2.3 Data collection:

Data was taken on leaf-damage visual-rating score two weeks after infestation on a scale of 1-9 on an individual plant basis, where 1 = no visible leaf damage and 9 = plants dying as a result of leaf damage (Tefera *et al*., 2011). At harvest, the numbers of stem exit holes were counted and the cumulative tunnel length (cm) was measured after splitting the stems. Ear diameter (ear with grains), cob diameter (cob without grains) and ear length (cm) taken from the fully-developed grain at the base to the last tip grain were measured. Plant height (cm) was taken during mid grain-filling stage as the distance from the soil level to the base of the flag leaf. Stem diameter (cm) was measured on the internode below the top or primary ear, and root and stem lodging counts were done at harvesting. Grain yield (t/ha) was computed from shelled grain weight and standardized to 12.5% moisture content.

3.2.4 Data analysis

Data was subjected to analysis of variance (ANOVA) and where significant differences were found, means were compared using Fisher's protected least significant difference test (LSD) at ($P < 0.05$). Genotypes were considered as fixed effects. Pearson's correlation coefficients were computed from among the stem borer-damage parameters and other parameters measured. A selection index based on leaf damage score, number of borer exit holes and cumulative tunnel length was computed by summing up the ratios between values and the overall mean and dividing by the number of damage parameters evaluated. Germplasm with selection-index values less than 0.8 were regarded as resistant, 0.8-1.0 as moderately resistant, 1.0-1.2 as moderately susceptible and above 1.2 as susceptible as described in Tefera *et al* (2011). The index is versatile to different pests where selection is based on susceptibility parameters i.e. storage pests. The superiority of the index is that it combines all the putative susceptibility traits to define the resistance/susceptibility responses of the crop being investigated. However, the decision on

genotypes to advance is best guided by the breeder's desired selection intensity for the trait. The data were analyzed using PROC GLM of SAS 2007 package. The variables associations were further explored through multivariate analysis using Genstat software (Genstat, 2009). Principal component analysis (PCA) biplot which reduces multiple testing in the association analyses by summarizing the variables over the various entries and by combining correlated traits into single PCA indices was generated using the genotype means (Dudley and Lambert, 2004).

3.3 Results

3.3.1 Grain yield and ear characteristics

There were highly significant differences (P<0.0001) in grain yield among the landraces. Grain yields ranged from 1.80 t/ha in BRAZ 4 to 8.52 t/ha in GUAT 1100 (Table 3.1). The grain-yield variations among the hybrid checks were less variable and ranged from 7.08 t/ha in CKPH09001 to 9.33 t/ha in CKIR07013. The mean yield for landraces was 5.29 t/ha compared to the hybrid checks' whose mean yield was 8.32 t/ha. Principle component one (PCA-1) which explained 71.21% of the variation in the biplot further indicated that grain yield was most closely related to ear length, stem diameter and ear diameter in that order. The most stable of these parameters in explaining yield were ear diameter and stem diameter, though there were variations in their contributions to yield (Figure 3.1). Grain yield was positively and significantly correlated with ear diameter (r=0.40***), cob diameter (r=0.25**) and ear length (r=0.45***). Among the ear traits measured, ear length contributed most to grain yield, probably through improved kernel number and kernel weight under borer infestation.

3.3.2 Stem borer damage indices, stem exit holes and cumulative tunnel length

The landraces showed significant differences (P < 0.0001) for borer-damage traits number of exit holes and cumulative tunnelling length (Table 3.1). The overall trial means for damage parameters were 9.0 for number of stem borer exit holes and 43.10 cm for tunnel length. The 10 most resistant landraces based on the selection index had stem exit holes mean of 5.50 and tunnel length of 24.80 cm, the same as the hybrid checks. Fourteen landraces were resistant, with a selection index of between 0.56 and 0.81, twenty five were moderately resistant with indices of 0.8-1.0, twenty were moderately susceptible with indices of 1-1.2, and 16 were susceptible with

indices of above 1.2. Thirty eight (38) landraces were, susceptible with a damage-selection index between 1.00 and 1.67, and 22 landraces were moderately susceptible with a selection index of 0.81-0.99. The most resistant landrace was GUAT 1050 with 3.87 exit holes and 16.54 cm tunnel length, while the most susceptible landrace was BRAZ 2179 with 11.51 exit holes and 52.68 cm tunnel length. The most resistant check was the hybrid CKIR06009 with 1.60 holes and 4.37cm tunnel length, while the most susceptible check was the post-harvest pest-resistant hybrid CKPH09001 with 6.90 exit holes and 35.60 cm tunnel length. Tunnel length, however, increased with increasing number of exit holes and so did the selection index, an indication that the two parameters were highly correlated (r=0.76) (Table 3.2). Stem lodging increased with increasing plant height and tunnel length. Reduced tunnelling resulted from lower damage by *C. partellus* in the resistant than in the susceptible landraces. The mean selection index for the resistant landraces was 0.70, which was almost as low as that of the resistant hybrid checks (0.69). Most of the GUAT accessions exhibited resistance, while most BRAZ accessions were susceptible. GUAT accessions were mostly early maturing and shorter in height, while BRAZ accessions were taller and late maturing, probably allowing stem borers more time for stem feeding and thus increasing tunnelling.

3.3.3. Correlation between pest damage assessment traits

Leaf damage, number of exit holes and cumulative tunnel length were all positively correlated with each other i.e. r=0.26***, r=0.18**, r=0.76***, respectively (Table 3.2). The highest correlation coefficient was between the number of exit holes and tunnel length (r = 0.76***). Leaf damage, number of exit holes and tunnel length were all negatively and significantly correlated with grain yield i.e. r=-0.17**, r=-0.17**, r=-0.14**, respectively. The high correlation between stem exit holes and tunnel length was an indication of the interrelatedness between the two parameters, and the possibility of the use of either of the two to measure resistance in maize to *C. partellus*. Tunnel length was also significantly and positively correlated with plant height and ear height (r=0.40***, r=0.38***, respectively), suggesting that the taller the genotype, the greater was the stem damage. Previous studies have reported that taller genotype have longer internodes and portrays increased susceptibility through increased stem feeding (Afzal *et al.,* 2009; Singh *et al.,* 2011). Ear morphological characteristics were

negatively correlated with damage parameters (leaf damage, stem exit holes and cumulative tunnel length), and positively and significantly correlated with grain yield even though the correlation coefficients were low (Table 3.2). There were some highly significant correlations among ear traits. Ear length was correlated with ear diameter (r=0.31***) and cob diameter with ear diameter (r=0.55***), while cob diameter was not significantly correlated with ear length.

3.3.4 Classification of resistance status of landraces

Principal component biplot analysis was used to classify reaction of the landraces to *C. partellus* infestation. The analysis explained 93.61% of the variation in resistance and grouped the damage parameters, the resistant germplasm and the yield components into different quadrants. All resistant landraces were grouped in quadrant one (1) while the hybrid checks were grouped in quadrant two (2) next to grain yield, ear length, stem diameter, and ear diameter, which could be an indication of the contribution of these traits to the yield of these hybrid checks (marked RC in figure 1). Tunnel length, stem lodging, leaf damage, exit holes, plant height and selection index were grouped as indicators of susceptibility in the landraces in quadrants three (3) and four (4), though there were variations among these parameter's contribution to damage. Based on the principal component biplot, the selection index and the three damage parameters exit holes, leaf damage score and cumulative tunnelling had similar response profiles in regards to susceptibility in the landraces. However, cumulative tunnel length was the most stable in expressing resistance while selection index was most variable.

Table 3.1 Reaction of tropical maize landraces to infestation by the spotted stem borer *C. partellus*, under field conditions in Kiboko Kenya during the Oct/Feb 2009 and Mar/Sept 2010 cropping seasons

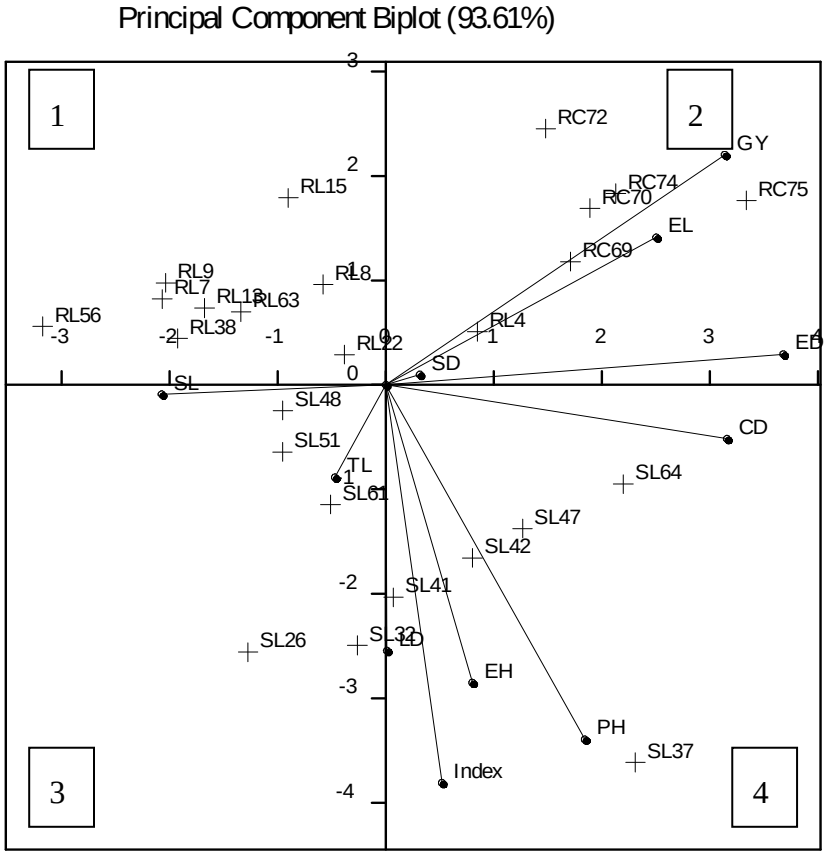
Category	Genotype	Index	Number of exit holes	Tunnel length (cm)	Leaf damage (score)	Ear diameter (cm)	Cob diameter (cm)	Ear length (cm)	Grain yield (t/ha)	plant height (cm)
Resistant	GUAT 1050	0.60	3.90	16.50	2.40	3.80	2.50	9.70	4.70	174.10
Landraces	GUAT 280	0.80	5.20	23.20	2.50	3.80	2.20	10.40	5.40	192.70
	GUAT 1093	0.70	5.30	22.10	2.50	3.60	2.10	13.80	6.70	223.30
	CHIS 114	0.60	5.50	24.10	2.50	4.20	2.60	12.40	5.10	227.50
	GUAT 1082	0.70	6.00	31.20	1.40	3.80	2.40	11.50	6.60	209.40
	GUAT 1014	0.70	6.50	23.10	2.60	4.30	2.40	14.10	7.40	241.40
	GUAN 34	0.60	6.70	22.30	2.00	3.60	2.30	10.40	3.60	214.00
	CAQU 321	0.60	4.00	24.30	2.30	3.90	2.20	12.10	4.40	207.80
	GUAT 1034	0.80	6.20	31.20	1.80	4.00	2.50	10.90	3.00	185.30
	GUAT 1038	0.80	6.10	30.00	2.00	4.10	2.40	12.30	6.10	208.80
Mean		**0.70**	**5.50**	**24.80**	**2.20**	**3.90**	**2.40**	**11.80**	**5.30**	**208.40**
Resistant	CKIR07013	0.70	4.70	19.60	2.00	4.90	3.00	15.60	9.30	229.70
Hybrid check	CKIR06009	0.70	4.40	20.40	1.60	4.70	2.70	13.60	7.90	194.10
	CKIR07001	0.70	5.80	24.50	1.90	4.60	2.80	14.70	8.80	223.80
	CKPH09001	0.80	6.70	35.30	2.10	4.40	2.70	16.60	7.10	222.80
	CKPH08032	0.60	5.90	24.30	2.20	4.60	2.80	15.80	8.50	225.00
Mean		**0.70**	**5.50**	**24.80**	**2.00**	**4.60**	**2.80**	**15.30**	**8.30**	**219.10**
Susceptible	BRAZ 2179	1.70	11.50	52.70	2.30	4.20	2.60	11.00	3.70	271.70
Landraces	VENE 897	1.50	10.40	45.30	2.90	4.30	2.50	14.30	6.50	257.60
	BRAZ 1384	1.40	13.10	63.80	2.50	4.60	2.60	9.20	4.70	247.40
	NAYA 129	1.40	11.90	68.50	2.30	4.10	2.50	14.50	4.60	232.10
	VENE 414	1.40	50.20	11.20	2.60	4.30	3.40	11.00	5.40	329.30

	BRAZ 1346	1.30	10.90	48.40	2.10	3.90	2.20	14.00	4.60	235.80
	BRAZ 1371	1.30	48.50	9.70	2.20	4.70	2.80	12.10	5.90	247.70
	PARA GP3	1.30	40.10	8.10	2.20	4.00	2.20	13.20	5.00	189.10
	BRAZ 4	1.30	68.50	10.90	2.30	3.60	2.10	12.20	3.90	271.70
	BRAZ 222	1.30	58.60	11.80	2.10	4.80	2.60	14.50	7.20	250.30
Mean		**1.40**	**32.40**	**33.10**	**2.40**	**4.30**	**2.60**	**12.60**	**5.20**	**253.30**
Overall Trial Mean			**9.00**	**43.10**	**2.50**	**4.20**	**2.50**	**13.20**	**5.60**	**237.70**
CV			**24.40**	**27.40**	**23.80**	**5.60**	**9.50**	**11.30**	**45.20**	**9.80**
Pr > Value			**<.0001**	**<.0001**	**0.30**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
LSD (0.05)			**3.60**	**19.10**	**0.70**	**0.40**	**0.40**	**2.40**	**3.00**	**37.70**

Table 3.2 Pearson's correlation coefficient (r) between damage parameters and ear characteristics among tropical maize landraces evaluated at Kiboko Kenya during the 2010 and 2011 seasons.

	Leaf damage (score)	Exit holes (#)	Tunnel length (cm)	Plant height (cm)	Ear length (cm)	Stem lodging (#)	Grain yield (t/ha)	Ear diameter (cm)	Cob diameter (cm)
Exit holes (#)	0.26***								
Tunnel length (cm)	0.18**	0.76***							
Plant height (cm)	-0.01ns	0.27***	0.40***						
Ear length (cm)	0.01ns	0.31***	0.38***	0.77***					
Stem lodging(#)	0.19**	0.15**	0.13ns	-0.04ns	0.05ns				
Grain yield (t/ha)	-0.17**	-0.16**	-0.14**	0.07ns	0.02ns	0.22**			
Ear diameter (cm)	-0.15**	0.03ns	-0.01ns	0.21**	0.15**	0.05ns	0.40***		
Cob diameter (cm)	-0.07ns	-0.02ns	-0.03ns	0.33***	0.13ns	0.03ns	0.25**	0.55***	
Ear length (cm)	-0.26***	-0.01**	0.02ns	0.22**	0.24**	0.15**	0.45***	0.31***	0.06ns

* significant at 10%,** significant at 5%, *** significant at 1%, ns; not significant



Key:

GL, grain yield; EL, ear length; ED, ear diameter; CD, cob diameter; PH, plant height; EH, exit holes; TL, tunnel length; SL, stem lodging; RL, resistant landraces; RC, resistant checks; SL, stem lodging;

Figure 3.1 Biplot from a principal component analysis of pest resistance variables assessed on maize accessions evaluated for reaction to *C. partellus* stem borer infestation at Kiboko for two seasons. The biplot identified relationships between damage parameters and yield components in the test germplasm.

3.4 Discussions

Leaf damage scores were not significantly different among the landraces. This was unexpected and could have been an indication of the unreliability of using leaf damage to measure resistance to stem borers in maize. In contrast, Singh *et al*., (2011) had reported that reduced leaf feeding resulted in lower damage effects by *C. partellus*. Number of exit holes and tunnel lengths were highly significantly different among the landraces and probably more reliable in expressing resistance. The greater variation in the selection index value indicated by the vector lengths in the biplot when compared with the other damage parameters was attributed to the use of overall trait mean values in the selection index calculation. The selection index was, however, an accurate method of categorizing the genotypes into resistant and susceptible categories; but the effect of the extreme traits values (lowest and highest damage trait values) may have contributed to the variation observed in the biplot. The similar vector direction for all damage values and the selection index confirms that all these traits are correlated and explained similar profiles for the trait of interest.

In the biplot, tunnel length and stem lodging were nearest to the axes and origin, and thus were more stable in expressing resistance than the other parameters, but tunnel length was the most stable of the two (Figure 3.1). This suggested that the two parameters could be used as stem borer damage-measurement parameters. Stem tunnelling reduces stem strength and predisposes the plant to stem lodging, breakage and ear drop, further compounding the yield losses. Stem lodging especially during the green stages of maize growth contributes to reduced maize grain yield through reduced photosynthetic area in the canopy and destruction of vascular bundles. According to Afzal *et al* (2009), the most damage in maize from stem borers is due to stem tunnelling which lowers plant growth and reduces potential yield by interrupting the flow of photosynthates, minerals and water. The early maturing genotypes are more resistant than the late maturing genotypes as they might go through important physiological stages before the stem borer matures and, therefore, escapes a second generation of stem borer attack. Late maturing varieties were more attractive to second generation moths, thus increasing their susceptibility in comparison with early maturing genotypes (Dicke and Guthrie, 1988).

Among the ear characteristics measured, ear length contributed most to grain yield. Khayatnezhad, *et al.,* (2010) observed that ear length was the most important ear aspect in the determination of grain yield in maize. Increased ear length influences grain yield through increased number of kernels and kernel weight (Nemati *et al.*, 2009). The positive and significant correlation between ear length and grain yield could be attributed to the effect of the ear on grain yield through increased grain number, size and weight. However, yield is affected by many other factors within and outside the plant environment including the genotype, the environment, genotype x environment interactions, and management.

Grain yield was negatively correlated with stem tunnelling, the longer the tunnels were, the lower the yield. Stem tunnelling disrupts nutrients and water uptake resulting in grain-yield penalties in susceptible germplasm (Kumar, 1988). In other studies it has been shown that the direct effect of stem tunnelling on grain yield loss in maize is greater than the effect of leaf feeding (Odiyi, 2007; Singh *et al*., 2011). Leaf damage score was low throughout the trial and may not have appreciably affected photosynthesis and hence lowered grain yield. Better yields may be attributed to lower stem-tunnelling damage and less disruption to water- and nutrient uptake in the resistant germplasm than in the susceptible germplasm, leading to bigger ears with more grains. The correlations between damage assessment parameters (leaf damage, exit holes and tunnel length) and ear characteristics (cob diameter, ear diameter and ear length) were mostly negative, not significant and very low. This was an indication of a probable low influence on ear characteristics by stem borer damage.

3.5 Conclusions

Resistant landraces were identified among the CIMMYT tropical gene-bank accessions evaluated. The resistance of these landraces was comparable to that of CIMMYT resistant hybrid checks. Among the resistant landraces, GUAT accessions were the most resistant (GUAT 1050, GUAT 280, GUAT 1093, GUAT 1082, GUAT 1014, CHIS 114, GUAN 34). Ear length, ear diameter and stem diameter were the traits that had most influence on grain yield. Stem tunnelling and the numbers of stem exit holes were better indicators of resistance to *C. partellus* damage than was leaf damage. The resistant landraces identified could be used to develop *C.*

partellus-resistant maize genotypes for tropical regions, or improved for yield and stability of other favourable traits. It is recommended that these resistant landraces be analysed for the active mechanisms of resistance, and also be incorporated in breeding programs as novel sources of resistance.

3.6 References

Afzal, M., Nazir, Z., Bashir, M and Khan, B. 2009. Analysis of host plant resistance in some genotypes of maize against *Chilo partellus* (Swinhoe) (Pyralidae: lepidoptera). Pakistan Journal of Botany 41:421-428.

Dicke, F. F and Guthrie, W.D. 1988. The most important corn insects, In G. F. Sprague and J. W. Dudley, (eds). Corn and corn improvement, 3rd ed. American Society of Agronomy. Madison, Wisconsin.

Dudley, J. and Lambert, R. 2004. 100 generations of selection for oil and protein in corn. Plant Breeding Review 24:79-110.

Genstat. 2009. Genstat Release 12. VSN International, Hemel, Hempstead, HP1 1ES, United Kingdom.

Khayatnezhad, M., Gholamin, R., Jamati-e-Somarin and Zabihi-e-Mahmoodabad 2010. Study of genetic diversity and Path analysis for yield in corn (Zea mays L.) genotypes under water and dry water conditions. World Applied Science Journal 11:96-99.

Kumar, H. 2002. Resistance in maize to larger grain borer, *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae). Journal of stored food products Research 38: 267 – 280.

Kumar, H. 1988. Effect of stalk damage on growth and yield of certain maize cultivars by the maize stalk borer *Chilo partellus*. Entomologia Experimentalis *et* Applicata 46:149-153.

Kumar, H. 1997. Resistance in maize in *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): an overview. Crop Protection 16:243-250.

Mbapila, J.C., Overholt, W.A and Kayumbo, H.Y. 2002. Comparative development and population growth of an exotic stem borer, *Chilo partellus* (Swinhoe), and an ecologically similar congener, *C. orichalcociliellus* (Strand) (Lepidoptera: Crambidae). Insect Science Applicata 22:21-27.

Ministry of Agriculture, National Agriculture Laboratories. 1987. Fertilizer use recommendation project (Phase 1). Vol 26.

Nemati, A., Sedghi, M., Seyedsharifi, R. and Seidi, N. 2009. Investigation of correlation between traits and path analysis of corn (*Zea mays* L.) Grain yield at the climate of Ardabil Region (Northwest Iran). Notulae Botanicae Horti Agrobotanici Cluj 37:194-198.

Odiyi, O.P. 2007. Relationships between stem borer resistance and grain yield reduction in maize: Correlations, path analysis and correlated response to selection. Agricultural Journal 2:337-342.

Painter, R.H. 1951. Insect resistance in crop plants Macmillan, New York.

Pressoir, G., and Berthaud, J. 2004. Population structure and strong divergent selection shape phenotypic diversification in maize landraces. Heredity 92:95-101.

Singh, B.U., Sharma, H.C. and Rao, K.V. 2011. Mechanisms and genetic diversity for host plant resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. Journal of Applied Entomology 135:333–392.

Tefera, T., Mugo, S., Beyene, Y., Karaya, H. and Tende, R. 2011. Grain yield, stem borer and disease resistance of new maize hybrids in Kenya. African Journal of Biotechnology 10:4777-4788.

CHAPTER 4

RESISTANCE TO SPOTTED STEM BORER AND AFRICAN STEM BORER IN MAIZE HYBRIDS, OPEN POLLINATED VARIETIES AND INBRED LINES

4.1 Introduction

Stem borer damage affects plant growth and specifically ear development impacting negatively on grain yield (Afzal *et al*., 2009). Stem borer larvae feed on young maize leaves reducing photosynthetic area and continues to destroy the stem as they feed on the pith compounding photosynthates translocation and thus reducing grain yields (Tefera *et al*., 2011). The spotted stem borer, *Chilo partellus* Swinhoe (Crambidae) and the African stem borer *Busseola fusca* Fuller (Noctuidae) are the most important lepidopteran stem borer species in Kenya accounting for significant grain yield losses (Tefera *et al*., 2011). The spotted stem borer (*C. partellus*) is the most widespread and damaging field pest of maize in Kenya. It is a highly invasive pest that has become the most important maize stem borer in Kenya since its introduction in the 1930s (Mbapila *et al*., 2002). *Busseola fusca,* which is indigenous and restricted to Africa has been the most economically important and widely spread stem borer pest of cereals in sub Sahara Africa (SSA) for a long time (Kfir *et al*., 2002). *Busseola fusca* maize stem borers cause up to 10% yield losses in Kenya (Ong'amo *et al*., 2006).

Host plant resistance usually deployed as improved varieties is the most viable option for resource constrained farmers and an important component of integrated pest management (Afzal *et al*., 2009). Resistant varieties to maize stem borer have been recommended in several studies (Bosque-Pérez, 1995; Mugo *et al.,* 2005; M'mboyi *et al*., 2010). Whereas intensified efforts to manage stem borers in Kenya have focused on *C. partellus*, indigenous species such as *B. fusca* still remains a major challenge to cereal production (Muturi *et al*., 2012). Developing maize genotypes that are resistant to *B. fusca* is thus still a major development issue that requires investments in research and development to secure harvests and fight extreme poverty. A starting point for improving commercial maize varieties currently cultivated by farmers in Kenya and the SSA region for resistance to *B. fusca*, is by exploiting the variability in resistance that is resident in tropical germplasm (Ahmad and Sharma, 2011; Wen *et al*,. 2012). There is paucity of

information on resistance to stem borers in commercial maize hybrids, open pollinated varieties (OPVs) and a large set of tropical landraces and genebank accessions. The objective of this study was to determine resistance to *C. partellus* and *B. fusca* maize stem borers in tropical inbred lines, commercial varieties and open pollinated varieties.

4.2 Materials and Methods

4.2.1 Study sites and materials used

The evaluations were conducted at the Kenya Agricultural Research Institutes (KARI) Kiboko (*C. partellus*) and Embu (*B. fusca*). The materials included (i) eighty five (85) commercial hybrids including hybrids developed under the Insect Resistant Maize for Africa (IRMA) Project, (ii) 15 improved OPVs and (iii) 120 CIMMYT maize inbred lines. They were evaluated under artificial infestation as described in chapter 3 for two growing seasons i.e. the October-February 2009 and March-September 2010. All the commercial varieties were obtained from commercial suppliers of certified maize seeds in Kenya while the stem borer resistant checks were hybrids developed for Kenyan agro-ecologies but not yet released and were obtained from CIMMYT-Kenya. The inbred lines included CIMMYT experimental materials for drought tolerance, post harvest pests resistance and striga resistance, and were mainly of medium to early maturity.

4.2.2 Experimental design, infestation, data collection and management

Experiments were setup following an α- lattice design with 3 replications in 2 x 5 m row plots spaced at 75 cm between rows and 25 cm between plants. Plant infestation, agronomy and assessment were carried out as described in chapter 3. All data were subjected to analysis of variance and where significant differences were found, means were compared using Fishers protected least significant difference test (LSD) at ($P < 0.05$). An index based on the damage parameters evaluated was computed and used to group the cultivars into susceptible and resistant as indicated in chapter 3. Data were analyzed using SAS 2007 package (SAS Institute Cary NC).

4.3 Results

4.3.1 Reaction of inbred maize lines to *C. partellus* infestation

The genotypes showed significant differences to stem borer infestation ($P<0.05$). The selection index categorized test lines into 26 resistant, 47 moderately resistant, 25 moderately susceptible and 22 susceptible. The top five resistant inbred lines to *C. partellus* were CKSBL10039, CKSBL10025, CKSBL10026, CKSBL10025, and CKSBL10014. The most susceptible inbred lines were CML395, followed by CML312, CZL01005, CML197 and CML204 with indices range of 1.51 to 1.85 (Table 4.1). All the damage parameters were significantly and positively correlated. The highest correlation was between number of exit holes and tunnel length ($r=0.89$). Plant height and grain yield were negatively correlated to the damage parameters, the correlation were low. Grain yield was negatively correlated to all damage parameters including stem lodging but was significantly and positively correlated to plant height ($r=0.54$).

Table 4.1 Reaction of CIMMYT inbred lines to *C. partellus* infestation under field conditions in Kiboko Kenya. The experiments were conducted during the Oct/Feb 2009 and Ma/Sept 2010 cropping seasons.

Resistant lines	Indices	Leaf damage (score)	Number of exit holes (#)	Cumulative stem tunnelling (cm)	Stem lodging (#)	Grain yield (T/ha)	Protected plant height (cm)	Infested plant height (cm)
CKSBL10039	0.5	1.9	1.29	3.61	0.67	2.85	164.72	163.08
CKSBL10025	0.52	1.8	1.86	3.12	2.65	5.97	160.13	161.08
CKSBL10026	0.53	1.89	1.5	4.07	1.54	4.8	159.38	150.27
CKSBL10014	0.57	2.08	1.54	4.44	7.54	2.97	143.77	128.83
CML488	0.67	2.14	1.59	7.88	0.02	2.66	142.03	124.92
CKSBL10004	0.69	1.97	1.85	8.55	0.45	3.95	138.37	133.59
CKSBL10027	0.71	2.18	1.87	8.02	1.82	2.79	142.16	133.43
CKSPL10206	0.72	2.35	2.42	5.67	0.22	2.12	133.36	127.78
CKSBL10040	0.72	2.13	2.08	8.12	1.3	2.79	162.89	146.04
CKSBL10007	0.73	1.85	2.05	9.99	-0.06	4.61	194.35	177.09
CKSBL10035	0.74	1.82	2.28	10.04	3.12	4.45	173.04	159.31
CKSBL10045	0.76	2.06	2.48	8.47	0.66	3.54	142.88	129.84
CKSBL10043	0.76	2.18	2.76	6.93	0.95	3.73	136.93	139.82
CKSBL10034	0.76	1.72	2.77	9.7	4.35	3.54	141.99	134.93
CKSBL10001	0.77	1.69	3.08	9.21	1.66	4.87	170.43	155.25
Mean	**0.68**	**1.98**	**2.1**	**7.19**	**1.79**	**3.71**	**153.76**	**144.35**
Susceptible lines								
CKSBL10041	1.35	2.61	4.98	19.36	0.95	5.58	173.73	169.95
CZL00003	1.35	2.23	5.4	20.11	0.85	4.65	179.54	159.57
CKSPL10224	1.37	2.64	5.28	19.08	0.19	1.65	162.07	144.44

DTPWC9-F115-1-4-1-1-B-B-	1.39	2.42	6.2	17.59	1.41	3.5	138.44	117.67
CKSPL10136	1.4	2.43	4.97	22.17	2.14	1.57	184.12	168.95
CKSPL10218	1.44	2.73	6.7	16.11	1.76	2.52	143.03	132.69
CML442	1.44	2.12	6.72	19.66	0.37	4.54	154.61	148.01
CML264	1.47	2.33	5.81	22.79	1.41	5.73	170.52	159.41
CKSBL10013	1.48	2.45	5.68	22.62	1.99	3.46	169.94	163.15
CML202	1.49	2.33	5.79	23.33	1.01	3.8	155.16	139.16
CML204	1.51	2.42	5.89	23.53	1.43	5.3	187.86	196.41
CML197	1.53	1.95	6.52	24.85	0.6	2.79	179.19	174.45
CZL01005	1.59	2.59	6.5	23.56	1.33	2.01	156.29	146.13
CML312	1.64	1.89	7.23	26.77	0.32	3.39	175.55	170.56
CML395	1.85	2.85	8.12	26.12	1.54	4.47	185.99	175.45
Mean for susceptible	**1.49**	**2.4**	**6.12**	**21.84**	**1.11**	**3.66**	**167.74**	**157.73**
Trial Mean		**2.22**	**3.69**	**13.7**	**1.74**	**3.55**	**156.41**	**145.42**
CV		**21.9**	**31.01**	**34.86**	**67.65**	**27.65**	**7.84**	**5.49**
Pr > Value		**<.0001**	**0.0002**	**<.0001**	**<.0001**	**0.055**	**<.0001**	**<.0001**
LSD		**0.6**	**0.64**	**9.42**	**0.74**	**1.11**	**19.76**	**12.87**

Table 4.2 Pearson's correlation coefficient (r) between damage parameters, plant height and grain yield among CIMMYT tropical inbred lines evaluated at Kiboko Kenya against *C. partellus* resistance.

	Leaf damage	Exit hole	Tunnel length	Plant height	Stem lodging
Exit hole	0.14**				
Tunnel length	0.09*	0.89***			
Plant height	-0.15**	0.01ns	0.24***		
Stem lodging	0.07ns	-0.02ns	-0.08ns	-0.05ns	
Grain yield	-0.27***	-0.15**	-0.11*	0.54***	-0.12*

*significant at 10%, ** significant at 5%, *** significant at 1%, ns; not significant

4.3.2 Reaction of inbred lines to *B. fusca*

There were significant differences in the inbred lines reaction to *B. fusca* infestation in all parameters measured (P>0.05). The trial means were 2.97, 0.91, and 3.01 for leaf damage, number of exit holes and tunnel length, respectively (Table 4.3). The selection index categorized the inbred lines into 33 resistant (<0.8), 31 moderately resistant (0.81-1.0), 28 moderately susceptible (1.1-1.2) and 28 susceptible (1.2-2.09). The CIMMYT multiple borer resistant (MBR) inbred lines were the most highly resistant with the inbred line CKSBL10008, being the most resistant with a selection index of 0.29. The inbred line CZL01005 was the most susceptible with a selection index of 2.09. Other highly resistant lines were CKSBL10005, CKSBL10025, CKSBL10027, CKSPL10273, CKSBL10026, CKSPL10080 and CKSPL10090. Mean plant height in the susceptible plots was 134.73 cm and 146.54 cm in protected plots, a reduction of 11.81 cm in plant height under infestation. Albeit insignificant, all the damage parameters (leaf damage, exit holes and cumulative tunnel length) were negatively correlated to yield, while number of exit holes was highly and significantly correlated to tunnel length (r=0.83) (Table 4.4)

Table 4.3 Reaction of CIMMYT tropical maize inbred lines to *B. fusca* infestation under field conditions in Embu Kenya. The experiments were conducted during the Oct/Feb 2010 and Mar/Sept 2011 cropping seasons.

	Pedigree	Index	Leaf damage	Exit holes (#)	Tunnel length (cm)	Infested plant height (cm)	Protected plant height (cm)
Resistant	CKSBL10008	0.29	2.16	0.05	0.28	144.26	157.68
Inbred lines	CKSBL10005	0.32	2.82	0.05	0.17	122.82	133.95
	CKSBL10025	0.33	2.38	0.09	0.29	140.41	143.14
	CKSBL10027	0.55	2.14	0.47	1.27	120.06	125.82
	CKSPL10273	0.56	2.70	0.34	1.20	118.23	132.2
	CKSPL10090	0.58	2.61	0.45	1.09	139.42	142.43
	CKSBL10026	0.58	2.59	0.51	0.95	135.94	148.79
	CKSPL10080	0.6	2.83	0.43	1.16	125.73	134.36
	CML78	0.61	3.4	0.43	0.61	125.61	135.81
	CKSBL10034	0.61	2.23	0.69	0.97	109.43	107.99
	CKSPL10086	0.63	3.3	0.52	0.62	120.14	125.56
	La Posta Seq C7	0.65	2.51	0.7	0.99	135.56	142.22
	CKSBL10021	0.68	2.61	0.52	1.74	138.35	148.21
	CKSBL10020	0.68	2.79	0.6	1.31	114.59	117.68
Mean for resistant lines		**0.55**	**2.66**	**0.43**	**0.88**	**128.06**	**134.86**
Susceptible Inbred lines							
	P300C5S1B-2-	1.33	3.26	1.35	4.26	117.05	125.49
	CML444	1.36	3.88	1.36	3.85	120.71	129.13
	CKSPL10164	1.37	3.3	1.22	4.99	126.79	143.78
	DTPWC9-F115-1-	1.39	3.18	0.98	6.15	123.61	132.49

	CKSPL10224	1.4	3.56	1.26	4.83	138.05	155.03
	CKSPL10341	1.41	2.67	1.21	5.99	152.12	160.25
	CML202	1.44	3.45	1.26	5.38	127.83	146.84
	CML488	1.45	3.22	1.15	6.03	138.95	145.94
	CML254	1.5	3.82	1.44	4.91	129.96	156.26
	CKSPL10112	1.5	3.62	1.45	5.11	143.35	157.54
	CML441	1.51	3.14	1.25	6.33	119.79	129.54
	CKSBL10046	1.57	3.73	1.26	6.21	131.28	138.9
	CKSPL10074	1.64	3.28	1.41	6.81	151.98	150.59
	CKSPL10229	1.8	3.48	1.6	7.43	155.52	171.94
	CZL01005	2.09	3.37	1.66	9.92	143.94	154.38
Mean		**1.52**	**3.4**	**1.32**	**5.88**	**134.73**	**146.54**
Trial mean			**2.97**	**0.911**	**3.01**	**134.6**	**143.66**
CV			**13.86**	**46.51**	**44.29**	**9.85**	**8.57**
P>value			**<.0001**	**0.016**	**0.022**	**<.0001**	**<.0001**
LSD (0.05)			**0.66**	**0.68**	**3.63**	**21.35**	**19.83**

Table 4.4 Pearson's correlation coefficient (r) between damage parameters and grain yield among CIMMYT tropical inbred lines evaluated at Embu Kenya against *B. fusca* resistance.

	Leaf damage	Exit holes	Tunnel length	Grain yield
Leaf damage	1			
Exit holes	0.08ns			
Tunnel length	0.11ns	0.83***		
Grain yield	-0.03ns	-0.09ns	-0.09ns	1

*significant at 10%, ** significant at 5%, *** significant at 1%, ns; not significant

4.3.3 Reaction of hybrids and OPVs to *C. partellus infestation*

The genotypes differed significantly in their reaction to stem borer infestation ($P<0.05$). The mean scores for leaf damage, borer exit holes and cumulative tunnel length were 2.23, 6.52 and 23.61 cm, respectively (Table 4.5). Selection index categorized the hybrids and OPVs into 20 highly resistant (<0.8), 37 moderately resistant (0.8-1.0), 27 moderately susceptible (1.0-1.2), and 16 as highly susceptible (>1.2). The most resistant genotypes were, CKIR09007, CKIR06009, CKIR09006, CKIR06006 and CKIR09005, with susceptibility indices of 0.50, 0.59, 0.62, 0.66 and 0.67, respectively. The most resistant commercial hybrids and OPVs were DH01, PH1, ECA-STRIGOFF-VL-102-#-#, KDV1 - 2-# and PH 3253 with indices of 0.67, 0.76, 0.77, 0.79 and 0.8, respectively. The most susceptible commercial hybrids were H628, H6213, H626, 611D and H6210 with indices of 1.85, 1.72, 1.61, 1.55 and 1.48, respectively. Mean plant height in protected plots was 224.52 cm and 215.69 cm in infested plants, a reduction of 8.83 cm (4%) under infestation. Mean ear height was 135.15 cm in protected plots and 126.31 cm in infested plots, a reduction of 8.84 cm (7%), while the mean yield was 5.94 t/ha in infested plots and 7.20 t/ha in protected plots, a 18% reduction (Table 4.5).

The greatest yield loss of 13% was recorded in the hybrid checks CKIR09007, CKIR06009, CKIR09006, CKIR09007, CKIR06006 and CKIR09006. The highest grain yield under infestation was in hybrid CKIR09005, which gave 7.62 t/ha, while the lowest yield was in OPV KDV1-4-# (2.65 t/ha) which was among the most highly susceptible OPVs. It also had the lowest yielding under protected conditions at 3.28 t/ha. The highest yielding genotype under protected conditions was the hybrid H629 that gave 10.03 t/ha, albeit a susceptible commercial hybrid to stem borers with a selection index of 1.47. Under infestation H629 still gave the highest yield at 9.44 t/ha. The lowest yield loss was, however, unexpectedly in the highly susceptible commercial hybrids category (7%). CIMMYT checks had greatest plant height reduction of 23.73 cm, followed by the susceptible hybrids with 11.58 cm and the resistant hybrids and OPVs with 8.78 cm. Grain yield under infested conditions was negatively and significantly correlated to leaf damage. Plant height was positively and significantly correlated to tunnel length and grain yield, but negatively correlated to leaf damage and number of exit holes.

The highest correlation between damage parameters was between number of borer exit holes and cumulative tunnel length (r=0.80) (Table 4.6).

4.3.4 Reaction of hybrids and OPVs to *B. fusca* infestation

There were significant differences in the Hybrids and OPVs against resistance to *B. fusca* in all traits measured (P>0.05). The trial mean scores were 2.01, 0.87 and 2.97 for leaf damage, number exit holes and tunnel length, respectively (Table 4.7). The selection index categorized 29 genotypes as highly resistant (0.31-0.80), 24 as moderately resistant (0.80-1.00), 22 as moderately susceptible (1.00-1.20), and 24 as highly susceptible (1.20-2.09. Among the highly resistant genotypes were five OPVs; KDV1-3-# EEQPM-8-EA-#-#-, KDV1-2-#, DKC8053, POOL15QC7-, KDV1-1-#, EEQPM-9-EA-#-#-# and ZM521-IR##, and three hybrids DHO2, PH4 and WH405. The most highly resistant hybrid was CKIR09008 with an index of 0.31, while the most highly susceptible was commercial hybrid H6210 with an index of 2.09. Mean plant height was 213.39 cm in infested plots and 218.57 cm in protected plots, a reduction of 5.18 cm (2.4%).

Table 4.5 Reaction of hybrids and OPVs to *C. partellus* infestation under field conditions at Kiboko Kenya. The experiments were conducted during the Oct/Feb 2009 and Mar/Sept 2010 cropping seasons.

CIMMYT Hybrid checks	Leaf damage (score)	Exit holes (#)	Tunnel length (cm)	Infested grain yield (t/ha)	Protected grain yield (t/ha)	Infested plant height (cm)	Protected plant height (cm)
CKIR09007	2	2	7.31	7.21	7.96	187.9	204.52
CKIR06009	1.49	3.84	11.99	6.83	8.19	188.37	198.72
CKIR09006	1.74	3.28	13.76	5.74	7.72	201.6	205.17
CKIR06006	1.38	4.11	17.09	6.88	7.46	215.96	294.59
CKIR09005	2.18	3.33	12.49	7.62	8.18	202.63	212.12
Mean for hybrid checks	**1.76**	**3.31**	**12.53**	**6.86**	**7.9**	**199.29**	**223.02**
Resistant Hybrids and OPVs							
DH01	2.55	3.35	8.42	4.44	6.16	216.89	184.39
PH1	2.21	4.05	16.02	4.66	4.66	173.54	184.98
ECA-STRIGOFF-VL-102-#-	2.24	4.34	15.21	5.39	5.55	185.07	202.14
KDV1 - 2-#	2.46	4.99	12.05	3.42	3.86	182.6	204.2
KDV1 - 1-#	2.09	4.54	18.96	4.6	4.57	173.09	187.28
PH 3253	2.6	4.55	14.25	7.07	7.9	201.56	216.41
Means for resistant hybrids and OPVs	**2.36**	**4.3**	**14.15**	**4.93**	**5.45**	**188.79**	**196.57**
Susceptible genotypes							
H628	2.59	14.21	52.23	7.77	7.9	254.2	274.97
H6213	2.38	13.7	47.06	8.04	9.23	255.86	266.09
H626	2.5	11.22	47.04	8.54	9.05	271.02	281.26
611D	3.09	10.68	38.63	7.45	7.93	257.8	283.49
H6210	2.31	10.38	42.69	7.78	8.55	269.31	276.61
H629	2.34	10.34	41.12	9.44	10	261.1	280.35
Means for susceptible genotypes	**2.54**	**11.76**	**44.8**	**8.17**	**8.78**	**261.55**	**277.13**
Trial means	**2.23**	**6.52**	**23.61**	**5.94**	**7.2**	**215.69**	**224.52**
Coefficient of variation	**21.5**	**36.3**	**43.8**	**18.61**	**15.77**	**12.42**	**9.16**
P>value	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
LSD (0.05)	**0.55**	**2.69**	**11.77**	**1.79**	**2.16**	**43.23**	**33.19**

Table 4.6 Pearson's correlation coefficient (r) between damage parameters, plant height and grain yield among hybrids and OPVs evaluated at Kiboko Kenya against *C. partellus* resistance.

	Leaf damage	Exit holes	Tunnel length	Plant height	Ear height	Stem lodging	Grain yield
Leaf damage	1						
Exit holes	0.39***						
Tunnel length	0.2939***	0.80***					
Plant height	-0.0329ns	-0.29***	0.29***				
Ear height	0.05ns	0.45***	0.48***	0.54***			
Stem lodging	0.06ns	0.06ns	0.02ns	-0.08ns	0.03ns		
Grain yield	-0.17**	-0.05ns	-0.07ns	0.45**	0.36***	-0.11ns	1

*significant at 10%, ** significant at 5%, *** significant at 1%, ns; not significant

Table 4.7 Reaction of hybrids and OPVs to *B. fusca* infestation under field conditions in Embu Kenya. The experiments were conducted during the Oct/Feb 2010 and Mar/Sept 2011 cropping seasons.

	Pedigree	Index	Leaf damage (score)	Exit holes (#)	Tunnel length (cm)	Infested plant height (cm)	Protected plant height (cm)
Highly resistant	CKIR09008	0.31	1.56	0.07	0.25	191.86	203.71
CIMMYT checks	CKPH09001	0.39	2.04	0.12	0.01	216.24	216.57
	CKIR09001	0.41	1.85	0.22	0.18	213.38	208.97
	CKIR06009	0.49	1.72	0.36	0.61	186.17	198.75
	CKIR04002	0.50	1.76	0.27	0.91	187.28	197.35
Mean		**0.42**	**1.78**	**0.21**	**0.39**	**198.99**	**205.07**
Resistant	KDV1 - 3-#	0.61	2.20	0.39	0.85	181.67	187.25
Hybrids and OPVs	EEQPM-8-EA-	0.61	2.05	0.48	0.78	185.53	182.24
	DH02	0.63	2.07	0.56	0.64	165.17	184.09
	KDV1 - 2-#	0.64	2.09	0.49	0.91	170.00	184.23
	DKC8053	0.65	1.90	0.47	1.41	208.25	208.76
	POOL15QC	0.66	2.02	0.46	1.29	175.26	186.24
	KDV1 - 1-#	0.67	2.09	0.49	1.18	166.15	167.96
	PH4	0.72	1.80	0.56	1.85	207.27	230.02
	WH403	0.76	2.18	0.56	1.64	217.24	219.39
	EEQPM-9-EA-	0.77	2.41	0.51	1.53	190.13	185.28
Mean			**2.08**	**0.50**	**1.21**	**186.67**	**193.55**
Highly susceptible	CKPH08009	1.63	2.11	1.46	6.47	242.04	239.45
Genotypes	H629	1.68	2.32	1.58	6.14	240.33	249.78
	H6213	1.70	2.23	1.52	6.72	249.03	252.28
	531A	1.80	2.65	1.56	6.82	221.99	230.95
	H6210	2.09	2.18	1.61	9.92	257.18	262.36
Mean		**1.78**	**2.30**	**1.54**	**7.21**	**242.12**	**246.96**
Trial mean			**2.01**	**0.87**	**2.97**	**213.39**	**218.57**
CV			**13.06**	**54.78**	**82.36**	**7.98**	**7.03**
LSD (0.05)			**1.49**	**0.76**	**3.94**	**27.45**	**24.77**
P>value			**0.001**	**0.007**	**0.033**	**<.0001**	**<.0001**

4.4 Discussions

4.4.1 Reaction of inbred lines to *C. partellus* infestation

There was great variation in resistance levels among the inbred lines for leaf damage score, number of exit holes and tunnel length. The fact that 73 of 120 inbred lines were categorized as either resistant or moderately resistant indicates superiority of these germplasm in resistance to stem borers. Fifteen of the 26 resistant inbred lines were the CIMMYT multi-borer resistant lines. A few multi-borer resistant selected lines where, however, categorized as susceptible and the findings may necessitate more evaluations in the future to confirm the true status of these CIMMYT lines. External factors may also have affected these multi-borer resistant selected lines's unexpected reaction to *C. partellus* as genotype x environment effects may not be ruled out.

The number of exit holes an indicator of the number of borers that completed their life cycle successfully in the plant, increased with susceptibility. An average of two (2) borer exit holes per genotype were recorded in the resistant inbred lines compared to six (6) in the susceptible lines. Since only five neonates were artificially infested per plant, the higher numbers of exit holes may suggest that susceptible inbred lines also attracted natural infestations, or there were larvae movement between plants after infestations. Thus, resistant varieties may have caused increased larval mortality, slow growth rate, and or delayed development. Similar findings on reduced feeding on resistant maize germplasm were reported in Afzal *et al* (2009) and Arabjafari and Jalali (2007).

Similarly, reduced stem tunnelling was recorded in resistant inbred lines. Stem tunnelling is a good measure of the extent of larval feeding on stem tissues which ultimately destroy plant vascular systems curtailing growth and yield (Tefera *et al*., 2010). One centimetre of stem borer tunnel was reported to reduce yield by approximately 3 g/plant (Songa *et al*., 2001). The longest tunnel was in CML312 and measured 26.71 cm, while the shortest tunnel was in the inbred CKSBL100025 and measured 3.12 cm. This later genotype was, therefore, less preferred for borer feeding and development than the former. Average grain yield in resistant inbred lines was

10% more than in susceptible lines, indicative of yield penalty associated with stem borer tunnelling and feeding (Songa *et al.,* 2001; Singh *et al.,* 2011).

The high correlation (r=0.89) between the number of exit holes and tunnel length was an indication of the interrelatedness of the two parameters in measuring borer damage. Correlations of similar magnitude between the two parameters have been reported (Arabjafari and Jalali. 2007). These two parameters could thus be used interchangeably to evaluate resistance to borers in maize. Grain yield was significantly correlated to plant height correlation (r=0.56), suggesting that an increase in plant height similarly improved grain yield. Similar results have been reported in sorghum (Muturi *et al.,* 2012). Plant height was also significantly and positively correlated to tunnel length, supporting previous reports that taller germplasm are preferred for stem feeding as they have longer internodes (Afzal *et al.,* 2009; Singh *et al.,* 2011). This argument supports previous findings in maize that have indicated that longer stem internodes are favourable for borer stem feeding, compared to shorter varieties that are in general resistant to borers (Parvez *et al.,* 2000).

4.4.2 Reaction of inbred lines to *B. fusca* stem borers infestation

The CIMMYT multi-borer inbred lines were equally (as in *C. partellus*) the most resistant to *B. fusca* damage. Out of the 15 highly resistant lines, eight (8) were CIMMYT multiple borer resistant lines (CKSBL10008, CKSBL10005, CKSBL10025, CKSBL10027, CKSBL10026, CKSBL10034, CKSBL10021, CKSBL10020, CKSPL10273 and CKSPL10090). This shows their superiority in resistance to stem borer damage among other tropical inbred lines against *B. fusca* stem borer damage. Resistant inbred lines CKSPL10273 and CKSPL10090 which are superior storage pests experimental materials were coincidentally also resistant to stem borers, this could be indicative of dual resistance to lepidopteran and coleopteran pests in the germplasm. Susceptibility increased with increasing leaf damage, number of borer exit holes and cumulative tunnel length. Stem borer damage reduced plant height by 11.8 cm (8.1%). The reduction suggests that plant height has a major effect on grain yield as previously reported (Zsubori *et al.,* 2002). More than half of the inbred lines were categorized as either highly

resistant, or moderately resistant to *B. fusca*, therefore, CIMMYT tropical inbred lines form a strong breeding pool from which resistance to *B. fusca* can be obtained from for breeding.

4.4.3. Reaction in hybrids and OPVs to *C. partellus* infestation

The CIMMYT hybrids, bred specifically for stem borer resistance had the highest resistance levels. Some commercial hybrids, however, performed better in spite of the high borer susceptibility with less grain yield loss penalty of 7%. These commercial hybrids appear to have been tolerant to stem borer infestation, possessing the ability to compensate for borer damage. The mean yield for the resistant OPVs was much lower at 4.93 t/ha than that obtained in the resistant hybrid checks which gave 6.86 t/ha under infestation. Open pollinated varieties give lower yields than hybrid varieties due to their lack for hybrid vigour (Morris, 2002; Malik *et al.,* 2010). The low yields notwithstanding, improved OPVs were more resistant to *C. partellus* than commercial hybrids perhaps due to their broad genetic background.

In this study, stem borer damage reduced the mean plant height by 8.5 cm, and reduced grain yield. Several studies elsewhere have identified plant height as the most important trait influencing maize grain yield (Zsubori *et al*., 2002). The negative correlation between plant height and number of exit holes and leaf damage in this study was an indication that as stem borer damage increased, plant growth is significantly reduced consequently reducing grain yield. The positive and significant correlation between tunnel length and plant height could be interpreted from two perspectives; that the plant compensated for increased stem damage through tunnelling by growing taller, or, that taller plants were more susceptible, i.e. the taller the plant the more was the damage. Previous studies have similarly associated shorter plants with less stem borer damage (Parvez *et al*., 2000). This trend was also similar in inbred lines, a strong indication that shorter germplasm suffer less stem borer damage effects than taller germplasm.

Most of the genotypes resistance to *C. partellus* were OPVs from the low attitude zones (PH1), to mid-attitude zones (DH1, ECA-STRIGOFF-VL-102-#-, KDV1-1, KDV1-2, and KDV1-3). The only resistant hybrid identified for the transitional to highland ecologies was P3253. Susceptible varieties clustered in the late maturing transitional to high altitude ecology varieties,

but these varieties exhibited low grain yield losses under infestation. For instance, commercial hybrid H629 that was highly susceptible with a selection index of 1.46, was also highly yielding under both protected (10 t/ha) and infested conditions (9.44 t/ha) suggesting the presence of tolerance mechanism to borers. Similar observations have been found in sorghum, a close relative of maize (Muturi *et al.*, 2012). Tolerance in these genotypes could be attributed to increased size of new leaves, delayed senescence of infected tissues, and or increased nutrient uptake which may lead to low penalties on yield. Tolerant genotypes are able to offset damage effects of pests or pathogens by adjusting their physiology to buffer the effects of infestation (Lattanzio *et al*., 2006).

4.4.4 Reaction to hybrids and OPVs to *B. fusca* infestation

The high significance in all the damage parameters evaluated was in agreement with previous studies on effective indicators of borer resistance in maize (Santiago *et al*., 2003; Odiyi, 2007; Singh *et al*., 2011). Over half (53) of the hybrids and OPVs evaluated were categorized as either highly resistant or moderately resistant, an indication that resistance existed in commercial hybrids and OPVs, and CIMMYT experimental hybrids. The CIMMYT hybrids exhibited the highest resistance levels to *B. fusca* compared to commercial hybrids and OPVs. The OPVs KDV1-3-#, EEQPM-8-EA-#, KDV1-2-#, DKC8053, POOL15QC, KDV1-1-# and EEQPM-9-E were most highly resistant among the commercial varieties being cultivated in Kenya. The only commercial hybrids that were highly resistant were DH02 and PH4, and, all are recommended for production in the low to mid-attitude ecologies. Most of the identified resistant hybrids and OPVs are therefore important genotypes in low to mid altitude maize growing ecologies, except DKC8053 and WH403 which are recommended for the medium to mid-late. These findings underscore the importance of OPVs as good sources of resistance to stem borers and for production in the relevant ecologies for reduced yield losses.

The most highly susceptible genotypes were mostly commercial hybrids recommended for the transitional to high altitude moist ecologies i.e. H6210, 531A, H6213 and H629 (Table 4.7). Interestingly, these high altitude hybrids yielded highly under both infested and protected conditions compared to resistant genotypes, a suggestion that these genotypes possess a

physiological capacity to offset losses in grain yield. These genotypes were late maturing and vigorous in growth and they may have been able to recover and outgrow damages inflicted at earlier growth stages and avoid grain yield losses. Thus, under field conditions such genotypes may still be deployed especially when resistant materials are not available.

4.5 Conclusions

This study showed that among tropical maize germplasm (commercial hybrids, OPVs, CIMMYT experimental hybrids and inbred lines) there exists variability for resistance to *C. partellus* and *B. fusca*. Resistant maize germplasm were less preferred for feeding, growth and development of the larvae, leading to less plant damages through antibiosis and/ antixenosis mechanisms of resistance, while tolerant varieties though exhibiting high damage levels were physiologically able to compensate for damage, leading to little or no yield losses under infestation (tolerance). Resistant inbred line CKSBL10039, open pollinated varieties KDV1-1-#, KDV1-2-#, KDV1-3-#, EEQPM-8-EA-#, POOL15QC and EEQPM-9-E which were incidentally not developed as a multiple borer resistance germplasm, exhibited dual resistance suggesting that they are new source of resistance to both stem borer species. These germplasm needs to be evaluated further for use in breeding programmes. CIMMYT MBR lines CKSBL10025, CKSBL10026, CKSBL10027, CKSBL10014, CKSBL10034 and CKSBL10039 were very highly resistant to both stem borer species (dual resistance) and are recommended for use in breeding for resistance in Kenya and the SSA region against both insect species. CIMMYT experimental hybrids checks developed for stem borer resistance CKIR09001, CKIR06009, CKIR06001, CKIR06006, CKIR09006, CKIR09007, and CKIR09002 exhibited dual resistance to both species and are recommended for release as superior stem borer resistant hybrids. The resistant OPVs and inbred lines which were highly resistant to both stem borer species are recommended as new sources of resistance in maize improvement for resistance to stem borers breeding. The resistant, moderately resistant and tolerant commercial hybrids and OPVs should consequently be promoted for production in the relevant maize production ecologies for improved yields. Though commercial hybrids recommended for high altitude areas (H6210, H6213, 531A, H6213, H611D, H628 and H629) had the highest selection index values they had lowest yield losses

under infestation, exhibiting tolerance mechanism of resistance to both stem borers and are recommended for production in the relevant ecologies for reduced grain yield losses.

4.6 References

Afzal, M., Nazir, Z., Bashir, M. and Khan, B. 2009. Analysis of host plant resistance in some genotypes of maize against *Chilo partellus* (Swinhoe) (Pyralidae: lepidoptera). Pakistan Journal of Botany 41:421-428.

Ahmad, R., and Sharma, J.P. 2011. Genetic Variability Studies of Yield and Quality Traits in Tomato (*Solanum lycopersicum* L.). International Journal of Plant Breeding and Genetics 5:168-174.

Arabjafari, K.H., and Jalali, S.K. 2007. Identification and Analysis of Host Plant Resistance in Leading Maize Genotypes against Spotted Stem Borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae). Pakistan Journal of Biological Sciences 10:1885-1895.

Bosque-Pérez, N.A. 1995. Major insect pests of maize in Africa: Biology and control. IITA, Ibadan, Nigeria.

Hailea, A., and T. Hofsvang. 2002. Host plant preference of the stem borer *Busseola fusca* Crop Protection 21:227–233.

Kfir, R., Overholt, W.A., Khan, Z.R. and Polaszek, A. 2002. Biology and management of economically important lepidopteran cereal stem borers in Africa. Annual Rev Entomology 47:701-731.

Lattanzio, V., Lattanzio, V.M.T. and Cardinali, A. 2006. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects *In* F. Imperato, ed. Phytochemistry: Advances in Research, Bari, Italy.

M'mboyi, F., Mugo, S. Mwimali, M. and Ambani, L. 2010. Maize production and improvement in sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF), Nairobi, Kenya.

Malik, H.N., Ara, I., Naeem, M., Hussain, M., Manawa, M.H. and Yousaf, M. 2010. Comparisons of open pollinated varieties and newly developed hybrids for yield and yield contributing traits in maize. Pakistan Journal of Agricultural Research 23:37-41.

Mbapila, J.C, Overholt, W.A. and Kayumbo, H.Y. 2002. Comparative development and population growth of an exotic stem borer, *Chilo partellus* (Swinhoe), and an ecologically similar congener, *Chilo orichalcociliellus (*Strand) (Lepidoptera: Crambidae). Insect Science Application 22:21-27.

Morris, M.L. 2002. Impacts of international maize breeding research in developing countries, 1966-1998., Mexico D.F.:CIMMYT.

Mugo, S., De Groote, H., Bergvinson, D., Songa, M., J. and Gichuki. S. 2005. Developing Bt maize for resource-poor farmers - Recent advances in the IRMA project. African Journal of Biotechnology 4:1490-1504.

Muturi, P.W., Rubaihayo, P., Mgonja, M., Kyamanywa, S., Sharma, H.C. and Hash, C.T. 2012. Novel source of sorghum tolerance to the African stem borer (*Busseola fusca*). African Journal of Plant Science 6:295-302.

Ong'amo, G.O., Le Ru, B.P., Dupas, S., Moyal, P., Calatayud, P.A. and Silvain, J.F. 2006. Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. Annales de la Societe Entomologique de France 42:171-177.

Parvez, A., Haq, I. and Ashfaq, M. 2000. Some studies on physico-morphological characters contributing towards resistance in some maize cultivars against stem borer *Chilo Partellus* (S.). Pakistan Journal of Agriculture 3-4:178-180.

Santiago, R., Souto, X.C., Sotelo, J., Butrón, A. and Malvar, R.A. 2003. Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: Noctuidae) attack. Journal of Economic Entomology 96:1563-1570.

Singh, B.U., Sharma, H.C. and Rao, K.V. 2011. Mechanisms and genetic diversity for host plant resistance to spotted stem borer, *Chilo partellus* in sorghum, Sorghum bicolor. Journal of Applied Entomology 135:333–392.

Songa, J.M., Guofa, Z. and Overholt, W.A. 2001. Relationships of stem borer damage and plant physical condition to maize yield in a semiarid zone of eastern Kenya. International Journal of Tropical Insect 21:243-249.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2010. Mass rearing of stem borers, maize weevil, and larger grain borer insect pests of maize. CIMMYT: Nairobi, Kenya.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2011. Methods of screening maize for resistance to stem borers and post-harvest Insect Pests. International Maize and Wheat Improvement Centre (CIMMYT), Nairobi, Kenya.

Wen, W., Franco, J., Chavez-Tovar, V.H., Yan, J. and Taba, S. 2012. Genetic characterization of a core set of a tropical maize race tuxpeño for further use in maize improvement. PLoS One 7:e32626. doi:10.1371/journal.pone.0032626.

Zsubori, Z., Gyenes-Hegyi, Z., Illes, O., Pok, I., Raez, F. and Szoke, C. 2002. Inheritance of plant and ear height in maize (*Zea Mays* L.). Journal of Agricultural Science Univer Debrecen 8:34-38.

CHAPTER 5

MECHANISMS OF RESISTANCE IN TROPICAL MAIZE INBRED LINES TO *Chilo partellus* STEM BORERS

5.1 Introduction

Host plant resistance is important because it provides an easy way to apply and integrate technology for management of pests and diseases. Developing maize with durable resistance to stem borers could be enhanced by identifying genotypes with efficacious mechanisms of resistance that can be pyramided into high yielding but susceptible genotypes. There are three types of mechanisms of resistance to insect pests including stem borers, i.e. non-preference (antixenosis), antibiosis and tolerance (Sarwar, 2012; Pimentel, 2002). These three mechanisms may also be associated with metabolites produced by maize plants accounting for the nature of resistance observed. In maize, the hydroxamic acid, 2,4-dihydroxy- 7-methoxy- (2H)-1,4-benzoxazin-3- (4H)-one (DIMBOA) is efficacious against diverse pests including stem borers decreasing in amounts as the plant matures (Santiago *et al*., 2003; Smith, 2005). The amount of DIMBOA is not always necessarily in higher amounts in resistant germplasm than in susceptible ones, suggesting that other mechanisms may account for resistance in maize to stem borers (Santiago *et al*., 2003). It, therefore, appears that resistance to stem borers is complex and may involve different mechanisms that interact to determine the level of resistance for each germplasm.

Physical resistance to stem borers has been identified as one of the mechanisms of resistance to stem borer damage. Plant attributes such as trichome density, leaf toughness and stem penetrometer resistance are important components of antibiosis against maize stem borers. Stem penetrometer resistance is a type of physical resistance which results in reduced digestibility and/or increased hardness and abrasiveness of plant epidermal tissues (Arabjafari and Jalali, 2007). In addition, stem penetrometer resistance is important in the prevention of larvae feeding on the stem pith during plant development. Larvae stem feeding is an important cause of yield losses through stem weakening and lodging of cereal plants (Santiago *et al*., 2003). Leaf toughness negatively influences stem borer feeding behaviour, thereby, reducing yield losses

(Malvar *et al*., 2008). In many plant species trichomes of juvenile plant varieties contain compounds which imparts indigestibility to the herbivore pests (Kher *et al*., 2011). The presence of dense trichomes on plant surfaces also hinders the easy movement and feeding of pests on a susceptible host. Conversely, high levels of pith sugars increase palatability of the stem and other plant tissues to stem borers (Juma, 2010). Higher levels of pith sugars have been reported to contribute to increased stem borer susceptibility in sorghum and rice irrespective of the varieties (Kumar *et al.*, 2006; Sarwar, 2012).

Broadening the genetic pool for maize breeding requires characterization of the mechanisms of resistance in tropical inbred lines. The objective of this study was to study the nature of resistance in selected tropical maize germplasm to *C. partellus* using various resistance elements such as leaf trichome density, leaf toughness, stem penetrometer resistance, and pith sugar content.

5.2 Materials and methods

5.2.1 Experimental design

The experiment was conducted at the Kenya Agricultural Research Institute (KARI) Kiboko Farm. The site description and materials used were as described in chapter 4 for CIMMYT inbred lines. The inbred lines were planted in single row plots of seven meters i.e. 29 hills per row spaced at 75cm and 25cm between and within rows, respectively. All field activities were as described in chapter 3. The trial was designed to accommodate destructive sampling of five plants per row. The plots, were, therefore, marked with five strings in such a way as to separate the first one border plant, the next five plants (sampling plants), one protected plant, 10 plants to be infested, 10 pesticide protected plants, and two border plants in each row plot. The first plant in each row was the border plant; the five consequent plants were used for destructive sampling data collection. The next 10 plants were each infested with five second instar *C. partellus* neonates three weeks after planting.

5.2.2 Data collection

Data on leaf damage (LD) was taken on visual rating score two weeks after infestation on each of the 10 infested plants on a scale of 1-9 (where, 1= no visible leaf damage and 9 = plants dying) as a result of leaf damage as described by (Tefera *et al*., 2011). Concurrently at the time of leaf damage scoring, leaf toughness in kilogram-force was taken on five randomly selected plants per row using a penetrometer (Model FT011, ALFOSINE-Italy). This was done on the youngest leaf with fully developed ligule punched on the adaxial side 2-3 veins away from the mid-rib. Before the onset of flowering, 10 randomly selected leaf samples per plot were taken from the protected plants for trichome density count. The leaf below the first ear was cut at the centre of the blade and a cork borer of 1cm diameter was used to punch a maize leaf disk for which the number of trichome hairs was counted under a dissecting microscope at 10x magnification.

At the silking stage, stem penetrometer resistance was recorded using a Penetrometer (FHT-803 fruit firmness tester software) with a fabricated needle. Five plants per row were punctured at the centre of the second inter-node below the primary ear. The force was recorded in maximum kg-force. Stem pith sugar content was taken using a Brix Refractometer (r2mini Handheld Refractometer) after silking. The second inter-node below the primary ear was cut into ten (10 cm) pieces and a 1cm radius cork borer used to extract the pith. The pith tissue was squeezed to extract about two drops (1ml) of juice onto the Refractometer sensor, and the sugar content read as a percentage (%). At harvest, the numbers of stem borer exit holes (EH) were counted and the cumulative tunnel length (TL) in cm measured after splitting the stems across the middle.

5.2.3 Data analysis

A multivariate analysis of variance within a canonical variant analysis was performed using the SAS package (Canonical discriminant analysis tool) to determine the most variable mechanism trait among the genotypes (SAS, 2007). To secure error control, coefficient correlation analysis was done for the mechanisms of resistance (trichome density, leaf toughness, sugar content and stem penetrometer resistance) and the damage parameters (leaf damage, exit holes and

cumulative tunnel length) using canonical correlations. A selection index based on the significant damage parameters was computed as described before (chapter 3). All data collected were analyzed using PROC GLM of SAS package (SAS, 2007) as described in chapter 3.

5.3. Results

5.3.1 Variability in mechanism of resistance based on canonical discriminant analysis

The ANOVA univariate statistics showed significant differences among the inbred lines for leaf toughness, sugar content, trichome density, leaf damage, number of exit holes and cumulative tunnel length i.e. the variables class means were, not equal to zero at $P<0.05$. The most important resistance mechanisms in discriminating the inbred lines were trichome density, leaf toughness, and stem sugar content with canonical coefficient loadings in CAN1 of 1.59, CAN2 of 1.22, and CAN3 1.08, respectively (Table 5.1). The coefficients of determination (R2) were 0.80 for trichome density, 0.66 for leaf toughness and 0.61 for sugar content. Leaf damage, number of exit holes, and tunnel length with canonical coefficient loadings in CAN1 of 1.25, CAN2 of 1.13, and CAN3 of 1.42, respectively were most effective in discriminating the inbred lines resistance. The R^2 values for damage parameters were 0.64 for leaf damage, 0.48 for exit holes and 0.43 for tunnel length. All the multivariate's statistics, Wilk's Lambda, Pillai's Trace, Hotelling-Lawley Trace and Roy's Greatest root tests, showed that none of canonical correlation was zero ($Pr>0.0001$) implying that all the three damage parameters were highly correlated (Table 5.1).

Canonical correlation indicated that the two sets of variables, mechanisms of resistance and damage parameters were not correlated (Table 5.2). The first canonical correlation is the greatest possible multiple correlation within the classes that can be achieved by using a linear combination of the quantitative variables.

Table 5.1 Canonical correlations between damage parameters and mechanisms of resistance variables in 120 CIMMYT inbred lines evaluated at Kiboko Kenya.

CAN	Canonical Correlation	Eigen Values	Likelihood Ratio	Approx. F-Value	Num DF	Den DF	Pr>F
1	0.3656	0.1543	0.852	1.56	12	299.3	0.1036ns
2	0.1026	0.0106	0.9834	0.32	6	228	0.9268ns
3	0.0781	0.0061	0.9939	0.35	2	111	0.7035ns

ns = not significant at p>0.05 level

Table 5.2 Variability in the resistance mechanisms in tropical inbred lines grown during the Mar/Sept 2010 and Oct/Mar 2011/2012 seasons at Kiboko. Data generated using canonical discriminant analysis.

Mechanisms traits	Total SD	Pooled SD	Between SD	R^2 –Value	Pr>F	CAN1	CAN2	CAN3	Variation
Leaf toughness	0.044	0.036	0.036	0.66	0.002	-0.26	**1.235**	-0.219	24
Sugar content	2.474	2.193	1.929	0.61	0.008	-0.07	0.3605	**1.079**	16
Stem hardness	0.734	0.707	0.538	0.53	0.211**ns**	-0.14	-0.39	0.211	12
Trichome density	5.046	3.19	4.521	0.8	<.0001	**1.594**	0.121	0.029	**46**
Damage traits									
Leaf damage	0.805	0.594	0.643	0.64	<.0001	**1.251**	-0.535	-0.152	**59**
No. of exit holes	1.387	1.228	0.959	0.48	<.0001	0.135	**1.1304**	-0.988	24
Tunnel length	3.89	3.587	2.575	0.43	0.002	0.247	-0.0485	**1.417**	17

ns = not significant at P>0.05

Table 5.3 Variability in the mechanisms of resistance and reaction to *C. partellus* stem borer infestation assessed using physical damage and physiological parameters on the most resistant and susceptible CIMMYT inbred lines.

Pedigree	Index	Leaf damage	Exit holes (#)	Tunnel length (cm)	Trichome density	Leaf toughness (kg-force)	[a]Sugar content (%)	[b]Stem penetrometer resistance(kg-force)
Resistant lines								
CKSBL10039	0.5	1.9	1.29	3.61	13.81	0.33	6.27	3.17
CKSBL10025	0.52	1.8	1.86	3.12	2.92	0.2	10.07	2.93
CKSBL10026	0.53	1.89	1.5	4.07	10.58	0.24	9.21	2.95
CKSBL10025	0.56	1.61	2.06	5.2	5.96	0.19	9.93	2.59
CKSBL10014	0.57	2.08	1.54	4.44	8.76	0.18	7.9	3.12
CKSBL10026	0.58	1.79	1.74	5.93	10.35	0.22	9.39	2.86
CKSBL10014	0.64	2.41	1.78	4.59	8.17	0.2	8.31	3.04
CML488	0.67	2.14	1.59	7.88	10.65	0.16	11.82	2.45
CKSBL10004	0.69	1.97	1.85	8.55	7.92	0.24	8.15	3.72
Susceptible	**0.58**	**1.95**	**1.69**	**5.27**	**8.79**	**0.22**	**9.01**	**2.98**
CKSPL10218	1.44	2.73	6.7	16.11	10.29	0.16	8.83	2.8
CML442	1.44	2.12	6.72	19.66	22.07	0.24	10.01	2.45
CML264	1.47	2.33	5.81	22.79	13.15	0.19	8.68	3.01
CKSBL10013	1.48	2.45	5.68	22.62	10	0.22	10.88	2.99
CML202	1.49	2.33	5.79	23.33	13.92	0.19	8.9	3.27
CML204	1.51	2.42	5.89	23.53	8.92	0.14	10.69	1.97
CML197	1.53	1.95	6.52	24.85	3.48	0.2	7.53	2.68
CZL01005	1.59	2.59	6.5	23.56	7.76	0.2	8.21	2.38
CML312	1.64	1.89	7.23	26.77	7.62	0.11	6.01	2.47

CML395	1.85	2.85	8.12	26.12	10.12	0.19	9.86	2.33
Mean	**1.54**	**2.37**	**6.50**	**22.93**	**10.73**	**0.18**	**8.96**	**2.64**
Trial Mean		**3.23**	**2.63**	**6.29**	**9.67**	**0.19**	**8.91**	**3.13**
CV		**19.01**	**42.1**	**51.6**	**33.6**	**18.9**	**9.31**	**19.35**
LSD		**0.99**	**1.78**	**5.22**	**5.23**	**0.06**	**1.34**	**0.97**
F-test		**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0096**	**<.0001**	**0.0002**

[a]sugar content was assessed in brix

[b]stem penetrometer was assessed in kg-force using a fruit penetrometer

5.3.2 Variability in mechanism of resistance based on analysis of variance

The ANOVA showed significant differences in all traits measured (P<0.05). The selection index categorized the inbred lines into 33 resistant (<0.80), 29 moderately resistant (0.8-1.0), 31 moderately susceptible (1.0-1.2) and 27 as susceptible (>1.2). These figures deviate from the normal distribution curve as the lines were not all randomly selected since the most resistant multiple borer resistant inbred lines had been deliberately selected. The most resistant inbred lines were CKSBL10039 and multiple borer resistant inbred line CKSBL10025 with indices of 0.50 and 0.52, respectively. CML395 and CML312 were the most susceptible with indices of 1.85 and 1.65, respectively. The means for leaf damage, number of exit holes and tunnel length were 2.22, 3.69 and 12.70cm, respectively (Table 5.3). Leaf damage was highest in CML254 at 5.94, a susceptible inbred line with an index of 1.37. The lowest score was 2.05 in CKSBL10035, with an index of 0.59. Highest number of exit holes was 7.94 in CML395 which was also the most susceptible with an index of 1.85, while the lowest was 0.91 in CKSBL10039 which was also the most resistant inbred line. The cumulative tunnel length was shortest in CKSPL10256 (4.99 cm) and longest in CML197 at 32.47 cm which was highly susceptible with an index of 1.53. All the damage parameters were significantly and positively correlated, the highest correlation was between exit holes and tunnel length (r=0.70***), a suggestion that any of the two parameters could be a good measure for resistance.

Trichome density was highly significantly different for the inbred lines (P < 0.0001) and ranged from 1.23 in P300C5S1B-2-3-2-#-#-1-2-B-B-#-B which was susceptible with an index of 1.21, to 27.04 in CML511 which was unexpectedly also susceptible in spite of the high trichome density, with an index of 1.45. CML442, a susceptible inbred line, was second with a similarly high trichome density of 22.07. The mean trial trichome density was 9.66.

The inbred lines showed highly significant differences in sugar content levels (P < 0.0001). Sugar content ranged from 5.37 in CKSPL10343 which was moderately susceptible (1.12) to 12.61% in LPSC7-F86-3-1-1-1-BB-#-B which was also moderately susceptible, with a mean of 8.91%. The inbred lines showed highly significant differences in leaf toughness (P<0.0001)

ranging from 0.12-0.33kg-force, with a mean of 0.19kg-force. The highest leaf puncture force of 0.33kg was in CKSBL10039 which was also the most resistant inbred line with the lowest index of 0.49. CKSBL10020, a line with an index of 0.98 was second best with a 0.29 kg-force. The lowest leaf puncture-force (softest) was in CML312 (0.13kg-force a susceptible line with an index of 1.64.

The stem penetrometer resistance ranged from 1.91 to 4.79 kg. The highest penetrometer resistance was in DTPWC9-F104-5-4-1-1-B-B-#-# at 4.80kg-force, it was moderately resistance. The lowest penetrometer resistance (softest stem) was in LPSC7-F86-3-1-1-1-BB-#-B at 1.91kg-force; it was moderately susceptible, and also had the highest percentage sugar content. Its selection index was 1.06. It was followed closely by CML395 with 1.93 kg-force which was the most susceptible inbred line with an index of 1.85 (Table 5.3).

5.4. Discussions

5.4.1 Trichome density

Canonical discriminant analysis showed that among the traits evaluated, trichome density was the best in discriminating traits for resistance to stem borer damage among the inbred lines. Resistance to the stem borer increased with higher trichome density as has been reported in earlier studies (Kumar and Saxena, 1992; Dalin *et al.,* 2008). Trichomes may contribute towards resistance through their physical presence or through the presence of chemical deterrents. Oviposition non-preference by stem borer on maize genotypes is influenced by the presence of trichomes (Kumar *et al.* 2007). Trichomes may also contain secondary compounds which deter feeding by a wide spectrum of insect herbivores (Handley *et al.,* 2005). While trichome density discriminated the inbred lines better into different resistant categories, it is possible that each inbred line had other different mechanisms of defence, or a combination of several mechanisms that were physiologically active. Novoa and Russel (1988) reported that resistance was mostly a result of the interaction of several structural and non-structural factors; some of these were not evaluated in this study. Trichomes could be glandular or non glandular and may contain several types of secondary compounds i.e. methylketones which improve resistance to pests and diseases in plants (Eyal *et al.,* 2005). These differences in trichomes structures and sizes affect the

efficiency of the trichomes in deterring feeding. This trial should be repeated with attention given to the examination of the types and structures of trichomes in the resistant and susceptible inbred lines would shed more light on the most effective type of trichomes in conferring resistance to stem borers.

5.4.2 Leaf toughness

Leaf toughness was the second best trait that discriminated genotypes into resistant and susceptible categories. The most resistant inbred line, CKSBL10039, had the highest penetrometer resistance force (0.33 kg-force), an indication of resistance through antibiosis for leaf feeding. The multiple borer resistant inbred lines exhibited high leaf toughness, while most of the susceptible genotypes had lower leaf toughness, a clear suggestion of the trait's importance in conferring resistance through antibiosis. Increased leaf toughness, therefore, enhances maize resistance to the stem borer. This observation corroborates earlier reports of Chu and Horng (1991) and Sarwar (2012) who reported that leaf feeding by the stem borer was negatively correlated with leaf tissue hardness, an important factor in conferring resistance to stem borers.

5.4.3 Stem sugar content

Genotypes with low to moderate stem sugar content fell into the resistant to moderately resistant categories. The most resistant inbreds CKSBL10039 and CKSBL10027, with indices of 0.49 and 0.71 had stem sugar contents 6.27 % and 6.62 %, respectively. LPSC7-F86-3-1-1-1-BB-#-B, P300C5S1B-2-3-2-#-#-1-2-B-B-#-# and CML204 which ranged between moderately susceptible to susceptible had the highest sugar contents of 12.61%, 11.42%, and 11.03 %, respectively. While this was a possible indication that the sugar content played a key role in conferring resistance, several variations were noted where some inbred lines with low sugar contents were moderately susceptible such as CML312, and a few with high stem sugar content were either resistant or moderately resistant such as CKSBL10025 (Table 5.3). It may thus be deduced that since there are different types of sugars existing in plants, specific types of sugars could have contributed to antixenosis while others may have promoted feeding. Canonical discriminant analysis indicated stem sugar content as the third best trait that would discriminate the inbreds. A

further biochemical study categorizing the actual type of sugars present in resistant and susceptible germplasm would help categorize the specific sugars that contribute to stem borer resistance in maize and those that increase susceptibility as previously reported (Padhi, 2004; Arabjafari and Jalali, 2007; Sarwar, 2012).

5.4.4 Stem penetrometer resistance

Susceptibility increased with decreasing penetrometer resistance. The highest stem penetrometer resistance was in DTPWC9-F104-5-4-1-1-B-B-#-# (4.76) and DTPWC9-F16-1-1-1-1-BB-#-# (4.20) which were both moderately resistant with indices of 0.92 and 0.88, respectively. Others were multiple borer resistant lines CKSBL10004 (3.72) and CKSBL10015 (3.68), which were resistant with indices of 0.8 and 0.69, respectively as described in chapter three. The lowest penetrometer resistance was in LPSC7-F86-3-1-1-1-BB-#-B (1.91) which was moderately susceptible with an index of 1.06; followed by CML395 (2.33), a susceptible CIMMYT line with the highest index of 1.85. These results indicate that stem penetrometer resistance played a role in conferring resistance. As the larvae bore into the stem for more feeding and pupation, more damage is caused on softer stems than on harder stems. The results indicated that moderate resistance was achieved through higher stem penetrometer resistance. In wild rice, non-preference was attributed to very hard and tough stems (Padhi and Sen, 2002). High stem resistance has also been shown to prevent larvae from feeding on the stem pith during plant development and causing weakening and lodging of the plants (Santiago *et al* 2003; Arabjafari and Jalali, 2007). Interestingly, in this study, canonical discriminant analysis showed that stem penetrometer resistance did not significantly discriminate the inbred lines. The stem borer bores into the maize plant during the early stages of maize seedling growth. At this stage, the stem internodes are, however, soft, underdeveloped and undifferentiated into individual nodes thus penetrometer resistance measurement was not possible until later stages of growth. The canonical findings may, therefore, suggest that stem rind hardness at later stages of maize development had no bearing on stem borer resistance. The results indicate that stem penetrometer resistance at later stages of maize development may not be used as an indicator for stem borer resistance.

5.4.5 Damage parameters

Leaf damage score, number of borer exit holes and cumulative stem tunnelling were important in measuring resistance. Leaf damage was, however, the most important in discriminating the genotypes followed by the number of borer exit holes and the cumulative tunnel length. In canonical discriminant analysis, the high canonical correlation among the damage traits was a strong suggestion that any of the traits could be used to evaluate genotypes for resistance to the stem borer. Canonical correlations between the damage parameters and the mechanisms of resistance were, however, not significant, a suggestion that other mechanisms of resistance could have inherently been active in the inbred lines. The resistance mechanisms were, therefore, mostly germplasm-specific as the importance of variables sometimes varied with different inbred lines. This may imply that maize breeders must evaluate and consider each germplasm's resistance mechanisms independently as germplasm may hold different forms of resistance genes. It gives breeders the opportunity to use the different forms of resistance mechanisms from different germplasm to pyramid resistance genes.

5.5 Conclusions

Several mechanisms of resistance exist in tropical inbred lines with trichome density being the most promising indicator of resistance followed by leaf toughness and stem sugar content. More research is needed to classify the specific types of trichomes, the chemical composition and the specific sugars present in both resistant and susceptible inbred lines in order to identify the causes for some of the inconsistencies reported herein. While CIMMYT has developed several multiple borer resistant lines, the search for new and higher levels of resistance could be enhanced by combining different forms of resistance mechanisms into new inbred lines for hybrid production.

5. 6 References

Arabjafari, K.H. and Jalali, S.K. 2007. Identification and analysis of host plant resistance in leading maize genotypes against spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Pakistan Journal of Biological Sciences 10:1885-1895.

Chu, Y.I. and Horng, S.B. 1991. Infestation and reproduction of Asian cornborer on slag-treated corn plant. Chinese Journal of Entomology 11:19-24.

Dalin, P., Agren, J., Bjorkman, C., Huttunen, P. and Karkkainen, K. 2008. Leaf Trichome Formation and Plant Resistance to Herbivory., p. 89-105, *In* A. Schaller, ed. Induced Plant Resistance to Herbivory. Springer Science+Business Media.

Eyal, F., Jihong, W., Yoko, I., John, E.F., David, R.G., John, O. and Eran, P. 2005). Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. The Plant Cell 17:1252-1267.

Handley, R., Ekbom, B. and Agren, A, 2005. Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana.* Ecological Entomology 30:284-292.

Juma, G. 2010. Basis of host plant recognition and acceptance by *Busseola fusca* (fuller) (Lepidoptera: Noctuidae) larvae. Jomo Kenyatta University of Agriculture and Technology. Nairobi. PhD Thesis pp 188.

Kher, S.V., Dosdall, L.M., and Cárcamo, H.A. 2011. The Cereal Leaf Beetle: Biology, Distribution and Prospects for Control. Insects and Diseases 4:32-41.

Kumar, H., and Saxena, K.N. 1992. Resistance in certain maize cultivars to first and third-instar *Chilo partellus* larvae. Entomologia Experimentalis et Applicata 65:75-80.

Kumar, V.K., Sharma, H.C. and Reddy, K.D. 2006. Antibiosis mechanism of resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. Crop Protection 25:66-72.

Kumar, V.K., Reddy, K.D. and Sharma, H.C. 2007. Expression of antixenosis and antibiosis components of resistance to spotted stem borer *Chilo partellus* in sorghum under greenhouse conditions. eJournal 3:1-4.

Malvar, R.A., Burtron, A., Ordas, A. and Santiago, R. 2008. Causes of natural resistance to stem borers in maize. p. 57-100. E. N. Burton and P. D. William, (eds) Crop Protection Research Advances. Nova Science Publishers Inc, New York.

Novoa, A.D. and Russel, W.A. 1988. Harvest Index Analysis in three improved maize synthetics. Brazil Journal of Genetics 11:355-367.

Padhi, G. and Sen, P. 2002. Evaluation of wild rice species against yellow stem borer (*Scirpophaga incertulas* Walk.). *Journal of Applied Zoology Research* 13:147-148.

Padhi, G. 2004. Biochemical basis of resistance in rice to yellow stem borer, *Scirpophaga incertulas* Walk. Madras Agricultural Journal 91:253-256.

Pimentel, D. 2002. Encyclopedia of pest management. New York, NY; Marcel Dekker Inc: 481-487.

Santiago, R., Souto, X.C., Sotelo, J., Butrón, A. and Malvar, R.A. 2003. Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: Noctuidae) attack. Journal of Economic Entom*ology* 96:1563-1570.

Sarwar, M. 2012. Management of rice stem borers (Lepidoptera: Pyralidae) through host plant resistance in early, medium and late plantings of rice (*Oryza sativa* L.). *Journal of Cereals and Oil seeds* 3:10-14.

Smith C. M. 2005. Plant resistance to arthropods: Molecular and conventional approaches. Springer, Dordrecht, The Netherlands.

Tefera, T., Mugo, S. Beyene, Y., Karaya, H. and Tende, R. 2011. Grain yield, stem borer and disease resistance of new maize hybrids in Kenya. African Journal of Biotechnology 10:4777-4788.

CHAPTER 6

QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO SPOTTED STEM BORER AND AFRICAN MAIZE STEM BORER IN A TROPICAL MAIZE POPULATION

6.1 Introduction

Stem borer resistance is quantitatively inherited and progress in breeding for resistance through conventional methods has been slow (Jampatong *et al*., 2002). Stem borer resistance using conventional breeding methods has been elusive due to limited genetic variation, the difficulty in maintaining a quantitative trait, and dealing with two organisms; pests and hosts (Mugo *et al.,* 2002). The trait is controlled by many genes of small effects, thus, there has not been any immune inbred lines developed for its control. Mapping of quantitative trait loci (QTL) associated with stem borer resistance would be an important step towards improving efficiency of breeding by using marker assisted breeding (MAB). To date, there are several molecular markers available and coupled with the completion of sequencing of the sorghum genome, (Bedell *et al.,* 2005) provides opportunities to harness advances in genomics and genetics for resistance breeding. Such markers especially when tightly linked to resistance loci can aid the introgression and selection of associated traits in early generations of breeding, while minimizing the need extensive phenotypic analysis (Drinic *et al*., 2004). Quantitative trait loci (QTL) for insect resistance in some temperate and tropical maize germplasm against various maize stem-borer species have been detected in the past (Bohn et *al,* 2000). Such results lead to the conclusion that QTL too can be found for resistance to tropical stem borers including *C. partellus* and *B. fusca* and could underpin marker assisted selection (MAS) in the future. It should also be noted that marker-assisted breeding is an expanding breeding frontier to improve the efficiency of plant breeding through the transfer of specific genomic regions of interest and accelerating the recovery of the elite parent background (Robyn, 2008).

Several methods for QTL mapping have been used by scientists and include simple interval mapping, composite interval mapping (similar to multiple QTL mapping) and association mapping (Toure *et al.*, 2000). Both simple interval mapping and composite interval mapping are based on maximum likelihood regression and calculate the most likely position of QTL within a

certain interval between two flanking markers. Composite mapping is very similar to simple interval mapping but possesses improved power because it includes additional genetic predictors, called 'cofactors' that represent QTL elsewhere in the genome and which absorb background genetic noise (Van Eeuwijk *et al*., 2010). Multiple QTL mapping (MQM) method was used in this study because theoretically, it reduces the error variance and increases the power for detecting QTL. Association mapping (linkage disequilibrium mapping) is a recent and more reliable method of locating putative QTL. The method, however, does not deal with a fixed population like interval and multiple QTL mapping but is based on a random and larger population (Yan *et al*., 2011). Both linkage mapping and association mapping studies aim at identifying functional sequence variants (alleles) encoding changes in phenotype, or markers sufficiently closely linked to them to allow breeders to continuously select and manipulate these alleles in different populations for crop improvement. Given the fixed nature of the 203 F2:3 populations used in this study, multiple QTL mapping was applied for locating putative QTL. The objective of this study was to map QTL associated with resistance to maize stem borer in a tropical maize population using tunnel length, number of exit holes and leaf damage as putative stem borer-resistance indices.

6.2 Materials and methods

6.2.1 Field trials for phenotypic data

The F2:3 mapping population was developed from the cross between the susceptible CIMMYT inbred line CML442 and multiple borer resistant (MBR) inbred line CKSBL10026. These two parents, both developed by CIMMYT were genetically divergent and had great differences for the traits of interest (leaf damage, cumulative stem tunnelling and number of stem borer exit holes). F2 and F3 progenies were developed by self pollinating previous F1 and F2 materials, respectively. Concurrently, three male rows of single cross tester CML395 x CML444 were sown preceding the female F2:3 rows. These families were used for the purpose of harvesting leaves for molecular analysis and were also crossed with the single cross tester for seed increase. Leaf samples for molecular analysis were collected from the F2:3 generation. Tender leaves from 15 representative plants were picked at seedling stage and transferred to Biosciences east and central Africa (BecA) laboratory in Nairobi and preserved at -80°C. The testcross ears were

harvested and a population of 203 selected depending on amount of seed achieved for the purpose of multi-environmental phenotyping evaluations.

In March 2011, the 203 F2:3 testcrosses were planted for phenotyping across six environments that included Kiboko, Mtwapa, Kirinyaga Technical institute, Kakamega, Bukura and Embu KARI stations (Table 6.1). The α- lattice design replicated three times was used in a 2 x 5 m rows plot spaced at 75 cm between rows and 25 cm between plants. *Busseola fusca* stem borer larvae were used for infestation at Kakamega, Bukura and Embu sites, while *C. partellus* larvae were used for Mtwapa, Kiboko and Kirinyaga Technical Institute sites. Ten plants in each plot were each artificially infested with five borer neonates three weeks after planting. The remaining plants were concurrently treated with a pesticide to act as a control. All crop field management and disease assessment were done as described in chapter 3. Analysis of variance was performed using the PROC GLM procedure SAS package (2007) and the means compared using Fishers protected least significant difference test (LSD) at $P<0.05$. The F2:3 populations were considered as random effects. Calculation of heritability for both individual and combined sites was done using PROC mixed method of SAS 9 (BLUPS). Due to the zero heritability observed from the Mtwapa site, it was dropped from the analysis. A selection index was computed as described in chapter 3 and used to categorize the individuals into resistance and susceptible groups. Germplasm with selection-index values less than 0.8 were regarded as highly resistant, 0.81 to 1.00 as moderately resistant, 1.01 to 1.2 as moderately susceptible and >1.2 as highly susceptible (Tefera *et al*., 2011).

Table 6.1 Location and description of the six test sites where the testcrosses were evaluated during the Mar-Sept 2011 rainy season

Site	Longitude	Latitude	Max °C	Min °C	Rainfall mm	Altitude in m asl	Soils
1. Kiboko	37.75'E	2.15'S	35.1	14.3	530	950	Sandy clays
2. Kakamega	34.45'SE	0.16'N	28.6	12.8	1915	1585	Sandy loam
3. Mtwapa	39.219'E	4.347'S	29.0	12.8	965	30	Sandy
4. Embu	37.412'E	0.449'S	25.0	14.1	1200	1510	Clay loam
5.Kirinyaga Technical Institute	37.19' E	0.34' S	24.0	18.0	1500	1282	Clay loam
6. Bukura	34. 36' E	0.15' N	22.0	20.0	1800	1397	Sandy loam

6.2.2 DNA extraction and analysis

Leaf samples from 15 representative three week old seedlings for each of the 203 F2:3 families were collected in November 2010. DNA was extracted from the lyophilized leaf tissue from 15 F2:3 plants of each family in August 2011. DNA extraction was done using the 96-well format high throughput protocol (Mace *et al*., 2003). The DNA was shipped to a single nucleotide polymorphic DNA (SNP) service genotyping laboratory (KBiosciences) and the data used in subsequent data analysis.

6.2.3 Single nucleotide polymorphisms (SNPs) marker analysis

One thousand and sixty (1260) SNPs were initially screened for polymorphism between the parental lines, the F1s and F2s. Two hundred and seventy nine (279) out of 1230 SNPs (22.7%) were heterozygote in one or both parents, i.e. nine (9) were heterozygote in parent CML442, 265 (21.5%) were heterozygous in the multiple borer resistant parent, and five (5) were heterozygote in both parents. One hundred and ninety two (192) SNPs were homozygous and polymorphic, and 98.5% of these (184) were true to type for F1 and F2. Out of the 184 SNPs, 152 polymorphic were used to genotype the F2:3 plants of the 203 individuals, because the chi-square (χ^2) test of fit revealed several markers that had high significance deviations from the 1:2:1 ratio expected

for an F2:3 populations (P<0.001), such markers were, therefore, excluded from the linkage map which reduced the markers to 152 SNPs. The linkage map was constructed with the 152 SNP markers using JoinMap 4 software package (Van Ooijen, 2006). Information on the SNPs used is available on maize panzea database website (http://www.panzea.org/database). Segregation at each marker locus was analyzed using chi-square (χ^2) goodness of fit test for the expected Mendelian segregation ratio of an F2 population. The linkage map was developed using Kosambi's mapping function. A $\log_{10}$ of the likelihood odds ratio (LOD) value of 6.0 was used to construct linkage maps. QTL detection (mapping) was performed using MapQTL 6 (Van Ooijen, 2009). Interval mapping and multiple QTL mapping (similar to composite interval mapping) were used for QTL detection. Automatic cofactors selection function was used to set co factors for multiple QTL mapping (MQM), a process that allows markers used as cofactors to absorb the effects of nearby QTL and increases power and precision of QTL analyses. For declaration of linkage, a LOD score of 3.0 and a maximum recombination frequency of 0.50 were used. Interval mapping with LOD score of above 2.5 were indicative of QTL. Gene action for each QTL was calculated using the dominance ratio using absolute additive and dominance values as described in (Stuber *et al.*, 1987). Values of 0 to 0.20 were interpreted for additive gene action, 0.21 to 0.80 as partial dominance, 0.81 to 1.20 as dominance and ≥1.20 as over dominance. The source of resistant allele was detected by the +/- of the additive value with reference to the resistant parent CKSBL10026 where negative values showed alleles came from the resistant parent CKSBL10026, and positive additive values showed resistance came from the sensitive parent CML442 according to (Jampatong *et al.*, 2002).

6.3 Results

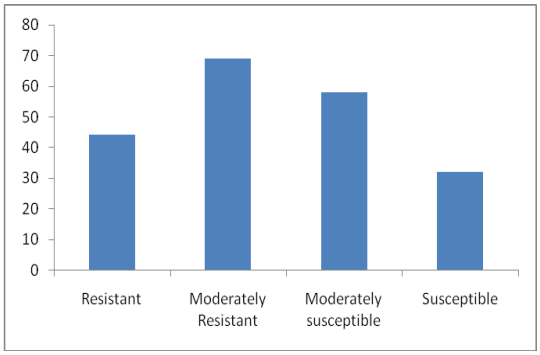
6.3.1 Phenotypic data

In the *C. partellus* infested sites (Kirinyaga Technical Institute, Mtwapa and Kiboko), only progeny evaluated at Kiboko showed significant differences (P≤0.05) for number of exit holes and tunnel length (cm). In *B. fusca* infested sites, only progeny evaluated at Bukura showed significant (P≤ 0.05) difference for leaf damage. Heritability for resistance traits based on combined analysis was low for both stem borer species but high when estimated for evaluations at individual sites except at Mtwapa (Table 6.2). The selection index computed for all sites and

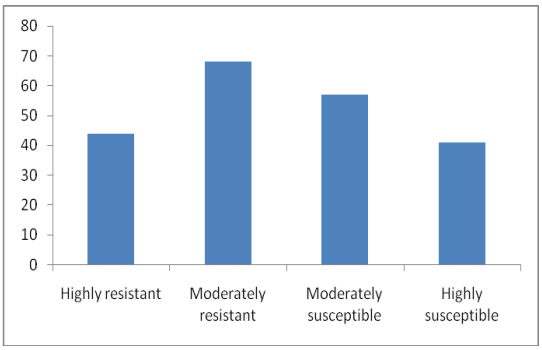
both borer species identified 44 individuals that were highly resistant, 69 moderately resistant, 58 moderately susceptible and 32 highly susceptible with normal distribution frequency (Figure 6.1). The selection index based on individual borer species such as *B. fusca* categorized 43 progeny as highly resistant, 68 as moderately resistant, 56 as moderately susceptible and 37 as highly susceptible. In the *C. partellus* infested sites, 44 progeny were highly resistant, 68 moderately resistant, 57 moderately susceptible and 41 highly susceptible (Figures 6.2). Eighteen progeny were highly resistant to both borer species across all locations.

Table 6.2. Heritability for the putative stem borer resistance traits generated through BLUPS (Best linear unbiased predictors)

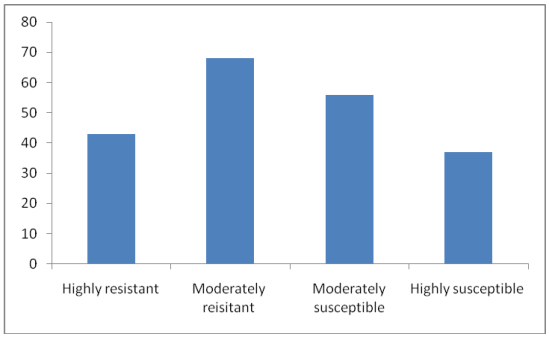
Heritability			
Individual Sites	**Leaf damage score**	**Exit holes (#)**	**Tunnel length (cm)**
Kiboko	0.34	0.70	0.84
KTI	0.69	0.88	0.90
Kakamega	0.62	0.12	0.11
Bukura	0.93	0.39	0.37
Embu	0.01	0.23	0.85
Combined *Chilo partellus* sites	0.13	0.0	0.0
Combined *Busseola fusca* sites	0.02	0.11	0.09



a)



b)



c)

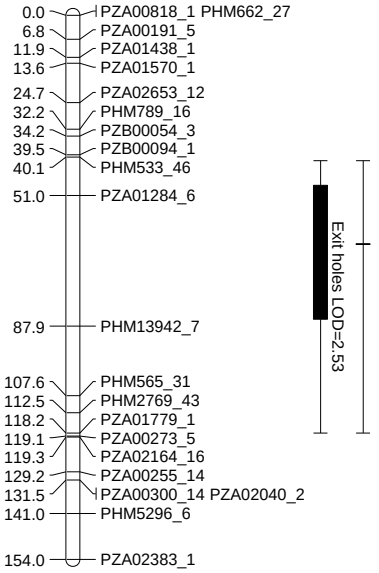
Key: Y-axis represents the actual number of genotypes per category, and the X–axis shows the genotype category names

Figure 6.1 a) Resistance frequency of the 203 F2:3 families from combined 6 sites against *C. partellus* and *B. fusca* b) Resistance frequency distribution of the 203 F2:3 families from combined two sites analysis against *C. partellus* c) Resistance frequency distribution of the 203 F2:3 families from combined three sites analysis against *B. fusca*

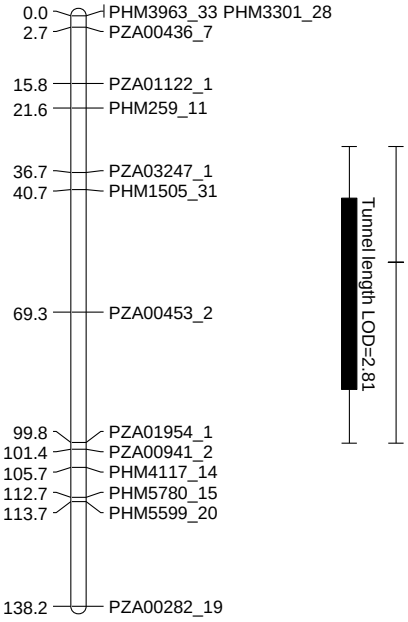
6.3.2 Mapping of quantitative trait loci

The genetic map was constructed with 152 SNP markers that spanned 1248.01 cM on 10 chromosomes of maize with an average interval length of 8.21 cM. Several QTL for resistance were detected on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9 based on individual sites and different species (Figure 6.2) (Appendix 1). QTL detection varied among sites and more QTL were detected for *B. fusca* than for the *C. partellus*. In the combined *B. fusca* site analysis, one QTL for resistance to stem tunnelling was detected on chromosome 4 (LOD 2.86) at position 76.33 and accounted for 6.2% of phenotypic variation. In the *C. partellus* combined sites, two QTL for stem tunnelling on chromosome 4 (LOD 2.81) and number of exit holes on chromosome 5 (LOD 2.53) were detected and accounted for 6.2% and 5.6%, respectively, of the phenotypic variation (Table 6.3).

QTL based on number of exit holes on chromosome 5



QTL based on tunnel length on chromosome 4 - Chilo partellus



QTL based on tunnel length QTL chromosome 4 - Busseola fusca

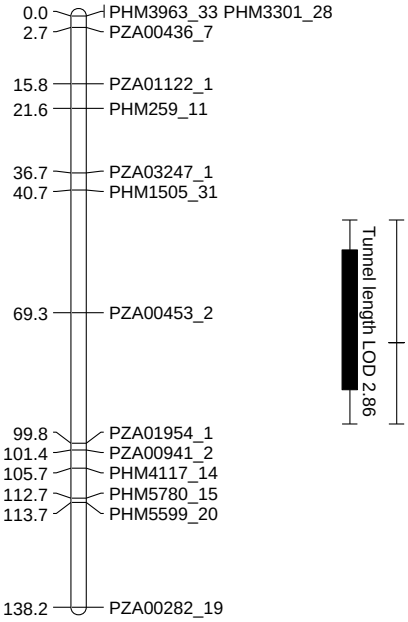


Figure 6.2 Linkage maps and QTL locations from specific stem borer species (*C. partellus* and *B. fusca*) analysis of the 152 SNPs on leaf damage, number borer exit holes and tunnel length. The line to the left of each QTL bar indicates the QTL peak.

6.3.3. Mapped QTL for resistance to other traits

6.3.3.1 QTL for resistance to leaf damage

Two QTL affecting leaf damage feeding score were detected on chromosome 2 for Embu site (LOD 3.37) and one indicative QTL on chromosome 1 for Kakamega site (LOD 2.68). The most significant was the QTL detected on the Embu site which explained 6.6 % of the phenotypic variation. Gene action was due to over dominance for both QTL.

6.3.4 QTL for resistance to number of exit holes

QTL mapped for *C. partellus* based on combined site analysis revealed 1 QTL (LOD 2.53) for number of exit holes on chromosome 4 but none for *B. fusca*. Conversely, three QTL for resistance to stem exit holes were detected in the individual sites. Several QTL were detected for progenies evaluated at the following sites; 1 QTL on chromosome 4 at the Kirinyaga Technical Institute site (LOD 3.73) for *C. partellus* species, 1 on chromosome 9 at Bukura site (LOD 2.97) and a minor QTL (LOD 2.56) on chromosome 1 at Kakamega site for *B. fusca* stem borer species. The most important was the QTL detected for *C. partellus* from Kirinyaga Technical Institute which explained 8% of the phenotypic variation. The QTL detected for *B. fusca* for Bukura site explained 6.5% of the total phenotypic variation while for Kakamega site; the QTL explained 5.5 % variation. The gene action for both Bukura and Kakamega sites were due to partial dominance while it was additive gene action for QTL detected for Kirinyaga Technical Institute site (Table 6.3).

6.3.5 QTL for resistance to stem tunnelling

Combined sites analysis for both stem borer species revealed stem tunnelling QTL on chromosome 4 (LOD 2.81 for *C. partellus* sites and LOD 2.86 for *B. fusca* sites). Five QTL for

reduced tunnel length were detected on different chromosomes on the individual sites analysis for the two stem borer species. The strongest QTL for *C. partellus* stem tunnelling resistance was detected at Kirinyaga Technical Institute site on chromosome 6 (LOD 3.24), while that for *B. fusca* stem tunnelling resistance was detected at Bukura on chromosome 6 (LOD 3.33), they explained 7.1% and 6.7% of the phenotypic variation, respectively. Suggestive QTL for resistance to stem tunnelling to *B. fusca* were detected at both Kakamega (LOD 2.99) and Embu (LOD 2.61) sites. A similar suggestive QTL for resistance to stem tunnelling against *C. partellus* was detected at Kiboko (LOD 2.67). Stem tunnelling QTL was conditioned by over dominance gene action except for the Bukura QTL which was due to partial dominance.

Table 6.3 Locations and QTL effects for *C. partellus* and *B. fusca* stem borer resistance mapped in F2:3 families from the cross between sensitive CML442 and CKSBL10026 multiple borer resistant inbred line parents.

Evaluation Sites	Trait	LOD	Chromosome No.	Locus	Position in cM	% variance explained	Gene effect Additive	Dominance	Gene Action
Embu	Leaf damage	3.37	2	PZA02890_4	106.9	6.6	-0.090	0.130	OD
	Tunnel length	2.61	7	PZA00795_1	98.01	5.8	-0.080	0.800	OD
Kakamega	Leaf damage	2.68	1	PHM14614_2	60.9	5.9	0.100	-0.110	OD
	Exit holes	2.6	6	PZA00571_1	39.02	5.5	-0.310	-0.120	PD
	Tunnel length	2.99	1	PZA03301_2	92.97	6.5	-0.150	-0.200	OD
Kirinyaga Technical Institute	Exit holes	3.73	4	PZA00453_2	69.33	8.1	-0.490	-0.090	A
	Tunnel length	3.24	6	PZA02478_7	57.43	7.1	-0.810	-1.170	OD
Bukura	Exit holes	3.15	9	PZA00152_1	55.41	6.5	-0.170	-0.070	PD
	Tunnel length	3.33	6	PZA00152_2	55.41	6.7	-0.410	-0.200	PD
Kiboko	Tunnel length	2.67	3	PZA03391_1	108.93	5.9	-0.240	-0.840	OD
Combined *C. partellus*	Exit holes	2.53	5	PZA01284_6-PHM13942_7	64.669	5.6	0.126	-0.668	OD
	Tunnel length	2.81	4	PHM1505_31-PZA00453_2	57.741	6.2	0.733	-0.104	PD
Combined *B. fusca*	Tunnel length	2.86	4	PZA00453_2-PZA01954_1	76.329	6.2	-0.0335	-0.837	OD

LOD, Log_{10} of likelihood odds ratio; OD, over dominance, PD; partial dominance, A; additive gene action

Table 6.4 List of SNP markers used to generate the genetic maps

No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome	No.	SNP-Chromosome
1	PHM13942_7Chr5	34	PZA00152_1Chr9	67	PZA03301_2Chr1	99	PZB01403_1Chr1	131	PZA00664_3Chr1
2	PHM4752_17Chr1	35	PZA00245_20Chr1	68	PZA03391_1Chr3	100	csu1171_2Chr1	132	PZA00750_1Chr3
3	PHM5794_13Chr6	36	PZA00255_14Chr5	69	PZA03409_1Chr4	101	PHM10621_29Chr1	133	PZA00795_1Chr7
4	PZA00191_5Chr5	37	PZA00273_5Chr5	70	PZA03461_1Chr6	102	PHM11946_19Chr9	134	PZA00838_2Chr8
5	PZA00424_1Chr7	38	PZA00300_14Chr5	71	PZA03527_1Chr3	103	PHM1218_6Chr9	135	PZA00910_1Chr6
6	PZA00892_5Chr3	39	PZA00418_2Chr7	72	PZA03577_1Chr2	104	PHM14475_7Chr1	136	PZA01122_1Chr4
7	PZA01257_1Chr8	40	PZA00453_2Chr4	73	PZA03605_1Chr10	105	PHM1505_31Chr4	137	PZA01210_2Chr7
8	PZA02117_1Chr1	41	PZA00498_5Chr8	74	PZB00901_3Chr2	106	PHM1511_14Chr2	138	PZA01374_1Chr2
9	PZA03713_1Chr10	42	PZA00706_16Chr8	75	PZB01009_2Chr6	107	PHM15331_16Chr10	139	PZA01462_1Chr6
10	PZD00027_2Chr3	43	PZA00942_2Chr6	76	PZB01062_3Chr1	108	PHM16125_47Chr2	140	PZA01470_1Chr8
11	PHM11114_7Chr8	44	PZA00978_1Chr1	77	PZB01647_1Chr1	109	PHM18513_156Chr10	141	PZA01542_1Chr7
12	PHM12830_14Chr7	45	PZA00986_1Chr7	78	PZD00022_5Chr2	110	PHM1968_22Chr1	142	PZA01570_1Chr5
13	PHM15449_10Chr3	46	PZA01028_2Chr7	79	sh1_12Chr9	111	PHM2518_28Chr4	143	PZA01591_1Chr6
14	PHM1932_51Chr1	47	PZA01241_2Chr10	80	PHM14614_22Chr1	112	PHM2658_129Chr6	144	PZA01642_1Chr10
15	PHM2487_6Chr8	48	PZA01246_1Chr1	81	PHM2691_32Chr7	113	PHM2691_31Chr7	145	PZA01779_1Chr5
16	PHM259_11Chr4	49	PZA01284_6Chr5	82	PHM2919_23Chr3	114	PHM3078_12Chr7	146	PZA01954_1Chr4
17	PHM2714_11Chr8	50	PZA01297_1Chr8	83	PHM3301_28Chr4	115	PHM3334_4Chr2	147	PZA01978_23Chr1
18	PHM2769_43Chr5	51	PZA01438_1Chr5	84	PHM3896_9Chr10	116	PHM4145_18Chr3	148	PZA02040_2Chr5
19	PHM3147_18Chr1	52	PZA01501_1Chr3	85	PHM3963_33Chr4	117	PHM4604_18Chr9	149	PZA02164_16Chr5
20	PHM3334_6Chr2	53	PZA01799_1Chr9	86	PHM4080_15Chr7	118	PHM4780_38Chr2	150	PZA02167_2Chr2
21	PHM3337_23Chr8	54	PZA01933_3Chr7	87	PHM5529_4Chr6	119	PHM4786_9Chr8	151	PZA02385_6Chr4
22	PHM3598_20Chr2	55	PZA02019_1Chr8	88	PHM595_30Chr1	120	PHM499_19Chr2	152	PZA02478_7Chr6
23	PHM3736_11Chr10	56	PZA02247_1Chr6	89	PZA00224_4Chr2	121	PHM5296_6Chr5		

24	PHM4117_14Chr4	57	PZA02383_1Chr5	90	PZA00282_19Chr4	122	PHM5359_10Chr5
25	PHM4620_24Chr2	58	PZA02423_1Chr3	91	PZA00381_4Chr1	123	PHM537_22Chr10
26	PHM4997_17Chr1	59	PZA02549_3Chr2	92	PZA00818_1Chr5	124	PHM789_16Chr5
27	PHM5306_16Chr1	60	PZA02653_12Chr5	93	PZA00860_1Chr9	125	PHM7953_11Chr2
28	PHM533_46Chr5	61	PZA02698_3Chr1	94	PZA00941_2Chr4	126	PZA00081_18Chr1
29	PHM5599_20Chr4	62	PZA02769_1Chr5	95	PZA01301_1Chr8	127	PZA00111_10Chr7
30	PHM565_31Chr5	63	PZA02872_1Chr7	96	PZA01445_1Chr1	128	PZA00256_27Chr7
31	PHM5780_15Chr4	64	PZA02890_4Chr2	97	PZA02129_1Chr1	129	PZA00436_7Chr4
32	PHM662_27Chr5	65	PZA02955_3Chr8	98	PZA02186_1Chr1	130	PZA00571_1Chr6
33	PHM7584_9Chr9	66	PZA03001_15Chr1	99	PZA02328_5Chr6	131	PZA00581_3Chr3

6.4 Discussion

6.4.1 Phenotypic data

The extremely low heritability in the combined sites analysis found in this study is an indicator of significant genotype by environmental interactions. The phenotypic data did not reveal distinct differences in resistance levels in the different sites except at Kiboko and Bukura. The high heterozygosity in the parents could have compounded phenotypic differentiation between resistance and susceptible progenies in the field. This phenomenon may have caused the low levels of trait significance for resistance traits in both individual and combined sites. High and significant differences were, however, recorded for the Kiboko site in tunnel length and exit holes number, and leaf damage at Bukura site. The selection index computed from the three resistance traits leaf damage, number of borer exit holes and cumulative tunnel length in the combined analysis revealed several individuals that were resistant to *C. partellus* or *B. fusca*, or both. Forty four (44) individuals were resistant to both borer species in all sites, a clear confirmation that the resistant parent carried genes for multi-borer resistance. The high correlation between the number of borer exit holes and stem tunnelling in the combined sites analysis was a strong indication of the two parameters reliability and consistency as a measure of resistance. The findings suggest that these parameters were not dependent on changing environments or the borer species used for in evaluation.

6.4.2 Quantitative trait loci for resistance to stem borers

Combined mapping of QTL based on data across sites for both species mapped resistance loci to chromosome 4 for stem tunnelling at position 57.74 (LOD 2.81) for *C. partellus* and at position 76.33 (LOD 2.86). One QTL for number of exit holes (LOD 2.53) was detected on chromosome 5 for *C. partellus* at position 64.67. The close proximity of stem tunnelling QTL within of 18 cM on chromosome 4 for the different borer species suggests that there could be a gene with significant effects on reduced stem tunnelling between positions 57.74 and 76.33. In other studies of a related lepidopteran pest (the European corn borer), QTL for resistance traits occurred in clusters (Papst *et al.,* 2005). It is thus possible that in the case of resistance to stem borer in tropical maize, similar genome setup may occur as found in this study. QTL for resistance to stem borers mapped based on data from individual sites were mostly inconsistent

with only two sites (KTI and Bukura) having consistently found QTL on chromosome 6 (Appendices 1). These inconsistencies in QTL detection may have been due to low levels of segregation in the mapping population, or it could underscore the enormous contribution and interaction of the environmental effects on QTL. Several QTL may, therefore, have been undetected in this study due to the environmental effects. Similar results have been reported in other studies for studies on the European corn borer (due to environmental effects (Jampatong *et al.,* 2002; Krakowsky *et al.,* 2004). The phenotypic variances associated with the QTL reported in this study were fairly low (slightly below 10%). Similar values have been reported in related studies on the European corn borer and storage insect pests (Jampatong *et al*., 2002; Garcia-Lara *et al.,* 2009). Small phenotypic variation values suggest that the QTL have only small effects, or have a larger effect but were only more loosely linked to the marker locus (Edwards *et al*., 1987; Bohn *et al.,* 2000).

The detected QTL in this study were conditioned by over dominance, partial dominance and additive gene actions. In 12 of the 13 QTL detected, resistance was conditioned by over dominance and partial dominance. Partial dominance was found on 3 QTL for number of exit holes and stem tunnelling whilst additive gene action accounted for 1 QTL for number of exit holes. In maize, resistance to the European corn borer is conditioned in a similar manner, albeit with additive gene action accounting for the majority of the QTL than dominance and over dominance gene actions (Guthrie and Russell, 1989; Bohn *et al.,* 2000; Krakowsky *et al*., 2004; Jampatong *et al.,* 2002). Scott (1966) showed that resistance to the European corn borer, a lepidopteran pest just like *C. partellus* and *B. fusca* was conditioned by a relatively large number of genes with small effects on chromosomes 1, 2, 4, 6 and 8. The caution, however, is that some QTL may not have been detected and, or, were dissimilar to those reported for related corn borers due to the low heritability of the putative traits, and differences in trait characterization, respectively (Khairallah *et al.,* 1998).

Overall these results show the presence of QTL for borer resistance in the tropical germplasm thus provides an opportunity to pyramid them into elite material as has been done for the European corn borer (Jampatong *et al.,* 2002). The low heritability for borer resistance indicative

of their polygenic nature cannot be considered as impediments to breeding activities (Stuber *et al*., 1987; Bohn *et al.,* 2000; Garcia-Lara *et al.,* 2009).

6.5 Conclusion

Quantitative trait loci for the three putative resistance traits were detected in the tropical population studied. Relative to other maize stem borer QTL mapping studies, fewer QTL were detected in this study. Among the three traits, QTL for stem tunnelling were the strongest and most detected in both individual and combined specific species environments. The variances explained by QTL-marker associations were, however, low indicative of many QTL with small variances that could have not been detected. Individual sites analysis revealed stronger QTL and it was noted that more QTL were detected in *B. fusca* sites than *C. partellus* infested sites. The low reproducibility of QTL across environments for both stem borer species underscores the need for finer mapping and need for larger populations in succeeding mapping activities.

6.6 References

Bedell, J.A, Budiman, M.A, Nunberg, A, Citek, R.W, Robbins, D. 2005. Sorghum genome sequencing by methylation filtration. PLoS Biol 3(1): e13. Doi:10.1371/journal.pbio.0030013.

Bohn, M. Schulz, B., Kreps, R., Klein, D. and Melchinger, A.E. 2000. QTL mapping for resistance against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European dent germplasm. Theoretical and Applied Genetics 101:907-917.

Drinic, S.M., Micic, D.I., Eric, I., Andelkovic, V., Jelova, D. and Konstantinov, K. 2004. Biotechnology in breeding. Genetika 36:93-109.

Edwards, M.D., Stuber, C.W. and Wendel, J.F. 1987. Molecular-Marker-Facilitated Investigations of Quantitative-Trait Loci in Maize. I. Numbers, Genomic Distribution and Types of Gene Action. Genetics 116:113-125.

García-Lara, S., Khairallah, M.M., Vargas, M. and Bergvinson, D.J. 2009. Mapping of QTL Associated with Maize Weevil Resistance in Tropical Maize. Crop Science 49:139-149.

Guthrie, W.D. and W.A. Russell. 1989. Breeding methodologies and genetic basis of resistance in maize to the European corn borer. *In* CIMMYT, ed. Toward Insect Resistant Maize for the Third World. Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. Mexico, D.F. pp 192-202.

Jampatong, C., McMullen, M.D., Barry, B.D., Darrah, L.L., Byrne, P.F. and Kross, H. 2002. Quantitative Trait Loci for First- and Second-Generation European corn borer Resistance Derived from the Maize Inbred Mo47. Crop Science 42:584–593.

Khairallah, M.M., Bohn, M., Jiang, C., Deutsch, J.A., Jewell, D.C., Mihm, J.A.., Melchinger, A.E., Gonzalez-de-leon, D. and Hoisington, D.A. 1998. Molecular mapping of QTL for south-western corn borer resistance, plant height and flowering in tropical maize. Plant Breeding 117:309-318.

Krakowsky, M.D., Lee, M., Woodman-Clikeman, W.L., Long, M.J. and Sharopova, N. 2004. QTL Mapping of Resistance to Stalk Tunnelling by the European Corn Borer in RILs of Maize Population B73 Ã— De81. Crop Science 44:274-282.

Mace, E.S., Buhariwalla, H.K. and Crouch, J.H. 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Molecular Biology Reporter 21 459-460.

Papst, C., Bohn, M., Utz, H.F., Melchinger A.E., Klein, D. and Eder, J. 2005. Evaluation of resistance factors against European corn borer (*Ostrinia nubilalis* HBn.) in central European dent material. Proceedings of the 54th Annual Meeting of the growers and the seed merchants Austria: Hybrid maize breeding and utilization, 25-27 November 2003, Gumpenstein Irdning, Austria.

Robyn, S. 2008. Prospects for using marker-assisted breeding to improve maize production in Africa. Journal of the Science of Food and *Agriculture* 88:745-755.

Scott, G.E., Dicke, F.F. and Pesho, G.R. 1966. Location of genes conditioning resistance in corn to leaf feeding of the European corn borer. Crop Science 6:444-446.

Stuber, C.W., Edwards, M.D. and Wendel, J.F. 1987. Molecular Marker-Facilitated Investigations of Quantitative Trait Loci in Maize. II. Factors Influencing Yield and Its Component Traits. Crop Science 27:639-648.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2011. Methods of Screening Maize for Resistance to Stem Borers and Post-harvest Insect Pests. International Maize and Wheat Improvement Centre (CIMMYT), Nairobi, Kenya.

Toure, A., B.I.G. Haussmann, N. Jonesc, H. Thomasd, and H. Oughame. 2000. Application of molecular markers in plant breeding, B. I. G. Haussmann, *et al.*, (eds.) Training manual for a seminar held at IITA, Ibadan, Nigeria from 16-17 August 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Van Eeuwijk, F.A., Bink, M.C., Chenu, K. and Chapman, S.C. 2010. Detection and use of QTL for complex traits in multiple environments. Current Opinion in Plant Biology 13:193-205

Van Ooijen, J.W. 2006. JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands.

Van Ooijen, J.W. 2009. MapQTL ® 6 Software for mapping quantitative trait loci in experimental populations of diploid species. Kyazama B.V., Wageningen, Netherlands.

Yan, J., M. Warburton, and J. Crouch. 2011. Association Mapping for Enhancing Maize (*Zea mays* L.) Genetic Improvement. Crop Science 51:433-449.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE PROSPECTIVES

7.1 General discussions

Maize stem borer control through chemical pesticide use remains elusive in SSA as it is expensive to the resource-poor farmers. This makes host plant resistance an important option for maize stem borer control. Resistance to stem borers is quantitatively inherited with a strong prevalence to additive gene action, and to a lesser extent dominance and over dominance (Scott *et al*., 1966; Jampatong *et al*., 2002), this study, however, found strong preference for partial dominance and over dominance gene actions, emphasizing the need for marker assisted selection. The search for high and durable resistance is ongoing in tropical maize germplasm; and the results will add fuel to the nascent breeding programs in the region. Tropical maize germplasm have not been fully exploited, and could offer a rich gene pool of new sources of resistance against maize stem borer damage (Malvar *et al.*, 2004; Mugo *et al*., 2005).

The four groups of germplasm evaluated i.e. hybrids, OPVs, inbred lines and landraces in this study revealed new sources of stem borer resistance. It also confirmed cross resistance to *C. partellus* and *B. fusca* maize stem borers in several inbred lines, landraces and commercial OPVs. CIMMYT experimental multiple borer resistant inbred lines and their stem borer resistant experimental hybrids exhibited high levels of resistance to both borer species. Some landraces (GUAT 1050, GUAT 280, GUAT 1093, GUAT 114 and GUAT 1082), however, exhibited higher resistance levels than CIMMYT's best experimental materials. Resistance in commercial varieties was revealed in genotypes recommended for the moist mid-altitude, dry transitional zones and the lowland zones (PH1, PH4, KDV-1#1, KDV-1#2, KDV-1#3, DH01 and PH3253). This was a significant finding because based on estimation given by farmers, potential grain yield losses were relatively higher in low to dry transitional zones in Kenya (De Groote, 2002). Several commercial hybrids recommended for the moist transitional and high altitude zones (H6213, H629, H6210, 611D, H628, H626 and 531A) were also tolerant to stem borer damage and exhibited low yield loss under infestation. These results suggest that resistance exists in selected commercial maize varieties for all the maize growing ecologies in Kenya. Advocacy for

adoption of these hybrids by farmers could reduce the current yield gap being experienced and move the country closer to food sufficiency. The superior landraces could be used for generation of new tropical inbred lines with high resistance levels or constituting new germplasm pools.

In order to understand the distinctiveness of resistance in the different tropical maize germplasm, the resistance mechanisms involved needed to be further explored. Trichome density, leaf toughness and percentage stem sugar content were the most important mechanism of resistance to maize stem borer. Leaf toughness was the most consistent in all germplasm with resistance increasing with increasing leaf toughness. Similar findings have been reported in maize against other stem borer pests (Bergvinson *et al*., 1995; Afzal *et al.,* 2009). The different resistance mechanisms active in the inbred lines suggest strongly that resistance could be improved through a combination of different plant resistance responses in favourable breeding materials. During the breeding process, when selecting for borer resistance the breeder is essentially selecting for mechanisms that produce the resistance response in the plant. Alotaiba and Elsayed, (2007) reported that the preference or non-preference of plants by insect feeding depends on the pest's behavioural responses to plant features which could be physical or chemical and that these factors could differ among genotypes. In this study for instance, higher levels of sugars did not necessarily translate to high susceptibility in all the germplasm, corroborating what has been reported in studies on several cereal crops (Padhi, 2004; Sarwar, 2012). This suggests that some types of sugars could be stem borer feeding deterrents rather than attractants. Indeed, nine types of sugar are found in maize plant extracts with the disaccharide turanose, an analog of sucrose being a deterrent to stem borer feeding while sucrose promoted feeding (Juma, 2010). Inbred lines with more turanose sugars than sucrose could have, therefore, displayed high resistance to stem borers while those with high sucrose levels displayed susceptibility.

Phenotypic correlations among damage parameters (leaf damage, number of stem borer exit holes and tunnel length) and the resistance mechanisms (trichome density, leaf toughness, percentage stem sugar content and stem penetrometer resistance) were not significant; which could be an indication that there were other inherent resistance mechanisms contributing to resistance besides the parameters evaluated. The morphological mechanisms though tedious to

measure, require less complicated equipment and are a more viable option for studying resistance in the SSA region. The findings in this study underscore the fact that the resistance in maize to stem borers is a manifestation of several mechanisms that are also germplasm dependent. In the search for durable resistance, it would be prudent to pyramid a combination of the different mechanisms in elite materials.

In an effort to understand the genetics of resistance, QTL for maize stem borer resistance were mapped in the F2:3 populations from a cross between CML442 (susceptible parent) and CKSBL10026 (resistant parent). More QTL (10) were detected from individual sites than in the combined sites (3). These QTL, however, had low heritability and phenotypic variances (< 10%) compared to what had been reported for European corn borer in other studies. The environmental effects, the small population size used (203), the degree of segregation, and the different underlying resistance mechanisms within the parents were suspected to have negatively lowered the power to detect more and stronger QTL in both the individual and combined sites analyses. This was not an isolated case as low phenotypic variations have been reported in QTL detected in several European corn borer studies (Krakowsky *et al*., 2004). The effectiveness of marker assisted breeding strongly depends on the accuracy of QTL mapping results and the QTL effects are usually inflated unless estimated from large mapping population or from an independent sample (Bohn, 2000). The efficiency of evaluating a large population through artificial infestations is challenging and may make the choice between markers assisted breeding and phenotypic selection for borer resistance quite complex for breeders in SSA region. The alternate hypotheses for the three studies were accepted; that variability in resistance exists in tropical maize germplasm, resistance is controlled by antibiosis, antixenosis and tolerance in maize stem borers, and QTL for resistance exists in the tropical maize population derived from CML442 x CKSBl00026.

7.2 Conclusions

Lepidopteran insect-related damages have contributed to maize yield losses and food insecurity in Kenya and the sub-Saharan Africa region. Improved insect resistant maize would contribute to increased and stabilized maize productivity in the region. Previous studies have indicated that

tropical germplasm remained a major source of genetic diversity that has not been fully exploited (Goodman, 1999; Larboda *et al*., 2005). This study was driven by the need for new, high and durable levels of resistance in tropical germplasm. The novel sources of resistance identified will be added to the list of existing experimental materials and widen the currently limited gene pool. Resistance was expressed through lower leaf damage scores, lower number of stem borer exit holes, and less cumulative stem tunnelling. The following germplasm, not previously developed or identified for stem borer resistance were found to be highly resistance and are recommended as novel sources of resistance for maize improvement in the region; CIMMYT landrace accessions GUAT 1050, GUAT 280, GUAT 1093, GUAT 114 and GUAT 1082 were resistant to *C. partellus* stem borers, OPVs KDV1-1-#, KDV1-2-#, KDV1-3-#, EEQPM-8-EA-#, POOL15QC, EEQPM-9-E, while dual resistance to both stem borer species was found in CIMMYT MBR lines CKSBL10025, CKSBL10026, CKSBL10027, CKSBL10034, CKSBL10014, and CKSBL10039. Most CIMMYT MBR lines exhibited high resistance levels, with CKSBL10008 being the most resistant *to B. fusca* and CKSBL10039 to *C. partellus*. CIMMYT experimental hybrids CKIR09001, CKIR06009, CKIR06001, CKIR06006, CKIR09006, CKIR09007, and CKIR09002 similarly exhibited highest resistance levels to both borer species and are recommended for release and commercialization.

These results indicated variability for resistance among tropical germplasm, and as such, the material identified as being highly resistant could be used as viable and economical means of stem borer control in SSA. Different levels of resistance were identified in the various germplasm with tolerance to the stem borer revealed among commercial maize hybrids as the resistance mechanism. Tolerance has, however, not been keenly studied in maize although it is the most desirable type of resistance in plants to insects (Kumar, 1997). These findings should stimulate breeders to widen their scope of maize improvement breeding objectives against the stem borers to incorporate the tolerance mechanism. The *C. partellus* infested sites recorded higher number of stem borer exit holes and longer cumulative stem destruction through tunnelling in the four types of germplasm studied. This extensive feeding could be a sign of aggressive feeding and fecundity by *C. Partellus* compared to the *B. fusca* stem borers. Reduced larvae feeding, pupation and completion of the pest's life cycle that were reflected in less leaf

damage, lower numbers of borer exit holes and stem tunnelling were manifestations of inherently active resistance mechanisms in the maize plant i.e. host plant resistance. Several CIMMYT genebank landrace accessions previously not evaluated for stem borer resistance in Kenya were found to be highly resistant to *C. partellus* stem borer and are recommended for incorporation in maize improvement programs. The results of this study will, therefore, contribute to the gene pool for stem borer-resistance breeding. Due to the consistently high correlation coefficients found between the number of stem borer exit holes and cumulative tunnel length across trials, it is recommended that coupled with leaf damage score, one of the two parameters be adopted for measuring resistance to maize stem borers. Such a step may contribute to reduced evaluation costs.

Plant morphological and biochemical properties have been reported to confer host plant resistance and are responsible for the suitability of a cultivar for insect feeding, oviposition and development which translates to amount of damage in terms of yield losses incurred (Kumar *et al*., 2006; Afzal *et al*., 2009). CIMMYT inbred lines; the main sources of breeding materials for maize improvement in the SSA region were assessed for one biochemical resistance mechanism (stem sugar content) and three morphological mechanisms (trichome density, leaf toughness and stem penetrometer resistance). Knowledge of the resistance mechanisms and associated factors are essential for effective utilization of resistant sources in the crop improvement programs. The observation that trichome density, leaf toughness and stem sugar content were the most important in discriminating the genotypes into resistant and susceptible suggests that breeders could use these traits to assess resistance and pyramid them to improve stem borer resistance.

This study, however, showed different findings from those that previously showed that susceptibility increases with increasing stem sugar content and resistance with increasing leaf trichome density across genotypes. While this argument held true in most of the inbred lines, there were differences in some genotypes. For instance inbred line CKSPL10343 had the lowest sugar content of 5.37%, while LPSC7-F86-3 had the highest sugar content of 12.61%, and both were moderately susceptible. Further, inbred line P300C5S1B had the lowest trichome density of 1.23, while CML511 had the highest trichome density of 27.04, and both were susceptible. The

different sugar analogs determine the extent of stem borer feeding on a plant. The shape of trichomes and their chemical composition were singled out as the most important factors in determining the ability of plants to deter herbivore feeding (Kang *et al*., 2010). These arguments could explain why some genotypes though exhibiting high trichomes densities were highly susceptible and some genotypes with high sugar contents were highly resistant.

Stem borer resistance is polygenically inherited and the need to quicken breeding for resistance could be achieved through identification of genetic markers associated with specific mechanisms of resistance. The discovery of chromosome regions controlling resistance mechanisms would accelerate breeding through MAB. While many studies have been conducted for European corn borer resistance QTL, less attention have been given to QTL mapping in tropical stem borers. In this study, 13 QTL were mapped for the resistance traits, but the phenotypic variances explained were low (<10). This study did not, therefore, achieve strong and compelling results to explicitly support MAB for stem borer resistance breeding. The results should, however, stimulate scientists and researchers to consider validating the QTL reported herein before carrying out fine mapping, or to continue using conventional breeding methods for this quantitative trait.

7.3 Future perspectives

The importance of maintaining a rich diversity for utilization by breeders towards crop improvement has been expressed in this study. The sources of resistance documented herein should assist maize breeders in enriching their lists of novel sources of resistance for exploitation in the search for stem borer resistance in SSA. It is envisioned that the documented resistant landraces will be utilized as superior sources of resistance to improve the current OPVs or in constituting new ones. The information on the resistant and tolerant commercial varieties needs to be disseminated for farmers' adoption in relevant production ecologies for reduced yield losses.

Leaf toughness was the most consistent mechanism of resistance as resistance increased with increasing leaf toughness in all genotypes and it is, therefore, highly recommended for use in identifying resistance in maize germplasm in the future. The inconsistencies found in some

germplasm exhibiting high resistance but high stem sugar contents, and a few others with high trichome density exhibiting high susceptibility needs to be resolved. It is recommended that further research be conducted to identify the specific types and chemical compositions of trichomes, and the actual structure of sugars responsible for conferring resistance to stem borers. Such clarification will enhance the clear choice for superior mechanism to target for breeding with accuracy especially for pyramiding the resistance genes for durable resistance. The findings of this study would have been richer and probably more conclusive had assays on other metabolites such as DIMBOA, silicon, leaf nitrogen and wax contents in resistant versus susceptible germplasm been carried out. Such kinds of studies are recommended in order to document the entire complex of factors responsible for conferring resistance against the stem borers in maize, how they interact between themselves and with the environment.

The detection of QTL with a high genotypic variance would have been the ideal outcome for the recommendation of the use of MAB. This study did not reveal such ideal QTL, and the current findings should be enhanced through validation of QTL positions using larger populations and different environments. Such a study would give more conclusive results and probably reduce bias of the variance explained by the QTL. Other QTL notwithstanding, the strong QTL for reduced stem tunnelling (LOD 3.38, 7% variance) points to the possible existence of stem borer resistance QTL and lays good ground for encouraging future research in this area. It is worth noting that ultimately marker assisted breeding will become profitable when cost-effective marker systems i.e. genotyping by sequencing will be made available and the costs of running insectaries and artificially infesting field trials proves higher. Research on molecular marker assisted breeding should not end with the near-miss results and should be encouraged as we edge closer to cheaper genotyping costs that could revolutionize maize breeding in SSA in the future.

7.4 References

Afzal, M., Nazir, Z., Bashir, M. and Khan, B. 2009. Analysis of host plant resistance in some genotypes of maize against *Chilo partellus* (Swinhoe) (Pyralidae: lepidoptera). Pakistan Journal of Botany 41:421-428.

Alotaiba, S. and Elsayed G. 2007. Recent knowledge about the relation between allelochemicals in plants and insects. World Journal of Zoology 2:01-08.

Bergvinson, D.J., Hamilton, R.I. and Arnason, J.T. 1995. Leaf profile of maize resistance factors to European corn borer, *Ostrinia nubilalis.* Journal of Chemical Ecology 21:343-354.

Bohn, M., Schulz, B., Kreps, R., Klein, D. and Melchinger, A.E. 2000. QTL mapping for resistance against the European corn borer (*Ostrinia nubilalis* H.) in early maturing European dent germplasm. Theoretical and Applied Genetics 101:907-917.

De Groote, H. 2002. Maize yield losses from stem borers in Kenya. Insect Science Applicata 22:89-96.

Goodman, M.M. 1999. Broadening the genetic diversity in maize breeding by use of exotic germplasm., p. 524. T. J. G. Coors and S. Pandey, (eds). The genetics and exploitation of heterosis in crops. ASA-CSSA-SSSA, Madison.

Jampatong, C., McMullen, M.D., Barry, B.D., Darrah, L.L., Byrne, P.F. and Kross, H. 2002. Quantitative trait loci for first- and second-generation European corn borer resistance derived from the maize inbred Mo47. Crop Science 42:584–593.

Juma, G. 2010. Basis of host plant recognition and acceptance by *Busseola fusca* (fuller) (Lepidoptera: Noctuidae) larvae. Jomo Kenyatta University of Agriculture and Technology. Nairobi. PhD Thesis pp 188.

Kang, J.H., Shi, F., Jones, A.D., Marks, M.D. and Howe, G.A. (2010). Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. Journal of Experimental Botany 61:1053-64.

Krakowsky, M.D., Lee, M., Woodman-Clikeman, W.L., Long, M.J. and Sharopova, N. 2004. QTL Mapping of Resistance to Stalk Tunnelling by the European Corn Borer in RILs of Maize Population B73 Ã— De81. Crop Science 44:274-282.

Kumar, H. 1997. Resistance in maize in *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): an overview. Crop Protection 16:243-250.

Kumar, V.K., Sharmaa, H.C. and Reddy, K.D. 2006. Antibiosis mechanism of resistance to spotted stem borer, *Chilo partellu*s in sorghum, *Sorghum bicolor*. Crop Protection 25:66-72.

Larboda, P.R., Garcia, A.A., Paterniani, M.E. and de Souza, A.P. 2005. Tropical maize germplasm: what can we say about its genetic diversity in the light of molecular markers? Theoretical and Applied Genetics 111:1288-1299.

Malvar, R.A., Butron, A., Alvarez, A., Ordas, B., Soengas, P., Revilla, P. and Ordas, A. 2004. Evaluation of the European Union Maize Landrace Core Collection for Resistance to *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Economic Entomology 97:628-634.

Mugo, S., De Groote, H., Bergvinson, D., Mulaa, M., Songa, J. and Gichuki, S. 2005. Developing Bt maize for resource-poor farmers - Recent advances in the IRMA project. African Journal of Biotechnology 4:1490-1504.

Padhi G. 2004. Biochemical basis of resistance in rice to yellow stem borer, *Scirpophaga incertulas* Walk. Madras Agricultural Journal 91:253-256.

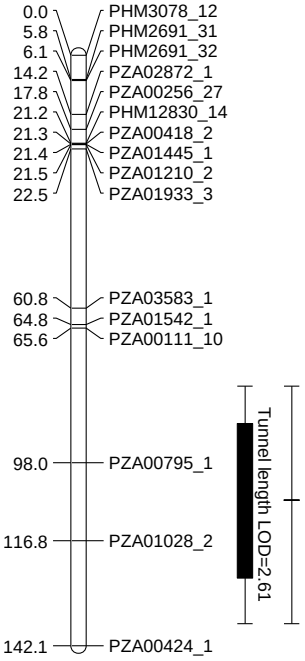
Sarwar, M. 2012. Management of rice stem borers (Lepidoptera: Pyralidae) through host plant resistance in early, medium and late plantings of rice (*Oryza sativa* L.). Journal of Cereals and Oil seeds 3:10-14.

Scott, G.E., Dicke, F.F. and Pesho, G.R. 1966. Location of genes conditioning resistance in corn to leaf feeding of the European corn borer. Crop Science 6:444-446.

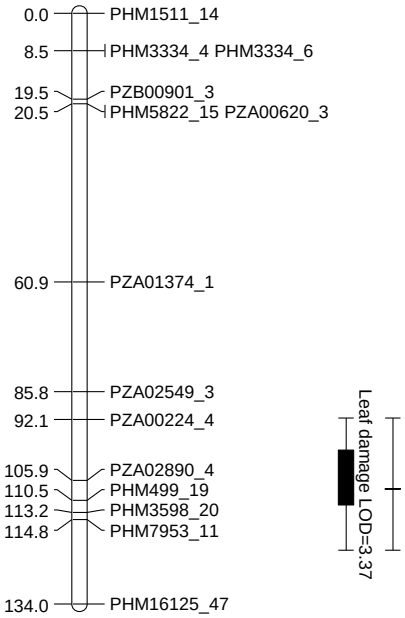
APPENDICES

Appendix 1. Linkage maps detected from individual sites analysis

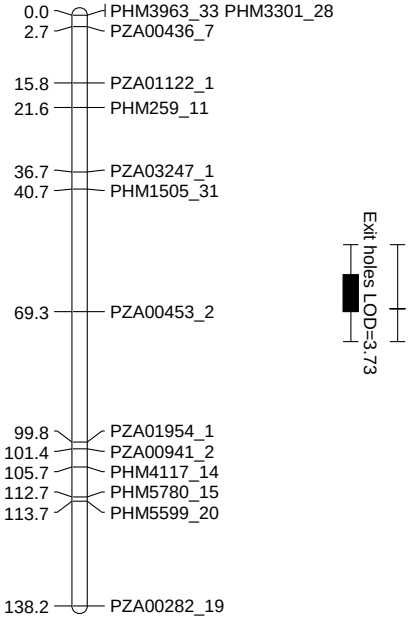
QTL based on tunnel length on chromosome 7



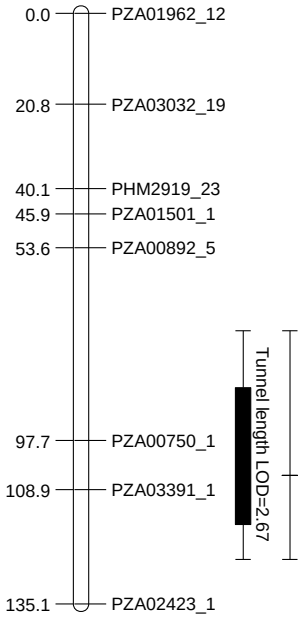
QTL based on leaf damage on chromosome 2



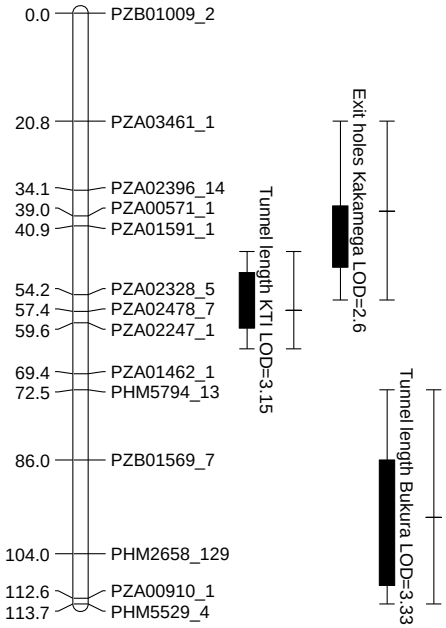
QTL based on exit holes on chromosome 4

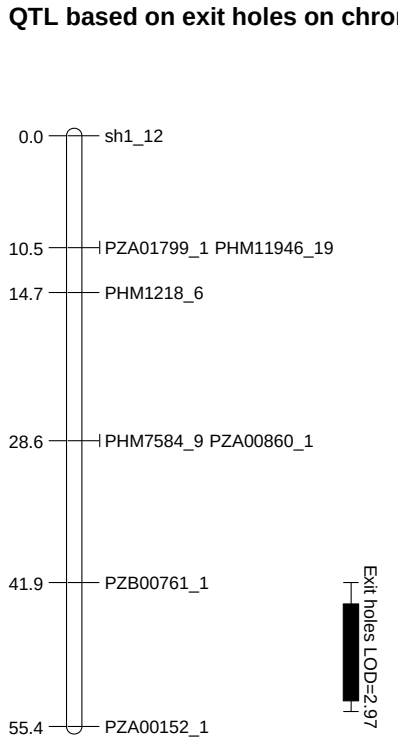


QTL based on leaf damage on chromosome 3



QTL based on tunnel length and exit holes on chromosome 6





Appendix 2. Figure 1.0 Linkage maps and QTL locations from individual sites analysis of the 152 SNP on leaf damage, number borer exit holes and tunnel length. The line to the left of each QTL bar indicates the QTL peak. The line to the left of each QTL bar indicates the QTL peak.

Appendix 3. List of germplasm used for evaluation of resistance to *C. partellus* and *B. fusca* maize stem borers among tropical maize germplasm.

No.	List of Inbred lines	List of Hybrids	List of Landraces
1	MBR C6 Bc F299-2-B-#-1-1-B-B-B-B-B-B -B	H6213	GUAT 1081
2	(200-6 x GUAT189)(51-2-1)F1-B-xP84c1 F26-2-2-4-B-2-B] F102-2-2-2-1 x [KILIMA ST94A]-30/MSV-03-2-10-B-1-B-B-xP84c1 F27-4-1-6-B-5-B]-1-2-B-B -B-B	H6212	GUAT 1008
3	CML395	H6210	GUAT 1010
4	MBR E.T(W)C3 S5/SINTxMBR F15-2-1-2-B-B-B-B -B-B	H629	GUAT 1014
5	MBR C5 Bc F60-2-1-2-B-B-BxCML 384-B-1-2-B-B-B-B -B-B	H628	GUAT 1100
6	Pob.SEW-HG"B"c0F39-1-1-1-1xMBR C5 Bc F22-2-1-4-B-B-B-B-2-2-B-B-B-B -B-B	H626	GUAT 1030
7	P501c1#-500-2-1-2-2-2-4-1-B-B-3-Bx200-6 x GUAT 189-F1-B-#-B-1-2-B-B-B-B -B-B	H513	GUAT 1034
8	CML254	PH4	GUAT 1038
9	MBR C5 Bc F13-3-2-1-B-4-2-B -B-B	PH1	GUAT 1050
10	CML312	DH02	GUAT 1155
11	CML311/MBR C3 Bc F65-1-2-2-B-B-B-B -B-B	DH04	GUAT 1162
12	MBR E.T(W)C3 S5/SINTxMBR F41-1-1-1-B-B-B-B -B-B	WH403	GUAT 1168
13	P590 C7 Blancos F11-1-1-1-B-B-B-B -B-B	SC Duma 41	GUAT 1093
14	(200-3 x GUAT189)(16xP84c1 F27-4-1-4-B-1-B59)F1-B-xP84c1 F26-2-2-4-B-2-B] F97-1-2-1-3 x CML312-B-xP84c1 F27-4-1-6-B-5-B]-2-1-B-B -B-B	SC Duma 43	GUAT 1096
15	P590 C7 Blancos F206-1-1-2-B-B-B-B -B-B	SC Simba 61	GUAT 1082
16	CML-264	PH 3253	OAXA 553
17	EMAP1A-233-B-6-1-B -B-B	611D	BRAZ 2454
18	MBR C5 Bc F108-2-3-1-B-5-2-B-B	KH 600-15A	SINA 30
19	MBR C5 Bc F14-3-2-8-B-4-2-B-B-B	DH01	BRAZ 2375

20	CML311/MBR C3 Bc F43-2-1-1-B-B-B-B -B-B	533A	SONO 72
21	CML311/MBR C3 Bc F95-2-2-1-B-B-B-B -B-B	531A	BRAZ 1470
22	P590 C7 Blancos F57-1-3-1-B-B-B-B -B-B	631Q	CHIS 114
23	MIRTC5 Bco F80-4-2-1-1-1-B-B-B -B-B	500Q	RIGS GP10
24	CML334	DK8031	PARA 151
25	CML202	CKIR07010	VERA GP24
26	MBR/MDR C3 Bc F1-1-1-1-B-3-2-B -B-B	CKIR07011	BRAZ 4
27	CML204	CKIR07012	BRAZ 1486
28	MBR-Et(W)/P590C3 F6-1-1-B-2x761B A1 Bco x 751B-B-3-B-1-2-B-B-B-B -B-B	CKIR07013	SINA 21
29	CML 380xMBR/MDR C3 Bc F21-1-1-2-B-B-B-B-3-1-B-B-B-B -B-B	CKIR07017	BRAZ 2017
30	MBR E.T(W)C3 S5/SINTxMBR F7-1-1-1-B-B-B-B -B-B	CKIR07018	GUAN 28
31	P591c4 F14-1-2-1-B-B-B-B -B-B	CKIR07001	BRAZ 2149
32	CML 384xMBR/MDR C3 Bc F58-2-1-3-B-B-B-B-3-1-B-B-B-B -B-B	CKIR07002	BRAZ 2179
33	MBR C5 Bc F60-2-1-2-B-B-BxCML 384-B-1-2-B-B-B-B -B-B	CKIR07003	VENE 352
34	CML442	CKIR07004	BRAZ 1364
35	MBR C5 Bc F8-1-1-1-B-2-2-B -B-B	CKIR07005	BRAZ 1832
36	MBR C5 Bc F4-1-2-1-B-1-2-B -B-B	CKIR07008	NAYA 130
37	CML444	CKIR07009	VENE 414
38	MBR C5 Bc F114-1-1-3-B-8-2-B -B-B	CKIR09001	GUAT 280
39	CKL06-1 = P100C5S1B-2-3-2#-#1-2-B-B-#-B	CKIR09002	BRAZ 1403
40	CML144	CKIR09003	BRAZ 1797
41	CML159	CKIR09004	BRAZ 1384
42	CML197	CKIR09005	VENE 897
43	CML440	CKIR09006	VERA 197
44	CML78	CKIR09007	GUAT 79
45	CML445	CKIR09008	PARA GP3
46	CZL03014	CKIR06001	VALL 385
47	CML511	CKIR06004	BRAZ 1371
48	DTPWC9-F104-5-4-1-1-B-B-#-#	CKIR06006	PARA GP3

49	DTPWC9-F115-1-4-1-1-B-B-#-#	CKIR06007	BRAZ 1442
50	La Posta Seq C7-F64-2-6-2-1-B-B-#-#	CKIR06008	BRAZ 1919
51	LPSC7-F86-3-1-1-1-BB-#-#	CKIR06009	BRAZ 1346
52	LPSC7-F180-3-1-1-1-BB-#-#	CKPH09001	GUAN 20
53	DTPWC9-F16-1-1-1-1-BB-#-#	CKPH08032	GUAN 36
54	P100C6-200-1-1-B***-#-#	CKPH09002	GUAN 84
55	P300C5S1B-2-3-2-#-#-1-2-B-B-#-#	CKPH08033	PERU 397
56	CML443	CKPH09003	GUAN 34
57	CML488	CKPH08035	VALL 380
58	CZL00003	CKPH08036	CHIS 94
59	CZL03007	CKPH08037	BRAZ 2451
60	CML441	CKPH08038	SAOP GP11
61	CML489	CKPH08039	NAYA 129
62	CZL01005	CKPH08040	JALI 43
63	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-69-1-B-1-B-B-B-B	CKPH08041	CAQU 321
64	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-69-1-B-3-B-B-B-B	CKPH08043	BRAZ 222
65	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-69-1-B-4-B-B-B-B	CKPH08044	BRAZ 1495
66	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-82-1-B-6-B-B-B-B	CKPH08002	BRAZ 1736
67	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-82-1-B-1-B-B-B-B	CKPH08003	BRAZ 2214
68	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-141-1-B-1-B-B-B-B	CKPH08004	BRAZ 2100
69	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-1-B-B-B-B	CKPH09004	CKPH09001
70	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-2-B-B-B-B	CKPH08009	CKPH08032
71	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-4-B-B-B-B	CKPH08010	CKPH09002
72	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-5-B-B-B-B	CKPH08012	CKIR06009

73	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-6-B-B-B-B	CKPH08014	CKIR07008
74	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-1-B-B-B-B	CKPH08020	CKIR07001
75	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-2-B-B-B-B	CKPH08024	CKIR07013
76	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-3-B-B-B-B	CKPH08025	
77	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-4-B-B-B-B	CKPH08026	
78	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-5-B-B-B-B	CKPH08028	
79	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-6-B-B-B-B	DKC8053	
80	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-7-B-B-B-B	ECA-QPOPE	
81	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-8-B-B-B-B	SUSUMA	
82	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-1-B-B-B-B	POOL15QC7-SRC1-F2-#-#-#-#	
83	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-2-B-B-B-B	EEQPM-9-EA-#-#-#	
84	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-3-B-B-B-B	EEQPM-8-EA-#-#-#	
85	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-1-B-B-B-B	KDV1 - 1-#	
86	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-2-B-B-B-B	KDV1 - 2-#	
87	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-3-B-B-B-B	KDV1 - 3-#	
88	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-1-B-B-B-B	KDV1 - 4-#	
89	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-386-1-B-1-B-B-B-B	KDV1 - 5-#	
90	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-393-1-B-2-B-B-B-B	ZM521-IR-#-#-#	
91	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-420-1-B-1-B-B-B-B	ZM421-IR-#-#-#	

92	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-420-1-B-3-B-B-B-B	ECA-STRIGOFF-VL-102-#-#
93	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-420-1-B-7-B-B-B-B	ECA-STRIGOFF-VL-125-#-#
94	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-445-1-B-1-B-B-B-B	BRAZ 2100
95	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-445-1-B-4-B-B-B-B	GUAT 1081
96	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-445-1-B-5-B-B-B-B	CML395/CML444
97	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-445-1-B-6-B-B-B-B	CML312/CML442
98	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-463-1-B-4-B-B-B-B	CKIR04002
99	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-463-1-B-5-B-B-B-B	CKIR04003
100	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-466-1-B-2-B-B-B-B	CML202 /CML204
101	MBR C6 Bc F299-2-B-#-1-1-B-B-B-B-B-B -B-B	
102	CML311/MBR C3 Bc F3-1-1-1-B-B-B-B -B-B	
103	CML311/MBR C3 Bc F43-2-1-1-B-B-B-B -B-B	
104	CML311/MBR C3 Bc F35-2-2-1-B-B-B -B-B	
105	MBR E.T(W)C3 S5/SINTxMBR F15-2-1-2-B-B-B-B -B-B	
106	MBR E.T(W)C3 S5/SINTxMBR F41-1-1-1-B-B-B-B -B-B	
107	MBR-Et(W)/P590C3 F35-1-3-B-1x1760B G1 Bco x Comp.-B-2-B-1-1-B-B-B-B -B-B	
108	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-2-2 x [KILIMA ST94A]-30/MSV-03-4-05-B-1-B-B-2xP84c1 F27-4-3-3-B-1-B] F4-1-1-2-2-1-B-B -B-B	
109	MBR C5 Bc F14-2-2-3-B-B-BxG16SeqC1F47-2-1-2-1-BBBBB-B-1-2-B-B-B-B-B	
110	P590 C7 Blancos F27-1-1-2-B-B-B-B -B-B	
111	P590 C7 Blancos F156-1-2-1-B-B-B-B -B-B	
112	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-1-1-7 x [KILIMA ST94A]-30/MSV-03-2-10-B-1-B-B-xP84c1 F27-4-1-6-B-5-B]-1-3-B-B -B-B	

113	MBR C5 Bc F60-2-1-2-B-B-BxCML 384-B-1-2-B-B-B-B -B-B
114	MBR/MDR C3 Bc F1-1-1-1-B-3-2-B -B-B
115	MBR C5 Bc F4-1-2-2-B-1-2-B -B-B
116	MBR C5 Bc F8-1-1-1-B-2-2-B -B-B
117	MBR C5 Bc F114-1-1-3-B-8-2-B -B-B
118	Pool B –36-B-4-3-B -B-B
119	MBR C5 Bc F13-3-2-1-B-4-2-B -B-B
120	Pob.SEW-HG"B"c0F39-1-1-1-1xMBR C5 Bc F22-2-1-4-B-B-B-B-2-2-B-B-B-B -B-B