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**RESISTANCE TO THE AFRICAN AND SPOTTED STEM BORERS
IN SORGHUM IN KENYA**

By

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DECLARATION

I declare that this study is original and has never been presented for examination for a degree or any award in any University. However, any sources of information are duly acknowledged.

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DEDICATION

This thesis is a special dedication to my cherished parents Charles and Elizabeth for their support in my life. My grandmother who played an immense role in my early life but passed on before witnessing this major achievement. Thank you all for seeing the potential in me and assisting me grow both spiritually and professionally. My dear siblings Hellene, Annah, Nellie, Peter, Joseph, Humphrey and my beloved daughter Elsie for your friendship, love and encouragement.

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PREAMBLE

This thesis is based on the following papers and draft manuscripts

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Muturi PW, Rubaihayo P, Mgonja M, Kyamanywa S and Sharma HC. Inheritance of resistance traits to spotted stem borer, *Chilo partellus* in sorghum. Draft manuscript

Muturi PW, Rubaihayo P, Mgonja M, Kyamanywa S, Sharma HC and Hash CT. QTL mapping for traits associated with resistance to the African and spotted stem borers in sorghum. Draft manuscript

ABSTRACT

Sorghum (*Sorghum bicolor*, L. Moench) is a key cereal crop to over 500 million people in semi arid tropics. Sorghum production in subsistence farming is low and ranges between 0.5– 0.8 t/ha compared to potential yields of 10 t/ha. Diseases, drought, insect pests and parasitic striga weeds are the most important causes of the low grain yield. Lepidopteran stem borers mainly *Busseola fusca* Fuller (Noctuidae) and *Chilo partellus* Swinhoe (Crambidae) are among the economically important insect pests of sorghum and maize in East Africa. Stem borers cause grain yield losses ranging between 15 % - 80% through leaf feeding, deadheart formation, exit holes and stem tunnelling damage. Considering multiple stem borer damage traits is useful since resistance to stem borers is quantitatively inherited thus selecting for resistance based on a single parameter would not be effective. Cultural strategies, biological control, as well as chemical pesticide application have been employed, but are either ineffective or uneconomical for resource constrained farmers. Host plant resistance is an economically viable strategy for stem borer management in cereal production. Sources of resistance to stem borers have been reported and the levels vary from low to moderate. Therefore, it is important to identify sorghum genotypes with higher levels of resistance and with different mechanisms of resistance to diversify the bases of resistance to these pests. In Kenya, little research attention has been accorded to indigenous economically important stem borers like *B. fusca* in cereals. The reason for chilo is because the pest is highly invasive and persistent and has expanded its distribution to areas where it had not been reported earlier. The objectives of this study were to (1) Identify sources of resistance to *B. fusca* and *C. partellus* in sorghum; (2) Establish the mode of inheritance of resistance traits to *B. fusca* and *C. partellus* in sorghum and (3) Map quantitative trait loci associated with resistance traits to *B. fusca* and *C. partellus* in sorghum.

The first study was conducted in two separate experiments in different agroecologies in Kenya. Evaluation of sorghum genotypes for resistance to *B. fusca* was conducted in the University of Nairobi, Kabete campus field station. Experiments to identify sorghum genotypes resistant to *C. partellus* were conducted at the Kenya Agricultural Research Institute (KARI), Kiboko. The reason for conducting these studies in these locations was that the pests causes epidemics on sorghum in these areas. The experiments were carried out in 2010 during long and short rain seasons. Seven East African commercial sorghum cultivars and twenty introduced cultivars from

India were evaluated in this study. Test material was sown in α -lattice design, consisting of nine plots in three blocks, replicated twice. Rows were 2 m long and 0.75 m apart, and the spacing between plants within rows was 0.25 m. First instar neonates of *B. fusca* and *C. partellus* were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi. At 30 days after sowing, five plants in each row were tagged and artificially infested with five larvae/plant using a camel hairbrush. Data collected on percentages was arcsin transformed while that of counts was log transformed before analysis of variance. Data was subjected to Genstat statistical software version 14 for analysis of variance. Treatment means were compared using protected Fishers' least significant difference test at $P = 0.05$. Selection index was calculated based on leaf damage, deadheart, stem tunneling and exit holes by adding the ratios between the values for each genotype and the overall mean for each parameter, and dividing by 4 (number of damage parameters considered). Pearson's correlation coefficients were computed to determine association between morphological characteristics with traits measuring plant reaction to *B. fusca* and *C. partellus* infestation. Susceptibility parameters leaf damage, deadheart incidence, stem tunneling and exit holes were employed to define the reaction of the sorghum genotypes to *B. fusca* and *C. partellus*. Sorghum genotypes namely ICSB 467, ICSA 473, Macia, ICSB 464 and ICSA 472 with cross resistance to *B. fusca* and *C. partellus* based on leaf damage, deadheart, stem tunneling and exit holes following artificial infestation with stem borer neonates were identified. The mechanism of this resistance to *B. fusca* and *C. partellus* was mainly tolerance since numerous genotypes produced substantial grain yield after supporting high leaf damage, deadheart, exit holes and stem tunnelling damages. Bloomy sorghum genotypes with highly glossy and non-vigorous seedlings suffered lower damage compared to the bloomless, non-glossy but highly vigorous plants. Highly vigorous sorghum genotypes tended to have higher total grain yields than those with low vigor perhaps because the whorls of the less vigorous genotypes provided a better environment for the stem borer larvae. Seedling vigor and trichome density could be used as morphological markers to select for sorghum resistance to both borers. The resistant sorghum genotypes can be used to increase the levels of resistance to SB in high yielding but susceptible genotypes.

The second study was conducted in two separate locations in Embu and Kiboko to investigate inheritance of resistance traits associated with *B. fusca* and *C. partellus*, respectively in sorghum. The experimental material consisted of sorghum lines with varying levels of resistance to *B.*

fusca and *C. partellus* identified in study one above. Classification of the sorghum lines into different resistance categories was based on leaf damage, deadheart, exit holes and stem tunneling in 2010 long and short rain seasons. North Carolina mating design 2 was employed where 15 lines were used as females and 2 as males. Test material was sown in α -lattice design, consisting of eleven plots in five blocks, replicated twice. Spacing and artificial infestation were carried out as in the first study described above. Genetic analysis was performed using line x tester analysis using Genstat version 14 statistical software. Major finding was that general combining ability and specific combining ability effects were significant indicating that both additive and non-additive gene effects conditioned resistance to both *B. fusca* and *C. partellus*. General combining ability and specific combining ability effects were significant for leaf feeding, deadheart, number of exit holes and stem tunnels damages indicating that additive and non-additive gene effects conditioned resistance to these stem borers. Additive gene action conditioned deadheart formation and leaf feeding damages. Number of stem borer exit holes and stem tunneling damages were controlled largely by additive and non additive gene action. Females ICSA 464, ICSB 474, ICSB 464 and ICSA 472 and male ICSB 473 exhibited high negative GCA effects for leaf damage, deadheart, exit-holes and stem tunneling, implying their good general combining ability for resistance to *B. fusca* and *C. partellus*. These parents could be utilized in development of stem borer resistant sorghum cultivars.

The objective of study three was to map quantitative trait loci (QTLs) associated with resistance traits to *B. fusca* and *C. partellus* in sorghum. The experiments were conducted in three locations namely Kabete, Embu (to phenotype for *B. fusca*) and Kiboko for *C. partellus*. QTLs associated with resistance to *B. fusca* and *C. partellus* were mapped in 243 F₉:₁₀ recombinant inbred lines (RILs) derived from a cross between ICSV 745 (S) and PB 15520-1 (R). The 243 RILs along with both parents were imported to Kenya from India and phenotyped in Embu and Kabete for *B. fusca* resistance and in Kiboko for resistance against *C. partellus* in 2011 and 2012 rain seasons. Each experiment was laid out in 24 × 10 alpha-lattice design consisting of twenty seven plots in ten blocks in two replications in plots of 2m length, 0.75m interspacing x 0.25m intraspacing. Data was subjected to analysis of variance using residual maximum likelihood model (ReML) in Genstat Version 14 statistical package. The predicted means for each genotype was estimated with genotypes as fixed and reps as random effects in the analyses. Sorghum linkage maps were

constructed using JoinMap 4.0 software. Composite interval mapping was performed on the data using PLABQTL software, version 1.2. Genetic maps and QTLs were drawn using MapChart program version 2.1. The identified QTLs on sorghum chromosomes 1,3,4,5,6,8,9 and 10 controlling *B. fusca* and *C. partellus* resistance against leaf damage, Deadheart, exit holes and stem tunneling are novel and had not been reported in earlier studies. Findings of the present study are also novel as QTLs governing more than one trait (pleiotropic QTLs) were identified. For example, chromosome 3 controlled stem tunneling and leaf damage; chromosome 4 conditioned deadheart, exit holes and stem tunneling and chromosome 6 conditioned exit holes and leaf damage. This study detected 2 QTLs for bloom waxiness, two for leaf glossiness, four for trichome density, five for leaf toughness and two for seedling vigour. Genomic regions governing trichome density detected on sorghum chromosomes 2, 3 and 6 are novel and have not been reported. Chromosome 9 was observed to possess pleiotropic effects and controlled seedling vigour, leaf toughness and bloom waxiness. The strong positive significant association observed in this study between exit holes and stem tunneling could be attributed to the fact that both damage traits are controlled by chromosomes 2, 4 and 9 and either of the traits can be used to predict the other. The positive significant relationship between deadheart formation and leaf feeding damages could be explained by the fact that chromosome 8 controlled both traits and they are governed by additive type of gene action. The identification of more than one QTL for different traits supports the hypothesis that several genomic regions condition expression of resistance to *B. fusca* and *C. partellus* in sorghum. The information generated in this study can be used in marker-assisted selection and the breeding of both *B. fusca* and *C. partellus* resistant sorghum cultivars. There is need for further studies to identify gene(s) underlying the mapped QTLs. Discovery of high through-put strategies that allow greater power and precision in utilization of QTLs conditioning sorghum resistance to stem borers will enhance sorghum improvement against these insect pests.

CHAPTER 1

1.1 INTRODUCTION

1.2 Botany and evolution of sorghum

Sorghum belongs to the family Poaceae, tribe Andropogoneae and subtribe Sorghinae (Dogget, 1988). Sorghum is self-pollinated with 2 – 20% outcrossing (Rai *et al.*, 1999). Genus Sorghum is categorized into three species; *Sorghum halepense* (L.) Pers., and *S. propinquum* (K.) Hitch., are native tetraploid perennials of India and South-Eastern Asia. *Sorghum bicolor* (L.) Moench, $2n = 2x = 20$ comprise of domesticated taxa derived from interbreeding domesticated sorghums and their closest wild relatives (Sally *et al.*, 2007). *Sorghum bicolor* has a small genome (735 Mbp), larger than rice (389 Mbp) but smaller than wheat (16 900 Mbp) and maize (2600 Mbp) (Sally *et al.*, 2007). The last genome duplication for *S. bicolor* genome could have occurred prior to the divergence of major cereals. The genome of *S. bicolor* was successfully sequenced and information is utilized in enhancing understanding of evolution in cereals and diversity studies (Paterson *et al.*, 2009). Information on whole-genome sequences propels development of molecular markers for precise genetic mapping and molecular study of genome structure and function (Elshire *et al.*, 2011).

Domestication of sorghum started in East Africa, Ethiopia and the surrounding countries in 1000 BC (Dogget, 1988). Improved sorghum types then spread to other regions of Africa, India, Middle East and America (Olembo *et al.*, 2010). Cultivated sorghums evolved from wild *Sorghum bicolor* subsp. *arundinaceum* (Dogget, 1988). Cultivated sorghums are divided into five basic races: *bicolor*, *guinea*, *caudatum*, *kafir*, and *durra*, and ten intermediate races of any two or more basic races (Harlan and de Wet, 1972). Sorghum being an indigenous crop has evolutionary benefits associated with wide adaptability and tolerance to abiotic and biotic stresses common in Africa.

1.3 Economic importance of sorghum

Sorghum is the fifth most important cereal crop after wheat, rice, maize and barley in the world (Markus and Gurling, 2006). The crop is a staple to more than 500 million people in arid and semi arid tropics in Africa and Asia (Charles *et al.*, 2006). In Africa, about 25 million

tons of sorghum are produced per annum and translates to one-third of the world crop (FAOSTAT, 2008). In sub Saharan Africa, sorghum is primarily a crop of resource-poor, small-scale farmers (Mace *et al.*, 2009). In East Africa, sorghum has recently become an important industrial crop for the manufacture of beer and its starch has potential in bio-energy production (Taylor, 2010). In Kenya, sorghum is ground into flour and mixed with other types of flour for baby food. Stalks are used for fuel, thatching huts and as animal feed (Charles *et al.*, 2006).

Sorghum is cultivated in East and Horn of Africa where rainfall is intermittent and characterized by short periods of high rainfall (Charles *et al.*, 2006). In East Africa, the crop grows well in a wide range of environments between 500 metres and 1700 metres above sea level with seasonal rainfall of 300mm and above. Sorghum is drought tolerant thus has become an alternative crop in several areas in Kenya like Eastern, Nyanza and Coast provinces where major staples like maize fail due to lack of enough rain (Taylor, 2010). Sorghum utilizes C4 photosynthetic pathway thus has greater efficiency of dry matter production relative to water use (Charles *et al.*, 2006). The crop also tolerates longer durations of water logging better than maize (Dillon *et al.*, 2007). These unique characteristics make sorghum an ideal crop in arid, semi arid and areas at risk of desertification. In the face of global warming and climate change, sorghum is a promising alternative for enhanced food and income security, compared to commodity staples such as maize that often fail due to drought. Sorghum improvement through breeding is essential to enhance the crop's potential in food and income security in sub Saharan Africa. The potential for sorghum to propel economic development in Africa is enormous.

1.3.1 Nutritional composition and health benefits of sorghum as food

Sorghum is an excellent source of energy, proteins, fibre, fat and vitamin B complex essential in energy metabolism (Charles *et al.*, 2006). Sorghum is rich in calcium, iron, zinc, copper, phosphorous, potassium, magnesium, sodium, manganese, folate and vitamins A, C and E (Mohammed *et al.*, 2010). Sorghum is gluten-free and has been recommended for people with diabetic, celiac disease or other gastrointestinal disorders (Ciacci *et al.*, 2007). Celiac disease is characterized by mal-absorption of nutrients as a result of gut sensitivity to gluten protein in

wheat, rye, barley and oats. Sorghum is an excellent source of phytochemicals such as phenolic acids, anthocyanins, phytosterols and policosanols which prevent colon cancer and reduce the risk of getting heart attacks by lowering cholesterol levels (Awika and Rooney, 2004; Dykes and Rooney, 2006).

Sorghum is processed into a variety of nutritious traditional foods in different parts of the world. Fermented bread such as 'kisra' and 'dosa' are found in Africa, Sudan, and India, while 'injera' is popular in Ethiopia (Charles *et al.*, 2006). Unfermented bread, such as 'chapatti' and 'roti' are common in East Africa and India (Taylor, 2010). Stiff porridge also known as 'ugali', 'tuwo', 'karo', and 'mato' are found throughout Africa, India and Central America (INTSORMIL, 2010). Thin porridges such as 'uji', 'ogi', 'koko', and 'akasa' are popular in East and West Africa. Boiled whole or pearly sorghums are consumed in Africa, India, and Haiti. Couscous, beer and other alcoholic beverages are popular in West and east Africa (INTSORMIL, 2010).

1.4 Sorghum production constraints

Sorghum grain output ranges between 0.5 – 0.8t/ha in low resource farming compared to potential yields of 10 t/ha (Singh *et al.*, 2011). The low sorghum productivity is due to abiotic and biotic stress factors. The main abiotic stress factors include drought, high temperatures, low yielding varieties, low soil fertility, poor agronomic practices and lack of markets (Olembo *et al.*, 2010). Drought during grain filling is the most devastating challenge that reduce sorghum grain yield by 45 – 49% (Charles *et al.*, 2006). Nitrogen deficiency is a major constraint in East and South Africa and accounts for about 1.2 kg/yr grain loss (Charles *et al.*, 2006). The key biotic production constraints include *striga* parasitic weeds, fungal diseases, birds and insect pests. In sub Saharan Africa, *striga* infests approximately 20 to 40 million hectares of land cultivated by sorghum and maize peasant farmers (Khan *et al.*, 2007). *Striga* is endemic in Eastern Uganda, Eastern Democratic republic of Congo, Western Kenya and South Africa (ICIPE, 2003). The parasitic weed is spreading to areas previously not infested thus increasing severity and infestation by *Striga* have resulted to abandonment of arable land in Africa (ICIPE, 2003). *Striga* is associated with more than 1000 kg/ha loss in sorghum production per year translating to annual loss of US\$ 7 to 13 billion (Charles *et al.*, 2006).

Economically important diseases of sorghum include downy mildew, anthracnose, grey leaf spot and leaf blight. Generally, sorghum diseases are responsible for more than 500 kg/ha yield loss in Eastern Africa (Ngugi *et al.*, 2002). *Quelea quelea* birds cause substantial sorghum grain damage in East and South Africa. Birds are associated with over 500 kg/ha sorghum grain reduction (Charles *et al.*, 2006). The most important arthropod orders and families of economic importance in sorghum include Diptera (Cecidomyiidae, Muscidae) and Lepidoptera (Crambidae, Noctuidae, Pyralidae) (Dhillon *et al.*, 2005). Insect pest damage in sorghum cause estimated loss of over 1 billion US\$ annually worldwide (Sharma *et al.*, 2005). Sorghum being an indigenous crop to Africa has co-evolved with native insect pests and it is likely that some varieties are tolerant or resistant to these insect pests (Muturi *et al.*, 2012).

Stem borers *Busseola fusca* Fuller (Lepidopteran: Noctuidae), *Chilo partellus* Swinhoe (Lepidopteran: Crambidae), *Chilo orichalcociliellus* Strand (Lepidopteran: Crambidae), *Eldana saccharina* Walker (Lepidopteran: Pyralidae), and *Sesamia calamistis* Hampson (Lepidopteran: Noctuidae) are economically important stem borers of sorghum and maize in sub Saharan Africa (Tende *et al.*, 2005). All these stem borer species are indigenous to Africa except *C. partellus* which was first reported in Africa in 1930s (Harris, 1990). *Chilo partellus* has expanded its distribution in the warm, low-altitude regions of eastern and southern Africa (Figure 1.1), has partially displacing some indigenous stem borers and strikingly, co-exist with *B. fusca* in moist mid-altitude zones of Kenya (Kfir *et al.*, 2002).

Busseola fusca is common in sub-Saharan Africa. In East Africa it occurs at altitudes of 1000 to over 2700 m while in Central Africa it is the predominant stem borer across all altitudes and in West Africa, it is common on sorghum in the dry-hot zones (Kfir *et al.*, 2002). *Chilo partellus* is predominant in India, Pakistan, Afghanistan, Nepal, Bangladesh, Sri Lanka, Thailand, Laos, Vietnam, Yeman and portions of Indonesia (CABI, 2007). In Africa, *C. partellus* is found in most eastern and southern sub-Saharan countries such as Botswana, Cameroon, Eritrea, Ethiopia, Kenya, Malawi, Mozambique, Somalia, Sudan, South Africa, Lesotho, Swaziland, Tanzania, Uganda, Zambia and the island, Comoros and recently reported in Madagascar (CABI, 2007). The distribution of *C. partellus* is highly influenced by altitude and moisture gradients. For example in Kenya, *C. partellus* populations are most common in the dry midaltitude and dry coastal areas, but the pest also occurs in the moist-transitional and moist mid-altitude more than

1500 metres above sea level (De Groote et al. 2003; Muhammad and Underwood 2004).

In Kenya, *B. fusca* and *C. partellus* are the most damaging to sorghum and maize (Karaya et al., 2009). *Chilo partellus* is a highly invasive and persistent Asian species (Figure 1.1) endemic in East and South Africa and no sorghum cultivar with high levels of resistance has been reported (Singh et al., 2011). *Busseola fusca* and *Chilo partellus* manifest similar damage symptoms (Plate 1). In East and southern Africa, *B. fusca* is a pest in altitudes higher than 600 m, but in Central Africa it occurs from sea level to over 2000 m, while in West Africa it is a key pest of sorghum in the dry savannah zone (Figure 1.2).

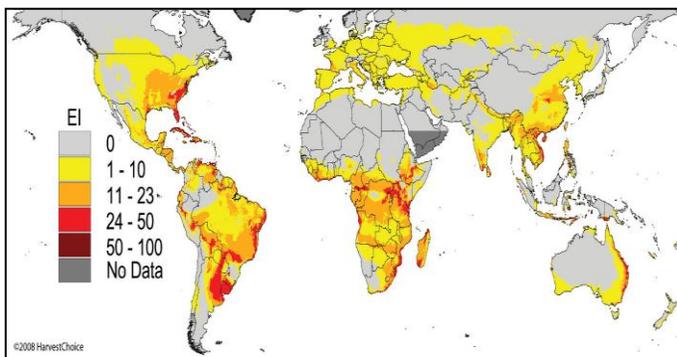


Figure 1.1: Distribution of *Chilo partellus* in the world according to harvest choice. The legend shows the density of *C. partellus* in the world. <http://harvestchoice.org> Accessed 20th May 2012.



Figure 1.2: *Busseola fusca* distribution in Africa according to biovision (in red). *Busseola fusca* has not been reported in the grey shaded areas. <http://www.info-biovision.org>. Accessed 20th May 2012

Busseola fusca and *Chilo partellus* manifest similar damage symptoms on sorghum (Plate 1).



a) Deadheart damage



b). Leaf feeding damage



c). Stem tunneling damage



d). Stem exit holes damage

Plate 1: Stem borer damage on sorghum

The larvae feed on the leaf whorls as they enter into the stem where they are protected from insecticides and natural enemies (Muhammad *et al.*, 2009). Leaf feeding by stem borers reduces the photosynthetic area of the leaves (Tende *et al.*, 2005). Infestation in seedling stage leads to damage of the growing point causing dead-heart which may lead to delayed maturity owing to tillering (Kishiore *et al.*, 2007). The larvae create stem tunnels through the vascular system as they feed thus reducing translocation of nutrients and assimilates (Odiyi, 2007). Stem borers cause grain yield reduction between 15% - 80% depending on crop variety, phenological stage and agro ecological environment (De groote, 2002; Karaya *et al.*, 2009).

1.5 Stem borer management approaches

There are several strategies methods for stem borer management. These include cultural practices such as intercropping, push and pull; biological control mainly introduction of parasitoids such as *Cotesia flavipes*; and use of chemical insecticides (Khan *et al.*, 2003; Tende *et al.*, 2005). Cultural control methods lower the insect pest infestation but do not effectively control the pests. Biological control methods are time consuming, laborious and the effects are obtained in the long run, when the insect has significantly caused damage to the crop (Mailafiya *et al.*, 2009). Chemical control is most effective if done before the damage is inflicted on the crop. Chemical insecticides are expensive to resource poor farmers and are

associated with health and environmental risks (Karaya *et al.*, 2009). Host plant resistance is a viable option to insect pests' management in cereals since it is cheap to farmers, environmentally sound and generally compatible with other strategies of pest control (Tadele *et al.*, 2011).

1.6 Statement of the problem

Area under sorghum cultivation in Africa has almost doubled but grain yields of 500 kg/ha - 800 kg/ha has not improved for the last three decades (Olembo *et al.*, 2010). Lepidopteran stem borers are among the key biotic constraints reducing sorghum grain yield. Grain yield losses due to stem borers range between 15% - 80% depending on the crop variety, locality and phenological stage at infestation (Khan *et al.*, 2003; Karaya *et al.*, 2009). Stem borer damage has been observed to greatly reduce grain yield with tunnels greater than 20 cm causing 40 % reduction of potential grain yield and one exit hole was associated with 33 % grain yield loss (Songa *et al.*, 2001). *Busseola fusca* and *C. partellus* infestation also lowers grain quality since these pests infest all sorghum parts except roots (Beyene *et al.*, 2011).

Knowledge of factors determining stem borer response differences in cultivars is scanty (Singh *et al.*, 2011). In East Africa, sorghum research interest to stem borers has been geared towards cultural, biological control methods and recently genetic engineering using *Bacillus thuringiensis* (*Bt*) *cry1Ac* gene against *Chilo partellus* (Chabi *et al.*, 2005; Mailafiya *et al.*, 2009). Cultural methods lower the pest population but do not effectively manage the pest. Biological control is expensive, laborious and benefits are realized in long run when the damage is already done. Enhancement of sorghum resistance to stem borers has been difficult owing to the complex inheritance of resistance traits and the strong genotype by environment interactions (Yueying *et al.*, 2010). Breeding to improve sorghum local landraces to *B. fusca* and *C. partellus* resistance has been limited partly due to inadequate understanding of the genetics of resistance (Karaya *et al.*, 2009). There is inadequate literature regarding inheritance and mechanisms of resistance to these two borers in East Africa where they cause devastating effects on sorghum.

1.7 Justification

Much of the existing sorghum germplasm in Africa and Asia has not been evaluated for resistance to stem borers (Dhillon and Sharma, 2012). Stem borer resistance levels in sorghum are low to moderate thus the need to increase the levels and diversify the bases of resistance through screening more germplasm (Singh *et al.*, 2011). Genetics of resistance to *B. fusca* and *C. partellus* has been investigated in maize (Karaya *et al.*, 2009). The concept of combining ability applied in maize could also be applied in sorghum to reveal the mode of gene action conditioning resistance to these borers. Current sorghum breeding efforts in sub Saharan Africa focus on high-yielding insect resistant genotypes (Olembo *et al.*, 2010).

Earlier studies have identified cultural and biological control strategies which can yield significant benefits to farmers if coupled with resistant cultivars (Khan *et al.*, 2003). There is need to comprehend inheritance mechanisms of resistance traits to *B. fusca* and *C. partellus* in order to identify molecular markers associated with different resistance mechanisms. Host plant resistance is easy to adapt and use by farmers. Host plant resistance through conventional breeding and modern biotechnology tools mainly use of molecular markers to identify genomic regions associated with resistance to stem borers can improve sorghum production (Sharma, 2008). Molecular markers linked with resistance factors associated with *B. fusca* and *C. partellus* resistance can be used to enhance additive type of gene action (Dhillon and Sharma, 2012).

Advancements in next-generation sequencing technology support whole genome re-sequencing in plant and animals species by allowing discovery and characterization of molecular polymorphisms. Genotyping-by-sequencing is utilized in association studies and genomics-assisted breeding. Genotyping-by-sequencing approach has been employed in whole genome sequencing to discover numerous single nucleotide polymorphisms (SNPs) for genome-wide association studies (Metzker, 2010). The technique is easy, fast, highly specific and reproducible and has been employed in sequencing and mapping studies in maize and barley (Elshire *et al.*, 2011). Identification and analysis of QTLs that influence both *B. fusca* and *C. partellus* resistance would improve understanding of the genetic and physiochemical mechanisms of sorghum defence against the stem borers (Yencho *et al.*, 2000).

1.8 Objectives

The overall objective of this study was to enhance breeding of resistance to stem borers in sorghum through identification of sources of resistance and establishing inheritance of resistance traits

1.8.1 Specific objectives were to;

1. Identify sources of resistance to *B. fusca* and *C. partellus* in sorghum
2. Establish inheritance of resistance to *B. fusca* and *C. partellus* in sorghum
3. Map QTLs associated with resistance to *B. fusca* and *C. partellus* in sorghum

1.9 Study hypotheses

1. Sorghum genotypes show varied levels of resistance to *B. fusca* and *C. partellus*
2. Resistance to both *B. fusca* and *C. partellus* is heritable and stable
3. Several genomic regions condition expression of resistance to *B. fusca* and *C. partellus* in sorghum

CHAPTER 2

2.1 LITERATURE REVIEW

2.2 Description, biology and distribution of *Busseola fusca* and *C. partellus*

The African stem borer, *Busseola fusca* eggs are round, flattened and about one mm in diameter. The eggs are laid in batches of 30 to 100 underneath leaves in stretching up the stem. They are white when freshly laid but darken as they age. Eggs hatch in about 7 to 10 days and larvae are light or dark violet to pinkish white in colour with a grey tinge (CABI, 2007). They lack noticeable hairs and look smooth and shiny, but have rows of small black spots along the body. On hatching larvae are black in colour. They move up the plant into the funnel feeding on leaves for two to three days and then either move to other plants or enter inside stem where they cannot be reached by chemical insecticides and predators. After boring into the sorghum stems, they feed and grow within the stems for 2 to 3 weeks where they grow to about 40 mm (Kfir *et al.*, 2002). When fully grown, they cut exit holes in side the stem before pupating within tunnels inside the sorghum stem.

The total larval period is about 35 days in favourable conditions during growing season, but during dry and cold weather larvae enter into diapause for six months in stems and other plant residues. At the beginning of the rains, the larvae pupate within the stems (Kfir *et al.*, 2002). Pupae are shiny yellow-brown to dark brown measuring approximately 25 mm long. After 7 to 14 days, adults emerge from the pupae and leave the stem. Adults have a wingspan of about 25 to 35 mm. Generally, females are larger than males. The forewings are light to dark brown with darker markings and the hindwings white to greyish-brown. Seasonal and geographic variations are common with darker coloration developing during cold wet conditions. Adult moths are rarely observed in the fields and they are inactive during daytime but lay their eggs during the night. They have numerous generations in a year and their populations increase towards the end of the season (CABI, 2007).

Chilo partellus (spotted stem borer) undergoes complete metamorphosis involving changes from egg, larval, pupal and adult phases. The borer undergoes 1, 2 or more generations annually, depending on the site and the availability of host ranges throughout the year (Muhammad and Underwood 2004). In warm low-altitude areas with abundant hosts to maintain larval

populations *C. partellus* cycle throughout the year. The borer starts diapauses in the larval stage, at higher elevations or during dry seasons (Kfir *et al.*, 2002). Female spotted stem borer moths typically prefer whorl stage plants for oviposition. Egg hatching range between 7 - 10 days, larvae complete development in 28 - 35 days, and pupae require 8 - 10 days depending on temperature. The eggs of spotted stem borer are oval-shaped, flattened and laid in clusters, with total fecundity averaging 100 - 166 eggs/female (Ofomata *et al.*, 2000). Majority of the stem borer eggs are on the lower leaf surfaces near the midrib. After hatching, early larval instars move upwards on plants and into the whorl to feed on leaf surfaces deep and late-larval instars bore into the stalk. In older plants, the larvae feed on the panicle.

The larvae of spotted stem borer look similar to those of the African stem borer, *B. fusca*. The larvae have a cream coloration, with dark spots on the dorsal surface and a brown head capsule. When mature the larvae measure approximately 25 mm long. *Chilo partellus* larvae can be distinguished from *B. fusca* by the hooks on the prolegs. In *C. partellus*, the hooks are arranged in complete circles, whereas in *B. fusca* the hooks are arranged in crescent shapes. *Chilo partellus* larvae pupate within the sorghum stalk. Preceding pupation, fully grown larva cut exit hole to allow the adult moth emerge from the plant (Kfir *et al.*, 2008). *Busseola fusca* and *C. partellus* are polyphagous. They feed on several plants including maize (*Zea mays* L.), Sorghum (*Sorghum bicolor* L.), Rice (*Oryza sativa*); Sugarcane (*Saccharum officinarum*) and several millets including Pearl millet (*Pennisetum glaucum*); several grasses including Sudan grass (*Sorghum vulgare sudanense*), Napier grass (*Pennisetum purpureum*), and *Sorghum arundinaceum* (Devs.) Stapf (CABI, 2007; Matama *et al.*, 2008).

2.3 Mechanisms of sorghum resistance to stem borers

Painter, (1951) recognized three mechanisms of resistance namely antibiosis, non-preference (antixenosis) and tolerance as described below.

2.3.1 Antibiosis

Antibiosis expressed in terms of larval and pupal mortality, decreased larval and pupal weights, prolonged larval and pupal development and reduced fecundity is an important component of resistance to stem borers in sorghum (Kumar *et al.*, 2006). Antibiosis factors function in leaf and stem tissues (Dhillon *et al.*, 2006). Resistance to leaf and stalk feeding by European corn borer

[*Ostrinia nubilalis* ((Hübner))] in temperate maize is conferred by 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) and increased concentrations of cell wall components mainly fiber and lignin (Krakowsky *et al.*, 2007). High levels of total phenols, orthodehydroxy phenol and silica have associated with resistance to yellow stem borer in rice (Padhi, 2004). Sorghum genotypes namely ICSV 705, ICSV 714, IS 1044, IS 2205 and IS 18573 have been observed to demonstrate antibiosis to *C. partellus* in terms of reduced larval survival, development and feeding (Kumar *et al.*, 2007).

2.3.2 Antixenosis (non-preference)

Presence of antifeedants such as glycosides, alkaloids, terpenoids contribute to antixenosis mechanism of stem borer resistance in sorghum (Sharma, 2008). Chemicals in the leaf surface waxes (benzaldehyde, p-OH benzoate, and -CN-ion metabolites) protect sorghum against desiccation, disease and insect feeding and movement (Sanford and Reinhard, 2002; Kishore *et al.*, 2007). High contents of proanthocyanidins (PAs), 3-deoxyanthocyanidins (3-DAs), and flavan-4-ols in sorghum have been associated with sorghum resistance to biotic and abiotic stresses (Abdel *et al.*, 2007). Trichomes and ligular hairs interfere with stem borers movement, feeding and oviposition (Muhammad *et al.*, 2009). Increased leaf thickness, fiber and epidermal cell wall toughness impede feeding by stem borer neonate larvae in maize (Bergvinson, 2002).

2.3.3 Tolerance

Tolerance is where the plant is capable of supporting, without loss of yield or quality, a population of insect pests which would damage a susceptible variety (John *et al.*, 1994). Sorghum tillering following stem borer damage and in response to shoot flies is a component of tolerance (Kishore *et al.*, 2007). Components of tolerance include vigour, compensatory growth in infested plants, rapid lesion healing, changes in photosynthate partitioning and tissue mechanical support (Dhillon *et al.*, 2006). Sorghum tolerance to *C. partellus* damage has been observed on IS 2205 after showing less grain yield reduction (Dhillon and Sharma, 2012).

2.4 Inheritance of traits associated with resistance to stem borers

2.4.1 Sorghum resistance traits

Information on mode of gene action for traits that contribute to *B. fusca* and *C. partellus* resistance is useful in developing an appropriate breeding strategy to produce stem borer resistant hybrids. Characters associated with resistance to cereal stem borers include early panicle initiation, trichomes, low sugar, high amino acids, high tannin, total phenols, neutral and acid detergent fibers, and high silica content (Taneja and Woodhead 1989; Singh, 1997; Padhi, 2004). Early panicle initiation was observed to be conditioned by non-additive type of gene action (Sharma *et al.*, 2007). Sorghum tolerance to shoot insect pests is generally governed by additive type of gene action (Dhillon *et al.*, 2006).

2.4.2. Polygenic inheritance

Resistance to stem borers is a quantitative trait with low heritability (Singh *et al.*, 2011). Additive genes are important for inheritance of stem borer resistance (Karaya *et al.*, 2009). Multiple traits mainly leaf feeding, deadheart, exit holes and stem tunneling damages are considered when selecting for *B. fusca* and *C. partellus* resistance (Tadele *et al.*, 2011). Resistance to stem borers is influenced by the interaction of factors in the cytoplasm of the maintainer lines and the nuclear genes (Sharma *et al.*, 2007). A high level of resistance is required in both male sterile and restorer lines to produce stem borer resistant hybrids (Dhillon *et al.*, 2006). The concept of combining ability has been employed in maize to detect superior combiners for disease resistance traits and is rarely used in breeding for stem borer resistance particularly *B. fusca* and *C. partellus* in sub Saharan Africa (Karaya *et al.*, 2009).

Hybridization between stem borer resistant lines with good combining ability for different stem borer resistance mechanisms would reduce damage in sorghum (Aruna and Padmaja, 2009). Sharma *et al.* (2007) observed that sorghum leaf feeding was conditioned by dominance gene action. Singh and Verma (1988) observed that GCA effect (additive gene action) was predominant for leaf damage, whereas specific combining ability SCA effect (non-additive gene action) was important for sorghum stem damage. Additive gene action has been reported to condition exit holes and stem tunneling in maize against *C. partellus* and *B. fusca* (Karaya *et al.*, 2009). Butron *et al.* (2009) reported additive– dominant model for stem and ear damage by the

pink stem borer (*Sesamia nonagrioides*) in maize. Singh and Verma (1988) observed additive gene effects to be predominant for deadheart and leaf injury as a result of *C. partellus* in sorghum. There is scanty information regarding genetics of resistance to *B. fusca* in sorghum.

Significant genotype by environment interactions (G x E) for leaf damage, exit holes and stem tunnels against *B. fusca* and *C. partellus* has been reported in different maize backgrounds (Karaya *et al.*, 2009; Beyene *et al.*, 2011). This observation explains why relatively few cultivars are reported to be resistant to both borers in maize and sorghum. Highly significant genotype by environment interactions results to different ranking of genotypes in diverse environments. This calls for conducting multi-environmental experiments under artificial infestation to identify genotypes with stable resistance to *B. fusca* and *C. partellus*.

2.5 Quantitative trait loci associated with resistance to insect pests

Features of quantitative trait loci (QTL) mapping include; a mapping population of 100 – 150 progenies from phenotypically contrasting parents; precise phenotypic data for the traits; genotype the population with markers spaced about 10 to 15 centimorgans (cM) apart; analysis of phenotypic and marker data with suitable statistical method executed in user-friendly software for discovery of few markers associated with each trait of interest (Deu *et al.*, 2005; Bernado, 2008). Quantitative trait loci analysis is undertaken in segregating population such as F2 derived populations, recombinant inbred lines, near isogenic lines and double haploids and back cross populations (Kassa *et al.*, 2007). Recombinant inbred lines are valuable for QTL mapping and trait analysis because they increase the resolution of QTL discovery and represent a more stable sample of progeny (Matthieu, 2008).

Quantitative trait loci mapping enhance the biological understanding of inheritance of quantitative traits and the markers identified can be used to select for complex trait (Bernado, 2008). Genetic linkage maps are essential for localization of genes conferring resistance/tolerance to stem borer damage in sorghum (Sally *et al.*, 2007). Several QTLs have been mapped for different stem borer and agronomic traits in cereals. Quantitative trait loci for resistance to European corn borer-stem tunneling have been mapped in RILs of ‘B73’ × ‘DE811’ (Krakowsky *et al.*, 2004). Favorable alleles at these identified QTL can be successfully used in breeding to improve preferred cultivars through the introgression of these alleles into the

preferred cultivars susceptible to stem borer damage. Five major regions conditioning resistance to European corn borer were reported on chromosomes 1, 2 and 4 and 6 (Scott *et al.*, 1966).

Four QTLs for resistance to generation I of European corn borer (ECB I) (leaf feeding) were reported on chromosomes 1, 4, 6 and 9 using 150 F₃ lines derived from a cross between Mo17 and H99 (Schön *et al.* (1991). Seven regions controlling resistance to second generation of European corn borer on leaf feeding and stem tunneling) on chromosomes 1, 2, 3, 7 and 10 were identified using F₃ lines of B73 x B52 between (Schön *et al.*, 1993). Sixteen major QTLs were identified using F₃ families derived from three crosses B73 × B52, B73 × DE811 and Mo17 × B52, Lee (1993) conferring resistance to ECB II. F₄ families derived from 112 F₂ plants of B73 x Mo17 and mapped three major QTLs affecting generation II of European corn borer (ECB II) on chromosomes 7, 8 and 9 (Beavis *et al.*, 1994). Two hundred and forty four F_{2:3} families of B73Ht × Mo 47 identified 9 QTLs for resistance to ECB I and 7 for resistance to ECB II respectively. Five of the identified QTLs were located at different positions on the genome suggesting different mechanisms of resistance to the two generation of ECB.

Ten QTLs were mapped on chromosomes 1, 2, 5, 7, 8, 9 and 10 on a mapping population of 171 F₃ families derived from a cross CML 131 × CML 67 contributing resistance to generation SCB I (leaf feeding damage). The QTL on chromosome 2, on chromosome 5 and QTL on chromosome 9 were mapped to genomic regions known to carry genes involved in cell wall biochemistry supporting the hypothesis of a major role of cell wall components (CWCs) in the resistance to SCB (Bohn *et al.*, 1996). A major QTL for ECB I mapped on the short arm of chromosome 4, near *bx1* locus validating the role of DIMBOA for resistance to leaf feeding by temperate stem borers (Simcox and Weber, 1985).

2.4.1. Approaches for QTL mapping

Methods for QTL mapping include single-marker analysis, interval mapping, joint mapping, multiple regression and composite interval mapping (Bernado, 2008). Interval mapping is the most popular for QTL mapping (Matthieu *et al.*, 2008). The method makes use of a genetic map of the typed markers, and like analysis of variance, assumes the presence of a single QTL (Bernado, 2008). Software packages for mapping with F₂ or backcross populations, selfed or

recombinant-inbred progenies, or germplasm collections include PLABQTL, MAPMAKER/QTL, JoinMap, QTL Cartographer, QGene and TASSEL (Buckler, 2007; Bernado, 2008).

JoinMap is useful for the calculation of genetic linkage maps in experimental populations of diploid species (Ooijen, 2006). The program is significantly enhanced thus ease of marker data management, charts and improved portability and has powerful analytical methods such as Monte Carlo maximum likelihood mapping function. PLABQTL is valuable for the detection and characterization of loci which affect the variation of quantitative traits. The program employs the interval mapping approach (Lander and Botstein, 1989). PLABQTL utilize a multiple regression approach with flanking markers (Haley and Knott, 1992). The linkage map of markers is assumed to be known and must be calculated with other programs such as JOINMAP (Stam, 1993). For detection of QTL, it is possible to utilize other identified QTL as cofactors (composite interval mapping) thus improving the power of QTL detection (Zeng, 1994). Cross validation lowers the tendency of high bias of explained variance by QTL (Utz *et al.*, 2000).

Simplest statistical method for QTL mapping is analysis of variance (ANOVA) also called marker loci (Broman, 2001). This approach suffers when there is appreciable missing marker genotype data and when the markers are widely spaced (Broman, 2001). Interval mapping, though more complicated and more computationally intensive, allows for missing genotype data. Logarithm of the odds (LOD) favoring linkage scores are used to measure the strength of evidence for the presence of a QTL with larger LODs corresponding to greater evidence (Bernado, 2008). The LOD curve for a chromosome indicates whether a QTL may be present and where it is likely to be located. The region where the LOD score is within one and half times of its maximum may be taken as the plausible region for the location of the QTL (Broman *et al.*, 2001). Considering multiple QTLs simultaneously have three advantages in that there is greater power to detect QTLs, ability to separate linked QTLs, and the ability to estimate interactions between QTLs (Bernado, 2008).

2.4.5. Molecular markers utilized in crop improvement

Randomly amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeats (SSR), amplified fragment length polymorphisms (AFLP), single nucleotide polymorphisms (SNP) and diversity arrays technology markers have increased the number and decreased the cost of markers in different crop species (Casa *et al.*, 2005; Bernado, 2008; Mace *et al.*, 2009). Single nucleotide polymorphisms (SNPs) are definitive genetic markers since they represent the finest resolution of a DNA sequence which is a single nucleotide. SNPs are the most common types of DNA polymorphism, are codominant, bi-allelic, highly polymorphic and have good reproducibility that render them as efficient biological marker. SNPs are generally abundant in the populations with a low mutation rate. SNPs are vital tools in studying complex genetic traits which are quantitative in nature and also in genome evolution (Bernado, 2008). Recently, next-generation sequencing approaches have been used for whole genome sequencing to discover large numbers of single nucleotide polymorphisms (SNPs) for exploring diversity, constructing maps and in genome-wide association studies (Metzker, 2010). Genotyping-by-sequencing (GBS) is a simple, highly multiplexed approach suitable for breeding and trait mapping in cereals (Elshire *et al.*, 2011).

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CHAPTER 3

Sources of Resistance to the African Stalk Borer in Sorghum

Introduction

Sorghum (*Sorghum bicolor* [L.] Moench [Poaceae]) production is affected by numerous insect pests such as shoot fly, *Atherigona soccata* Rondani (Diptera: Muscidae), midge, *Stenodiplosis sorghicola* Coquillett (Diptera: Cecidomyiidae), stem borers, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) and *Busseola fusca* Fuller (Lepidopteran: Noctuidae), green bug, *Schizaphis graminum* Rondani (Hemiptera: Aphididae), head bugs, *Calocoris angustatus* Lethierey (Hemiptera: Miridae) and *Eurystylus oldi* Poppius (Hemiptera: Miridae), and aphids, *Rhopalosiphum maidis* L (Homoptera: Aphididae) and *Melanaphis sacchari* Zhent (Homoptera: Aphididae) (Dhillon *et al.*, 2005). *Busseola fusca*, (Lepidopteran Noctuidae) is an economically important pest of maize, sorghum and pearl millet in sub-Saharan Africa (Kfir *et al.*, 2002). This pest is more important at high altitudes, but co-exists in mid-altitude zones of Kenya with *C. partellus*, another economically important stem borer introduced into Africa from Asia (De Groot *et al.* 2002; Wale *et al.*, 2006).

Stem borers reduce grain yield through leaf feeding, deadheart and stem damage (Karaya *et al.*, 2009). The larvae of *B. fusca* infest sorghum at the seedling stage and thrive till maturity, resulting in substantial loss in grain yield (Sally *et al.*, 2007). The larvae remain protected inside the stems, and thus, are less vulnerable to insecticides and natural enemies (Muhammad *et al.*, 2009). *Busseola fusca* reduce grain yield by more than 15 % depending on the pest population density at the time of attack, crop age, and variety (Karaya *et al.*, 2009). Generally, studies on insect resistance have lagged behind disease resistance due to the extensive use of pesticides and the complex nature of insect-host plant resistance interactions (Songa *et al.*, 2001). In Kenya, much attention has been accorded to *C. partellus*, an introduced pest from Asia consequently neglecting other indigenous economically important stem borers. *C. partellus* is highly invasive and persistent and has been observed to expand its distribution from warm low lands to moist mid altitudes zones of Kenya. Research on management of *C. partellus* and *B. fusca* in sorghum has mainly focused on cultural control, predominantly intercropping, fertilizer use, and recently genetic engineering (Markus and Gurling, 2006; Amsalu *et al.*, 2008).

This implies that host plant resistant has not been fully exploited as a method of stem borer control. Other *B. fusca* management components are host plant resistance, biological control, synthetic pheromones, and chemical insecticides (Songa *et al.*, 2001; Amsalu *et al.*, 2008). However, many sub-Saharan farmers have not adopted majority of these methods owing to their impracticability and cost in-effectiveness. A major component of integrated pest management strategy in cereals is the use of host plant resistance (Odiyi, 2007). Host plant resistance is an effective, economical and environmentally friendly approach to manage insect pests and diseases (Karaya *et al.*, 2003). Painter, (1951) recognized three mechanisms of resistance namely antibiosis, non-preference (antixenosis) and tolerance. Antibiosis refers to a situation where the plant exerts adverse influences on growth and survival of the insect. Antibiosis expressed in terms of larval mortality, slow growth, and delayed development is an important component of resistance to stem borers in sorghum (Kumar *et al.*, 2005).

Non preference is where the plant exerts adverse effects on the insect's behavior. Waxes protect plants against desiccation, insect predation, disease and may also physically prevent the movement of an insect across leaf surface. Trichomes affect stem borers behavior by providing a barrier that prevents the insects from landing on the plant, prevent movement and feeding (Muhammad *et al.*, 2009). Tolerance or recovery resistance is where the plant is capable of supporting, without loss of yield or quality, a population of insect pests which would damage a susceptible variety (John *et al.*, 1994). Tiller production in sorghum following damage to the main plant by stem borers is a component of recovery resistance (Kishore *et al.*, 2007). Efforts are continuing to identify sources of stem borer resistance, but high levels of resistance have not been reported (Kfir *et al.*, 2002; Singh *et al.*, 2011). The objective of this study was to identify sorghum sources of tolerance to the African stem borer.

Materials and methods

Experimental site

Evaluation of sorghum genotypes for tolerance against *B. fusca* was conducted in Nairobi at the University of Nairobi, Kabete campus field station. The experiments were carried out in 2010 during long and short rainy seasons. Kabete lies at latitude of 1⁰ 15" South and longitude of 36⁰ 44" East at an altitude of about 1940 meters above mean sea level (Franzel *et al.*, 1999). The

long rains fall from March to June, while the short rains occur from October through December. Daily maximum temperatures range between 16⁰ - 23⁰C (Franzel *et al.*, 1999).

Experimental material and design

Seven East African commercial sorghum cultivars and twenty introduced cultivars from India were used in this study as shown in Table 3.1.

Table 3.1: List of sorghum genotypes evaluated for tolerance to *B. fusca*

Genotype name	Pedigree	Origin
ICSA 464	[(ICSB 11 x ICSV 702) x PS 19349B]5-1-2-2	India
ICSB 464	[(ICSB 11 x ICSV 702) x PS 19349B]5-1-2-2	India
ICSA 467	[(ICSB 11 x ICSV 700) x PS 19349B] x ICSB 13]4-1	India
ICSB 467	[(ICSB 11 x ICSV 700) x PS 19349B] x ICSB 13]4-1	India
ICSA 472	(ICSB 51 x ICSV 702)7-3-1	India
ICSB 472	(ICSB 51 x ICSV 702)7-3-1	India
ICSA 473	(ICSB 102 x ICSV 700)5-2-4-1-2	India
ICSB 473	(ICSB 102 x ICSV 700)5-2-4-1-2	India
ICSA 474	(IS 18432 x ICSB 6)11-1-1-2-2	India
ICSB 474	(IS 18432 x ICSB 6)11-1-1-2-2	India
IS 21879	IS 21879	India
IS 21881	IS 21881	India
IS 27329	IS 27329	India
Swarna	Swarna	India
DJ 6514	DJ 6514	India
Tam 2566	Tam 2566	India
IS 2205	IS 2205	India
IS 1044	IS 1044	India
ICSV 700	(IS 1082 x SC 108-3)-1-1-1-1-1	India
IS 8193	IS 8193	India
Seredo	Seredo	Kenya
Kari Mtama - 1	Kari Mtama - 1	Kenya
Gadam	Gadam	Kenya
Macia	Macia	Kenya
IESV 91104 DL	IESV 91104 DL	Kenya
IESV 91131 DL	IESV 91131 DL	Kenya
IESV 93042 SH	IESV 93042 SH	Kenya

The reason for including exotic sorghum materials was to assess if they possess some level of resistance to this borer. Macia, Kari Mtama – 1 and Gadam have good processing qualities for making beer by Kenya Breweries Company. Along with Seredo, these varieties are preferred by the farmers, and their grain and stover are utilized for food and feed, respectively. The rest of the genotypes evaluated are breeding materials from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The test material was sown in an α -lattice design, consisting of nine plots in three blocks, replicated twice. The rows were 2 m long and 0.75 m apart, and the

spacing between plants within rows was 0.25 m. Recommended practices were followed to raise the crop. First instar neonates of *B. fusca* used in this study were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. For each sorghum cultivar, at 30 days after sowing, five plants in each row were artificially infested with five larvae/plant using a camel hairbrush. To avert drowning of larvae in the water held in leaf whorls, sorghum seedling whorls were tapped gently before infestation. Infestation was carried out early in the morning to encourage larval survival.

Parameters evaluated

Stem borer damage in plants

The observations on leaf damage were recorded on per plant basis at two and four weeks after artificial infestation. Percentages of plants with leaf damage were computed by expressing the number of plants showing pinholes damage as a percentage of the total number of plants sampled. Five plants within each row were tagged to indicate the plants for infestation and data was taken systematically from these five marked plants. Observations on deadheart were recorded at 2 and 4 weeks after infestation (Kumar *et al.*, 2005). Deadheart incidence was computed by expressing the number of plants showing deadheart as a percentage of the total number of plants sampled.

The tagged five plants within each row that were artificially infestation were monitored and data was taken systematically from the five marked plants. At harvest, the number of stem borer exit holes on the stem was counted on each of the five sampled plants. The main stem of plants infested with *B. fusca* larvae were split open from the base to the apex, and the cumulative tunnel length measured in centimeters. Susceptibility parameters (leaf damage, deadheart incidence, stem tunneling and exit holes) were employed to define the reaction of the sorghum genotypes to *B. fusca*. A selection index based on the four damage parameters considered that is, leaf damage, deadheart incidence, stem tunneling and exit holes was computed by adding the ratios between the genotypic values and the overall mean, and dividing by 4 (number of damage parameters considered) (Tadele *et al.*, 2011).

Morphological traits

These are characteristics of each genotype expressed independent to infestation. For these traits, sampling was done systematically and data was recorded from the five tagged sorghum plants within each row. Leaf toughness was measured at 45 days after sowing using a penetrometer (Model FT011, ALFOSINE-Italy). The penetrometer was fitted with a 1-mm diameter tip, which was positioned on the leaf lamina, and the peak force required to puncture the leaf blade was recorded. Sorghum leaves from each of the five tagged plants were assessed for hardness using the device mentioned above. The 5th leaf from the base of each of the tagged plants was selected and 5 regions on the leaf lamina randomly pierced using the penetrometer and the peak force recorded. Leaf trichome density was measured on the 5th leaf from the base of each the five tagged plants in each genotype at 30 days after sowing (Dhillon, 2005). The leaf samples were well labeled, separated from the main plant, and placed in plastic bags in a cool box for observations in the laboratory. In each leaf sample was removed from the plastic bag and put on a clean bench and was measured each at a time.

A cork borer with a diameter of 1 cm was used to cut randomly 5 samples of leaf discs on each leaf lamina of the harvested leaves. The leaf samples were observed under a dissecting microscope (Model S111Z, England). The number of trichomes on the adaxial (upper) surface of each 1 cm diameter leaf disc sample were counted and expressed as trichome density/cm². Leaf glossiness was recorded at 30 days after sowing on a scale of 1 - 5 where 1 = highly glossy, 3 = moderately glossy, and 5 = non glossy (Sharma and Nwanze, 1997). Seedling vigor was scored at 30 days after sowing on a scale of 1 – 5, where 1 = low vigor (plants showing minimum growth, less leaf expansion and poor adaptation; 3 = moderate vigor; 5 = high vigor (tall plants with expanded leaves and robustness (Kishore *et al.*, 2007). Bloom waxiness was recorded on a scale of 1 – 9, where 1 = no bloom, 3 = slightly present, 5 = medium, 7 = mostly bloomy, 9 = completely bloomy at 50% flowering (Dhillon, 2004). At physiological maturity, plant height was measured in centimeters from the base of the plant to the tip of the panicle.

Plant color was visually assessed on the leaf sheath on a scale of 1 – 2 where 1 = tan and 2 = pigmented. Time to panicle emergence was recorded as the number of days from the date of sowing to the date of panicle emerged in a plot. Time to flowering was recorded as the number

of days from the date of sowing to the date of anthesis of plants in a plot. Fifty percent flowering was considered since it is a distinct stage in flowering plants and in sorghum panicles flower from the top. The number of tillers with harvestable panicles was recorded on each plant sampled. After harvest, sorghum panicles were sun-dried and hand threshed. Total grain yield and hundred-grain mass were recorded for each of the sampled plants using a weighing balance (Mettler PM 6000, CH- 8606 GREIFENSEE-ZURICH, made in Switzerland).

Statistical analysis

Data on percentages was arcsin transformed while that of counts was log transformed before analysis of variance (Kishore *et al.*, 2007). The computed mean values of all the traits for each replicate were used to compute the analysis of variance (ANOVA 1) using Genstat statistical software (Genstat Release 12 Reference Manual, Part 1 Summary, 2009, VSN International, Hemel Hempstead HP1 1ES, United Kingdom). Treatment means were compared using a protected Fishers' least significant difference test at $P = 0.05$. Pearson's correlation coefficients were computed to determine association between morphological characteristics with traits measuring plant reaction to *B. fusca* infestation.

Results

Damages caused by *Busseola fusca* infestation

Results of damage parameters caused by *B. fusca* is presented in Table 3.2. The analysis of variance indicated highly significant ($P = < 0.01$) differences in leaf damage, deadheart, stem tunneling, exit holes, panicle length, dry panicle weight, total grain yield and hundred grain mass among the genotypes tested. Genotypes that showed low leaf damage during the first and second sampling period were ICSA 474, IS 27329 and IS 2205. Genotypes that suffered low deadheart damage were ICSA 467, ICSA 472, ICSA 473, IS 2205 and IESV 91131 DL.

Table 3.2: Reaction of sorghum genotypes to *Busseola fusca* at Kabete, Kenya during 2010 rains

Genotype	DH		LD		stem	No. of	Selection	Cate gory ²	Plant	Panicle	Dry panicle	Grain	100 grain
	1(%)	2 (%)	1 (%)	2 (%)	tunnel (cm)	exit holes	index ¹		height (cm)	length (cm)	weight (gm)	yield (gm)	mass (gm)
ICSB 467	13	20	45	51	0	0	0.4	R	113.4	20.9	35.5	24.8	2
ICSA 473	0	27	13	39	0.8	0.6	0.4	R	99.6	16	8.9	2	0.2
Macia	13	13	39	33	1.7	0	0.5	R	101.1	17.9	32.8	18	2.9
IS 21881	27	33	27	20	0	0	0.5	R	91.3	20.9	40.7	25.2	2.5
ICSB 464	13	31	33	20	1.4	0.5	0.5	R	85.6	15.6	51.8	36.2	3.1
Gadam	13	33	39	25	0	0	0.5	R	101.2	16	28.1	18.8	1.6
IS 2205	0	0	27	0	11.2	4.3	0.3	R	173.5	15.6	39.1	26.1	2.2
IS 1044	0	13	20	13	9.9	4	0.5	R	153.2	21.4	76.3	49.4	4.1
IESV 91131 DL	0	27	25	39	1.4	1.2	0.5	R	86.6	19.5	32.4	19.2	2.4
IESV 91104 DL	33	33	39	39	1.5	0.5	0.6	MR	136.7	18.2	62.6	47.4	3.4
ICSA 472	0	36	28	35	2.8	1.3	0.7	MR	151.7	14	7.2	3.8	1.7
ICSA 474	22	22	0	0	1.7	0.8	0.5	R	114.7	14.3	24.2	9.4	1.2
Seredo	33	33	27	33	1.1	0.3	0.7	MR	117.9	20.1	46.2	28.1	3.4
ICSB 474	18	27	39	28	1.5	1	0.7	MR	168.9	17.9	76.1	50.2	4.7
ICSA 464	39	28	57	48	4	0.4	0.9	MR	104.2	20	15.1	5.4	0.8
Kari Mtama - 1	13	27	39	26	6.4	1.4	1	MS	124.3	26.5	65.7	49.4	4.1
IESV 93042 SH	0	13	57	39	5.5	3	1.2	MS	138.5	18.9	57.4	42	3.9
IS 21879	33	25	13	20	4.5	2.5	1.2	MS	120.4	17.7	77.1	41.5	1.5
DJ 6514	57	51	27	33	4.1	2.1	1.4	MS	122.3	15.6	44.5	28.2	1.2
Tam 2566	13	39	39	13	7.6	4.3	1.6	S	77.6	17.7	32.9	21.8	2.7
Swarna	13	13	20	20	10.3	4.9	1.8	S	115	20.7	37.4	28.3	2.6
IS 8193	13	20	39	45	10.8	4.7	1.9	S	135.7	20.1	89.9	60.8	3.1
LSD	29.8	32.0	27.2	31.1	5.02	2.56	0.92		34.65	4.91	21.18	16.76	0.9
(P=0.05)	0.002	0.056	<.001	0.051	<.001	<.001	0.04		<.001	<.001	<.001	<.001	<.001
CV	14.6	15.7	13.4	15.3	71	65.9	47.3		62	42.5	73.8	89.6	1.6

DH 1(%) = percent deadheart at 44 days after sowing, DH2 (%) = percent deadheart at 58 days after sowing, LD 1 (%) = percent leaf damage at 44 days after sowing, LD2 (%) = percent leaf damage at 58 days after sowing.
¹Selection index was calculated based on leaf damage, deadheart, exit holes and stem tunneling damage;²R = Resistant, MR = moderately resistant, MS = Moderately susceptible, S = Susceptible. Genotypes with selection index values less than 1.0 were regarded as resistant and those with a selection index greater than 1.0 as susceptible (Adopted from Bergvinson *et al.*, 2004; Tadele *et al.*, 2011).

Genotypes that suffered low stem tunneling damage were ICSA 473, Seredo, ICSB 464 and IESV 91131 DL. The highest stem tunneling damage was observed in Swarna, IS 8183 and IS 2205. Genotypes with fewer exit holes were Seredo, ICSA 464 and ICSB 464 than Tam 2566, IS 8193 and Swarna. Plant height ranged from 181 cm to 78 cm on ICSV 700 and Tam 2566 with an average of 124 cm. Longer panicles were observed on IS 27329, Kari Mtama - 1, ICSA 467 and IS 1044 while IS 2205, ICSA 474 and ICSA 472 had shorter panicles. Highest and lowest dry panicle weight was recorded on ICSB 472 and Gadam respectively with an average of 46 grams. High grain yield (> 49 grams) were observed on IS 8193, ICSB 472, ICSB 474 and IS 1044 while lower grain yield was recorded on IESV 91131 DL, Gadam, Macia and IS 27329 (< 20 grams). Genotypes with high hundred grain mass were ICSB 474, Kari Mtama - 1 and IS 1044 compared to IS 21879, ICSB 473 and DJ 6514. Based on the selection index, 26% of genotypes evaluated were categorized as resistant, 30% as moderately resistant, 33% moderately susceptible and 11% as susceptible.

Morphological traits characterizing the sorghum genotypes during *B. fusca* infestation

The results of morphological traits characterizing the sorghum genotypes during *B. fusca* infestation are presented in Table 3.3. Leaf glossiness, seedling vigour, leaf toughness, trichome density and tillering varied significantly ($P = \leq 0.01$) among the genotypes tested. Leaf glossiness ranged from 1 to 4 (where 1 = highly glossy, 3 = moderately glossy and 5 = non-glossy). Genotypes Tam 2566, Swarna and Kari Mtama - 1 were non-glossy, while ICSA 474, ICSA 464, ICSA 472, their respective maintainer lines, ICSV 700 and IS 1044 were highly glossy. Seedling vigor scores ranged from 2 to 4 [where 1 = low vigor (plants showing minimum growth, less leaf expansion and poor adaptation), 3 = moderate vigor, and 5 = high vigor (plants showing maximum height, leaf expansion and robustness)].

Table 3.3: Morphological traits governing resistance to *Busseola fusca* in 27 sorghum genotypes evaluated in 2010 rains at Kabete, Kenya

Genotype	Leaf glossiness ¹ score (1-5)	Seedling vigour ² score (1-5)	Leaf toughness (Kg force)	Trichome density (No./cm ²)	Bloom waxiness ³ score (1-9)	Number of tillers
DJ 6514	4	2	0.10	0.2	6	0
ICSA 472	2	2	0.09	4.1	5	1
ICSA 473	3	2	0.08	6.7	7	2
ICSA 474	1	2	0.07	6.8	6	2
IS 27329	2	2	0.09	0.1	4	2
Gadam	3	3	0.07	3.4	6	2
ICSA 464	2	3	0.15	0.6	7	1
ICSB 464	2	3	0.10	0.1	6	1
ICSB 467	3	3	0.07	2.1	7	1
ICSB 473	4	3	0.10	0	6	1
ICSB 474	2	3	0.10	1.9	7	2
ICSV 700	2	3	0.10	2.1	6	2
IESV 91131 DL	4	3	0.07	1.4	7	0
IS 21879	2	3	0.08	0.2	7	0
IS 2205	2	3	0.10	0	5	4
Macia	3	3	0.09	3.6	5	1
Seredo	3	3	0.05	3.6	6	2
Swarna	4	3	0.09	0.3	6	1
Tam 2566	4	3	0.09	7.2	6	1
ICSA 467	4	4	0.09	10.5	6	4
IESV 91104 DL	4	4	0.10	10.1	6	1
IESV 93042 SH	3	4	0.09	0.6	6	1
IS 1044	2	4	0.09	0.2	5	2
IS 8193	3	4	0.09	2.5	6	3
Kari Mtama - 1	4	4	0.07	1.1	7	1
LSD	1.366	1.346	0.077	1.517	2.463	1.134
(P = 0.05)	<.001	<.001	<.001	<.001	NS	<.001
CV (%)	24.1	3	4.7	5.5	5.2	6

¹Leaf glossiness scale of 1 – 5, where 1 = highly glossy, and 5 = non-glossy; ²Seedling vigor scale of 1 – 5, where 1 = low vigor and 5 = high vigor; ³Bloom waxiness scale of 1 – 9, where 1 = no bloom, 3 = slightly present, 5 = medium, 7 = mostly bloomy, 9 = completely bloomy at 50% flowering

Genotypes ICSA 467, IESV 93042 SH, IS 1044, Kari Mtama - 1 and IS 8193 had greater vigor scores (i.e., were more vigorous) than ICSA 472, ICSA 474 and DJ 6514. Greatest leaf toughness (≥ 0.1 kg) was recorded on ICSA 464, IS 2205, ICSB 464, DJ 6514, IESV 91104 DL, ICSV 700 while the least force (< 0.05 kg) was recorded on Seredo and IS 21881. Genotypes

ICSA 467, IESV 91104 DL, Tam 2566, ICSA 474 and ICSA 473, had high density of trichomes while ICSB 473, IS 2205, ICSB 464 and IS 27329 were essentially trichomeless. Bloom waxiness was not significantly different among genotypes evaluated.

Correlations between morphological and damages parameters

The results of correlations between morphological and damage parameters are presented on Table 3.4. Positive correlations between hundred grain mass and seedling vigor score ($r = 0.6$) ($P = 0.002$), hundred grain mass and grain yield per plant ($r = 0.6$) ($P = 0.002$), hundred grain mass and dry panicle weight ($r = 0.6$) ($P = 0.004$) and, hundred grain mass and deadheart damage ($r = -0.5$) ($P = 0.03$) were highly significant. Highly significant positive correlations was observed between grain yield and leaf damage ($r = 0.5$) ($P = 0.03$), grain yield and plant height ($r = 0.6$) ($P = 0.007$) and, grain yield and seedling vigor ($r = 0.6$) ($P = 0.002$). Significant positive correlations were observed between bloom waxiness and grain yield ($r = 0.4$) ($P = 0.09$) and, bloom waxiness and leaf damage ($r = 0.5$) ($P = 0.03$).

Table 3.4: Correlation of means between between morphological and damages parameters

100 gm																		
FL	-0.1																	
BW	0.2	0.2																
DH	-0.5*	-0.2	-0.2															
DPW	0.6*	0.3	0.3	-0.2														
TGY	0.6*	0.2	0.4*	-0.3	0.9*													
LD	0.3	0.1	0.5*	-0.4	0.3	0.5*												
LG	-0.2	-0.3	0.3	0.2	-0.4*	-0.3	0.1											
LT	0.1	0.3	-0.3	-0.1	0.2	0.3	-0.1	-0.3										
EH	0.1	0.1	0.0	0.1	0.3	0.3	0.0	0.0	0.4*									
PL	0.1	-0.4*	-0.2	0.1	-0.1	-0.1	-0.5*	0.2	-0.4*	-0.1								
PH	0.4*	0.3	-0.2	-0.1	0.6	0.6*	-0.1	-0.6*	0.5*	0.3	0							
VG	0.6*	-0.2	0.3	-0.5*	0.5	0.6*	0.4*	0.1	-0.1	0.3	0.2	0.1						
SI	0.0	0.0	-0.1	0.3	0.3	0.3	-0.1	0.0	0.4*	1.0	-0.1	0.3	0.2					
TL	0.2	-0.3	-0.3	0.0	0.1	0.1	-0.1	-0.4*	0.1	0.3	-0.1	0.5*	0.2	0.3				
TD	0.2	-0.1	0.1	0.0	0.0	0.0	0.1	0.3	-0.1	-0.2	-0.1	-0.2	0.3	-0.2	-0.1			
ST	0.2	-0.1	-0.1	0.0	0.3	0.4*	0.0	0.0	0.3	1.0	0.0	0.3	0.4*	0.9*	0.4*	-0.2		
100 gm	FL	BW	DH	DPW	TGY	LD	LG	LT	EH	PL	PH	VG	SI	TL	TD	ST		

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

100 gm = hundred grain mass, FL = days to 50% flowering, BW = bloom waxiness, DH = deadheart damage, DPW = dry panicle weight, TGY = total grain yield, LD = leaf feeding damage, LG = leaf glossiness, LT = leaf toughness, EH = exit holes, PL= panicle length, PH = plant height, VG= seedling vigor, SI = selection index, TL= tillering, TD = trichome density, ST = stem tunnel damage

Negative correlations were observed between days to 50 % flowering and panicle length ($r = -0.4$) ($P = 0.04$). Negative relationship existed between leaf glossiness score and number of tiller ($r = -0.4$) ($P = 0.04$), and plant height ($r = -0.6$) ($P = 0.03$). There was positive correlation between tillering and stem tunneling ($r = 0.4$) ($P = 0.05$). Significant positive relationship was observed between seedling vigor score and stem tunneling ($r = 0.4$). A negative correlation was observed between deadheart damage and seedling vigor ($r = -0.5$) ($P = 0.01$) and leaf damage ($r = -0.4$) ($P = 0.03$). A significant correlation was observed between plant height and tillering ($r = 0.4$) ($P = 0.008$). A positive relationship existed between leaf damage and seedling vigor ($r = 0.4$) ($P = 0.06$), leaf damage and panicle length ($r = -0.5$) ($P = 0.03$). A highly significant relationship was observed between selection index and stem tunneling ($r = 0.9$) ($P = <0.001$). A significant correlation was observed between exit holes and selection index ($P = <0.001$) and exit holes and stem tunneling ($P = <0.001$).

Direct and indirect relationships by path coefficient analysis

The results of the direct and indirect relationships of damage parameters to grain yield are presented in Table 3.5. The relative contributions of the damage parameters to grain yield loss were partitioned into direct and indirect relationships by path coefficient analysis with grain yield being the resultant variable. Deadheart exerted a significant positive direct effect on grain yield supported by negative indirect effects through exit holes and stem tunneling. Number of exit holes damage had a positive effect on grain supported by negative indirect effects on leaf damage and stem tunneling. Leaf damage had a significant positive direct effect on grain yield supported by negative indirect effects on exit holes. Stem tunneling had a positive direct effect on grain yield supported by negative indirect effect on exit holes.

Table 3.5: Path analysis on direct and indirect effects of *B. fusca* damage parameters on sorghum grain yield

Character	Correlation with grain yield	Direct effect	Indirect effect via			
			DH	EH	LD	ST
DH	-.088*	37.5**	-	-.099	0.0076	-.0018
EH	-.088*	0.58	0.0118	-	-.0026	-.0015
LD	-.088*	50.2**	0.0008	-.014	-	0.008
ST	-.088*	1.69	-.057	-.0096	0.0246	-

DH = deadheart, EH = exit holes, LD = leaf damage and ST = stem tunneling
 *, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

Discussion

This study identified sorghum genotypes with resistance to *B. fusca* based on reduced leaf damage, deadheart, stem tunneling and exit holes following artificial infestation of seedling whorls with stem borer neonates. The mechanism of this resistance to stem borer damage was mainly tolerance since some genotype produced substantial grain yield after supporting high leaf damages, deadheart and stem tunnelling damages. Several studies have reported antixenosis and antibiosis to stem borers in sorghum (Kumar *et al.*, 2006; Singh *et al.*, 2011). Multiple stem borer damage were considered because resistance to stem borers is a multi-mechanism quantitative trait, and thus, selecting for resistance based on a single parameter would not be effective (Singh *et al.*, 2011). Sorghum resistant to *C. partellus* based on reduced deadheart damage reported (Sharma *et al.*, 2006). Genotype IS 2205, previously reported resistant to *Chilo partellus* and *Busseola fusca* in sorghum was also found be resistant in this study (Chinwada *et al.*, 2001; Sharma *et al.*, 2007) . This implies that the genotype IS 2205 is not influenced by genotype by environment interactions thus can be used as resistant check in screening trials.

Sorghum genotypes with highly glossy, non-vigorous seedlings suffered lower damage compared to the non-glossy but highly vigorous ones. This implies that high leaf glossiness and seedling vigor could be used as morphological markers to select for stem borer damage. Similar observations have earlier been reported for sorghum shootfly, wherein highly glossy lines were resistant to shoot fly attack (Dhillon *et al.*, 2005). A similar finding that highly trichomed and vigorous maize genotypes suffered less deadheart compared to less vigorous and trichomless

maize has been reported in maize against *C. partellus* (Muhammad *et al.*, 2009). Bloom waxiness influences insects' attachment, movement and feeding (Sanford and Reinhard, 2002). The positive correlation observed between grain yield and plant height implied that tall genotypes yielded significantly more than short genotypes. Under the artificially-infested conditions of this trial, sorghum genotypes with poor seedling vigor (i.e. low seedling vigor scores) tended to have lower grain yields than those with highly vigorous seedlings perhaps because the whorls of the less vigorous genotypes provided a better environment for the stem borer larvae. In addition, vigor meant more photosynthates were available for grain filling. Plants with low seedling vigor rates also had poor adaptation in the study site and this may have contributed to low grain yield among such sorghum genotypes.

Plants with high seedling vigor scores (i.e., highly vigorous seedlings) suffered less deadheart damage and were high yielding. This implies that improving seedling vigor could lead to development of sorghum genotypes that are more tolerant to *B. fusca* damage. This conforms to observations that vigorous maize plants suffered less damage by *Eldana saccharina* and *Sesamia calamistis*, and were high yielding (Odiyi, 2007). Seedling vigor as a result of lack of damage to seedlings is highly correlated with shoot fly resistance in sorghum as reported by Dhillon *et al.* (2005). Sorghum genotypes with high leaf toughness suffered less deadheart possibly because of poor palatability of such genotypes to the borer larvae. The leaf tissues may have been tough for the borer's mandibles. Leaf toughness is positively correlated with resistance to stem borer attack in maize (Bergvinson, 2002). An inverse correlation existed between deadheart incidence and hundred grain mass implying significant and direct negative relationship between these two direct measures of stem borer damage. This could be ascribed to the fact that deadheart causes the death of growing part of the plant and severely affected plants do not produce panicles. The affected genotype may tiller and this predisposes the tillers to other insect pests that come later in the season and may not produce harvestable panicles.

Trichomes are known to hinder movement of insect pests in many host plants. Most of the tolerant genotypes in this study possessed more trichomes, were highly glossy, and were more vigorous than the susceptible genotypes. Leaf trichomes is among the most important traits contributing resistance against stem borers in maize by deterring stem borer feeding and

oviposition (Muhammad *et al.*, 2009). High levels of resistance to shoot fly were identified when both glossy and trichome traits occurred together (Dhillon *et al.*, 2005; Aruna and Padmaja, 2008). There were positive and significant correlations between grain yield, leaf damage and stem tunneling. Leaf damage and stem tunneling may not have been severe to disrupt photosynthesis and translocation of nutrients and assimilates and thus did not influence grain yield. Grain yield was measured on plot basis and there might have been tendency to compensate by other plants which utilized more of the resources.

Leaf feeding and deadheart have been reported not to lead to significant reduction in maize grain yield due to stem borer damage (Odiyi, 2007). In the present study, deadheart significantly contributed to substantial grain yield loss. This could be explained by the fact that deadheart resulted in destruction in of apical shoot of the main stem thus production of unproductive tillers. Sorghum genotypes with high seedling vigour scores (i.e., high vigor) had high grain yield per plant under the conditions of this artificially-infested trial. A positive and significant correlation between grain weight and plant height implied that tall genotypes yielded significant more than the dwarf genotypes.

Conclusion and recommendation

Sorghum genotypes tolerant to *B. fusca* infestation were identified in this study based on reduced leaf damage, deadheart damage, exit holes and stem tunneling. Seedling vigour and trichome density could be used as morphological markers to select for sorghum resistance to the African stem borer. These identified sorghum genotypes can be useful in development of new cultivars tolerant to the African stem borer.

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CHAPTER 4

Sources of Resistance to Spotted Stem Borer in Sorghum

Introduction

Sorghum production especially in tropical Africa is curtailed by a number of important anthropod pests, with the stem borers belonging to Lepidoptera playing the most significant role. There are two groups of stem borers that infest sorghum, indigenous species such as African stem borer *Busseola fusca* (Fuller) and *Sesamia calamistis* Lepidoptera Noctuids and exotic ones such as the spotted stem borer *Chilo partellus* (Swinhoe) Lepidoptera Crambid introduced from Asia (Sharma *et al.*, 2005). *Chilo partellus* is reported the most important pest in East Africa and many countries of sub-Saharan Africa accounting over 49% yield losses (Seshu Reddy, 1998; Songa *et al.*, 2001). *Chilo partellus* is highly invasive, and has partially displaced some indigenous stem borers in Kenya attacking all cereals (Kfir *et al.*, 2002). Damage symptoms of *C. partellus* in sorghum include leaf feeding, deadheart, exit holes, stem tunnels and chaffy grain in case of extensive stem tunneling and peduncle damage (Jose *et al.*, 2001; Kishore *et al.*, 2007; Sally *et al.*, 2007).

Cultural pest management practices such as early planting, destruction of stover, biological control, developing insect-resistant cultivars, and the use of chemical insecticides are being used (Ofomata *et al.*, 2000; Rwomushana, 2005; Sharma *et al.*, 2006). Push and pull technology is a relatively new cultural technique of managing stem borers where by a repellent crop, in this case desmodium *spp* (Fabaceae) is planted around the cereal crop while napier grass (*Pennisetum Purpureum*) is utilized as a trap plant to the borers (Zeyaur *et al.*, 2007). The efficacy of pesticides is however limited especially when the larvae are feeding inside the stalks (Kfir *et al.*, 2002). Thus host plant resistance to insects remains the most viable option to manage the pest. Several sorghum accessions have been screened for resistance to *C. partellus*, and several sources of resistance identified, but the resistance levels range from low to moderate (Sharma *et al.*, 2003, 2006; Singh, 2011). Therefore, it is important to identify sorghum genotypes with higher levels of resistance with diverse mechanisms of resistance to diversify the bases of resistance to this pest.

Progress in breeding for resistance to this pest has been slow due to the complex inheritance of the trait and the strong influence of environmental factors on expression of resistance to stem borers. Screening for resistance to stem borer under natural conditions is ineffective because of non-uniform pest pressure over time and space, and thus, it is necessary to employ artificial infestation to identify sources of resistance to this pest (Songa *et al.*, 2001). Several other authors have screened sorghum under artificial infestation and genotypes with varying levels of resistance identified (Marulasiddesha *et al.*, 2000; Sharma *et al.*, 2006; Singh, 2011). Improvement for resistance to *C. partellus* requires identification of new sources of resistance to diversify the bases of resistance to this pest (Songa *et al.*, 2001). Therefore, the present studies were undertaken to identify new sources of resistance to *C. partellus*.

Materials and methods

Experimental site

Experiments to evaluate sorghum genotypes against *C. partellus* damage were conducted at the Kenya Agricultural Research Institute (KARI), Kiboko, Kenya. Kiboko is located at an elevation of 975 m above sea level with average minimum and maximum daily temperatures of 14.3⁰C and 35.1⁰C, respectively, with an overall annual mean temperature of 24⁰C (Franzel *et al.*, 1999). The long rains are received from March to June with a seasonal mean of 233 mm, while the short rains are more reliable and are received between October to January, with a seasonal mean of 328 mm (Franzel *et al.*, 1999). Spotted stem borer is known to result in severe damage to the crop thus the basis upon which this site was selected for the study (Sharma *et al.*, 2007).

Experimental material and design

Seven East African popular commercial cultivars and 20 introductions from India were evaluated during the long and short rainy seasons in 2010 as described in Chapter 3 of this thesis. At 30 days after planting, five plants in each row were artificially infested in the whorl with five larvae/plant using a camel hairbrush. To avert drowning of larvae in the water held in leaf whorls, sorghum seedling whorls were tapped gently before infestation. Infestation was carried out early in the morning or late in the evening to encourage larval survival.

Parameters measured

The observations were recorded on per plant basis at two and four weeks after artificial infestation. Leaf feeding damage was expressed as the number of plants showing pinhole damage as a percentage of the total number of plants sampled. Deadheart incidence was computed by expressing the number of plants showing deadheart damage as a percentage of the total number of plants sampled. At harvest, numbers of stem borer exit holes on the stem were counted on each sampled plant. The main stem of plants infested with *C. partellus* larvae were split open from the base to the apex, and the cumulative tunnel length measured in centimeters.

Numbers of larvae recovered alive or dead after cutting the stems longitudinally were also recorded. Bloom waxiness was recorded on a scale of 1 - 9, where 1= no visible bloom, 3 = slightly present, 5 = medium, 7 = mostly bloomy, 9 = completely bloomy at the 50% flowering stage (Dhillon, 2005). At physiological maturity, plant height was measured from the base of the plant to the tip of the panicle. At the same time, plant color based on leaf sheath coloration was visually assessed on a scale of 1 - 2 where 1= tan and 2 = pigmented. Days to panicle emergence was recorded as the number of days from the date of planting to the date of panicle emergence in 50% of plants in a plot. Days to 50% flowering was recorded as the number of days from planting to anthesis of 50% of the plants in a plot. The number of tillers with harvestable panicles was recorded on each plant.

Brix is a measure of the level of soluble solids in the sorghum juice (Nibouche and Tebere, 2008). All genotypes were harvested at the same time and sugar brix was measured using a hand held refractometer (Reichert Inc, 3362 Walden Avenue Depew NY 14043 made in Japan). The cane was squeezed by hand and the juice dispensed on the sample area of the refractometer. Samples were tested each at a time and the sample dip area was cleansed using methylated spirit to avert sample contamination. After harvest, sorghum panicles were sun-dried and threshed. Grain yield and hundred grain mass were measured for each of the 5 plants sampled using an electronic balance (Mettler PM 6000, CH- 8606 GREIFENSEE-ZURICH, made in Switzerland).

Statistical analysis

Data on percentages was angular transformed while that of counts was log transformed before analysis of variance. The mean values of all the traits for each replicate were used to compute the analysis of variance using Genstat (10th version). Treatment means were compared using a protected Fishers' least significant difference (LSD) test at $P = 0.05$. A borer damage selection index was calculated based on leaf damage, deadheart, stem tunneling and exit holes by adding the ratios between the values for each genotype from the overall mean for each parameter, and dividing by 4 (number of damage parameters considered). Genotypes were grouped into four categories namely resistant, moderately resistant, moderately susceptible and susceptible (Tadele *et al.*, 2011). Pearson's correlation coefficients were computed to determine the association of morphological characteristics with traits measuring stem borer damage.

Results

Relative susceptibility of different sorghum genotypes to *C. partellus* damage

Results of relative susceptibility of different sorghum genotypes to *C. partellus* damage are presented in Table 4.2. Susceptibility parameters deadheart, exit holes, stem tunneling damages varied significantly ($P = \leq 0.01$) among genotypes. Low deadheart damage was recorded in ICSV 930 SH, ICSB 472, ICSA 464 and ICSA 472 during the first sampling period. In addition to IESV 93042 SH, ICSA 472, IS 1044 (R), Seredo and ICSV 700 scored low deadheart damage in the second sampling period. Genotypes ICSA 472, IS 1044 (Resistant check), Seredo, ICSV 700 had low leaf damage during the first sampling period while Kari Mtama - 1, IESV 93042 SH, ICSA 464 and ICSB suffered low leaf feeding damage in the second sampling period.

The numbers of exit holes per plant in ICSA 472, ICSA 464, ICSA 473 and ICSV 700 were much lower than that of resistant check, IS 1044. Genotype IS 8193 suffered three times more damage compared to the resistant check IS 1044. Stem tunneling damage per plant was low in genotypes ICSA 472, ICSV 700, ICSA 464, ICSB 473, ICSB 472 and IESV 91131 DL (< 10 cm) as compared to Gadam, Seredo, and IS 8193 that suffered more than 24 cm long tunnels. IS 8193 suffered eight times more stem tunneling than the identified most resistant genotype ICSA 472. The stem borer damage index based on leaf damage, deadheart, exit holes and stem tunneling varied significantly.

The susceptibility index developed categorized genotypes into four groups namely resistant (26 %), moderately resistant (37 %), moderately susceptible (26 %) and 11 % as susceptible. In this scheme, some genotypes were better than the resistant check and it is noteworthy that some genotypes succumbed to *C partellus* damage distinctly more than the susceptible check. There were significant ($P = \leq 0.01$) differences in the numbers of larvae recovered either alive or dead among different genotypes. Dead stem borer larvae were recovered from ICSV 700, ICSB 474 and Gadam. The least number of larvae recovered alive were recorded on Kari Mtama - 1, ICSA 474, IS 1044 (R), ICSB 464 and ICSB 464. Genotypes that produced harvestable tillers were ICSB 473, IS 2205 and ICSA 464 produced highest number of productive tillers.

Table 4.2: Response of 27 sorghum genotypes to *C. partellus* damage during 2010 rain seasons at KARI Kiboko, Kenya.

Genotype					No. of exit holes	Stem Tunnel (cm)	larvae recovered alive	No. of larvae/plant recovered dead	No. of tillers /plant	¹ Selection index	Category	Plant colour
	DH 1	DH 2	LD 1	LD 2								
ICSA 473	20	5	25	40	2.8	10	1	0	0	0.6	R	T
ICSA 464	10	35	25	30	2.2	7.5	1	0	2	0.67	R	T
ICSV 700	20	20	20	55	2.3	6.6	1	3	0	0.7	R	T
ICSA 472	10	30	20	40	1.9	4.3	1	0	0	0.71	R	T
IESV 93042 SH	0	5	30	20	3.5	22.3	2	0	0	0.72	R	T
ICSB 473	20	20	25	35	3.4	8.6	1	0	1	0.72	R	T
ICSB 472	5	20	30	40	3	8.6	1	0	0	0.78	R	T
IS 1044 (R)	15	15	20	35	3.4	16.9	0	0	0	0.79	MR	P
Kari Mtama - 1	15	15	50	15	5	17.8	0	0	0	0.8	MR	T
ICSA 467	5	5	45	40	4.5	16.9	4	0	0	0.81	MR	T
Macia	15	25	45	50	3.4	13.7	2	0	0	0.84	MR	T
IESV91131 DL	40	35	50	60	2.4	9.9	1	0	0	0.85	MR	T
ICSB 464	35	45	25	25	3.1	11.3	0	0	0	0.87	MR	T
ICSB 474	20	30	45	65	6.3	20.4	0	5	0	1.23	MS	P
IESV91104 DL	55	60	50	40	5.8	14.9	2	0	0	1.27	MS	T
Seredo	15	45	20	40	6.3	24.8	2	0	1	1.3	MS	P
Tam 2566	25	35	55	65	7.1	18.8	3	0	2	1.3	MS	P
Gadam	15	35	50	45	6.5	24.3	2	5	0	1.3	MS	P
Swarna (S)	50	85	40	65	4.8	18.8	2	0	0	1.48	S	T
DJ 6514	50	75	15	55	5.9	24	0	0	1	1.53	S	T
IS 8193	20	25	65	60	9.5	34	3	0	1	1.62	S	P
LSD	29.6	33.66	36.82	38.12	2.508	8.041	1.726	0.4	0.819	0.402		
P=0.05	0.007	<.001	0.259	0.297	<.001	<.001	0.002	<.001	<.001	<.001		

DH 1(%) = percent deadheart at 44 days after sowing, DH 2 (%) = percent deadheart at 58 days after sowing, LD 1 (%) = percent leaf damage at 44 days after sowing, LD 2 (%) = percent leaf damage at 58 days after sowing, R = Resistant, Ms = Moderately Susceptible, MR = Moderately Resistant, S = Susceptible, T = Tan, P = Pigmented ¹Selection index was based on deadheart, leaf damage, exit holes and stem tunnels damage, Genotypes with selection index values less than 1.0 were regarded as resistant and those with a selection index greater than 1.0 as susceptible (Adopted from Bergvinson *et al.*, 2004; Tadele *et al.*, 2011).

Variation in morphological traits among different sorghum genotypes evaluated for resistance to *C. partellus*

Results of variation in morphological traits among different sorghum genotypes evaluated for resistance to *C. partellus* is presented in Table 4.3. Sugar brix level, plant height, days to 50 % flowering, waxy bloom, days to panicle emergence, agronomic score, panicle length, dry panicle weight, grain mass and a hundred grain mass varied significantly among the genotypes tested. Sugar brix levels ranged from 11.2 on IESV 91131 DL to 22.6 on ICSB 464, with an average of 17.2. The susceptible check, Swarna had a brix value of 19.1 while the resistant check, IS 1044 scored 16.7 brix. The tallest genotype, IS 27329 recorded 253.5 cm while the most dwarf, Tam 2566 reached 79 cm. Days to 50 % flowering ranged from 87 to 65 days on ICSV 700 and Gadam respectively. Bloom waxiness ranged from 2.8 (slightly present) to 6.5 (mostly bloomy), with an average mean of 4.6. Genotypes with highest bloomy wax included Swarna (susceptible check), Macia and Gadam in decreasing order while little bloom was recorded on the resistant check IS 1044 and IS 27329 in the same order.

Days to panicle emergence were longest for ICSV 700 with 87 days while Gadam took 58 days for the panicle to emerge. Agronomic score determined from a scale of 1-5 where 1 was poor while 5 excellent ranged from 3.6 - 2.5 on ICSA 474 and Seredo correspondingly. The longest (37 cm) and shortest (16 cm) panicles were recorded on genotypes IS 27329 and ICSA 472 respectively. The heaviest dry panicle on IESV 91131 DL weighed 4 times more than ICSA 472 which weighed the least. Grain weight ranged from 45.3 grams on IESV 91131 DL to 2.1 grams on ICSA 472 with an average of 20 grams. Highest hundred grain mass was recorded in Kari Mtama - 1, IESV 93042 SH, IS 27329 and Seredo in decreasing order. Despite the fact that Swarna was used as a susceptible check, it yielded one and a half times much as the resistant check, IS 1044.

Table 4.3: Agronomic traits of 27 sorghum genotypes evaluated for response to *C. partellus* damage during 2010 rain seasons at KARI Kiboko, Kenya.

Genotype	Sugar Brix	Plant height (cm)	Days to 50% flowering	Waxy bloom (1-9 scale)	panicle emergence	Agronomic score (1-5)	Panicle length	Dry panicle weight	Grain mass	100 Grain mass
Gadam	16.4	110.2	65.0	5.5	57.8	3.0	19.9	28.9	17.9	1.8
ICSA 464	20.5	151.8	79.0	3.5	68.0	3.5	26.7	15.8	3.8	0.1
ICSA 467	15.0	138.7	76.5	3.5	66.3	3.3	26.0	19.7	5.9	1.0
ICSA 472	16.2	193.2	81.0	5.0	68.3	3.5	16.1	13.6	1.8	0.2
ICSA 473	16.4	131.2	74.3	5.0	65.0	3.3	19.2	17.6	5.3	0.7
ICSA 474	17.0	189.5	74.0	4.5	64.0	3.6	22.9	21.3	3.8	1.0
ICSB 464	22.6	136.0	78.8	4.0	69.3	2.6	24.7	45.8	31.0	1.7
ICSB 467	17.8	145.9	74.0	4.0	64.8	3.0	26.0	36.5	23.7	2.1
ICSB 472	17.2	179.2	80.0	4.5	68.3	2.5	16.2	32.0	18.6	2.0
ICSB 474	18.3	188.0	74.8	4.5	65.0	2.5	21.5	38.2	19.8	2.4
ICSV 700	16.3	218.1	87.0	3.5	76.8	3.1	17.9	33.5	21.1	1.7
IESV 91104 DL	20.0	169.0	73.8	3.5	65.3	2.8	20.1	29.1	17.2	2.0
IESV 91131 DL	11.7	101.9	74.3	5.5	63.8	2.6	23.2	58.6	45.3	2.1
IESV 930 42 SH	20.1	151.4	76.8	4.5	67.5	3.0	21.3	41.9	30.0	2.9
IS 1044 (R)	16.7	159.6	68.3	3.0	59.8	3.0	22.0	25.0	12.3	2.0
IS 21881	13.5	87.8	75.8	6.0	63.0	2.8	23.8	42.0	28.6	2.0
IS 2205	18.6	211.2	80.0	4.0	68.8	3.1	16.1	16.2	5.7	1.0
IS 8193	15.0	132.4	73.8	4.5	63.0	3.3	19.2	34.5	27.0	1.9
Kari Mtama - 1	18.8	137.9	73.8	4.5	64.3	2.9	22.2	40.3	25.9	3.6
Macia	16.8	122.2	76.5	6.0	66.5	2.8	23.1	50.0	34.6	2.1
Seredo	18.5	129.4	73.5	5.5	62.8	2.5	22.2	41.7	28.6	2.6
Swarna (S)	19.1	104.1	69.8	6.5	61.5	3.3	22.5	32.2	16.6	2.2
Tam 2566	15.3	79.0	75.8	3.5	65.5	2.8	16.7	44.3	33.2	1.6
Mean	17.2	148.7	76.0	4.6	65.8	3.0	21.8	33.0	20.0	1.8
LSD	3.44	11.93	6.19	1.56	4.57	0.54	2.36	12.96	9.58	0.74
P	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Association of morphological parameters with resistance to *C. partellus* damage

Results of morphological traits associated with resistance traits to *C. partellus* are presented in Table 4.4.

Table 4.4: Correlation analysis among different characters in 27 sorghum genotypes grown at KARI Kiboko, Kenya

FL	-															
PE	0.97**	-														
DH	-0.21	-0.21	-													
DPW	-0.02	0	-0.01	-												
TGW	0.03	0.04	-0.05	0.98**	-											
LD	0.09	0.08	0.37*	0.07	0.1	-										
EH	-0.47	-0.52**	0.22	0.15	0.2	0.35	-									
LRA	-0.09	-0.06	-0.18	0.12	0.17	0.24	0.36	-								
TL	0.2	0.11	0.07	-0.07	0.03	0.27	0.17	0.02	-							
PL	-0.24	-0.21	0.26	0.11	0.01	-0.18	-0.07	-0.08	-0.06	-						
PH	0.22	0.24	0.18	-0.50**	-0.55**	0.01	-0.22	-0.38*	-0.15	0.14						
SI	-0.43*	-0.47*	0.65**	0.14	0.17	0.56**	0.86**	0.23	0.17	0.05	-0.11	-				
ST	-0.54**	-0.55**	0.13	0.21	0.26	0.2	0.88**	0.34	0.05	0.02	-0.26	0.77	-			
SB	-0.08	0.01	0.26	-0.19	-0.25	-0.40*	-0.07	-0.22	-0.06	0.18	0.37*	-0.03	-0.05	-		
BW	-0.01	-0.06	0.08	0.35	0.36*	0.19	0.02	0.04	-0.11	-0.21	-0.58**	0.14	0.14	-0.37*	-	
	FL	PE	DH	DPW	TGW	LD	EH	LRA	TL	PL	PH	SI	ST	SB	BW	

FL = days to 50% flowering, PE = days to panicle emergence, DH = deadheart damage, DPW = day panicle weight, TGW = total grain weight, LD = leaf feeding damage, EH = number of exit holes, LRA = number of larvae recovered alive, TL = number of tillers, PL = panicle length, PH = plant height, SI = selection index, ST = stem tunnel damage, SB = sugar brix level, BW = bloom waxiness

There was positive significant correlation between days to 50% flowering and days to panicle emergence ($r = 0.97$, $P < 0.001$). Leaf feeding damage had a marginal positive significant effect with deadheart damage ($r = 0.37$, $P = 0.05$). Selection index had a positive significant correlation with deadheart damage ($r = 0.65$, $P < 0.001$), with leaf damage ($r = 0.56$, $P = 0.002$), with exitholes ($r = 0.88$, $P < 0.01$), and with stem tunneling ($r = 0.86$, $P < 0.001$). Total grain weight had positive significant correlation with dry panicle weight ($r = 0.98$, $P < 0.001$). Days to 50% flowering had a negative significant effect on selection index ($r = -0.43$, $P = 0.02$). Stem tunneling had a negative significant correlation with days to 50% flowering ($r = -0.54$, $P = 0.004$). Days to panicle emergence had a negative significant association with exit holes ($r = -0.52$, $P = 0.0006$). Plant height had a negative positive association with total grain weight ($r = -$

0.55, $P = 0.003$). Sugar brix had a negative significant association with leaf damage ($r = -0.40$, $P = 0.03$). Bloom waxiness had a negative significant association with plant height ($r = -0.58$, $P = 0.002$), and marginally with sugar brix level ($r = -0.37$, $P = 0.05$).

Direct and indirect relationships by path coefficient analysis

The results of the direct and indirect relationships of damage parameters to grain yield are presented in Table 4.5.

Table 4.5: Path analysis on direct and indirect effects of *C. partellus* damage parameters on sorghum grain yield

Character	Correlation with grain yield	Direct effect	Indirect effect via			
			DH	EH	LD	ST
DH	-0.08	33.32**	-	-0.0039	0.0088	-0.0009
EH	-0.08	3.703**	0.0014	-	-0.0032	0.248
LD	-0.08	12.49**	0.0075	0.0018	-	-0.0067
ST	-0.08	39.93**	-0.0011	0.026	-0.016	-

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively
 DH = deadheart, EH = number of exit holes, LD = leaf feeding damage and ST = stem tunnels

The relative contributions of the damage parameters to grain yield loss were partitioned into direct and indirect relationships by path coefficient analysis with grain yield being the resultant variable. Deadheart had a significant positive direct effect with grain yield supported by negative indirect effects through exit holes and stem tunneling. Deadheart had a positive indirect effect on grain yield through leaf damage. Exit holes had a significant positive direct on grain yield supported by negative indirect effect through leaf damage. Exit holes exerted a positive indirect effect on grain yield through deadheart and stem tunneling. Leaf damage had a significant positive direct effect on grain yield supported by negative indirect effect on stem tunneling. Leaf damage had a positive indirect effect on grain yield through deadheart and exit holes damage. Stem tunneling had a significant positive direct effect on grain yield supported by negative indirect effects through deadheart and leaf damage. Stem tunneling had a positive indirect effect on grain yield through exit holes.

Discussion

This study suggested that sorghum genotypes showed varied levels of resistance to *C. partellus*. This study identified sources of resistance to *C. partellus* based on reduced leaf damage, deadheart, exit holes and stem tunneling under artificial infestation with stem borer neonates. Genotypes ICSA 473, ICSA 464, ICSV 700, ICSA 472 and IESV 93042 SH were the most resistant to *C. partellus*. Multiple stem borer damage traits were considered because resistance to *C. partellus* is a multi-mechanism, low-heritability quantitative trait, and thus, selecting for resistance based on a single parameter would not be effective and reliable (Singh *et al.*, 2011). The mechanism of resistance was mainly tolerance since some genotypes supported high leaf damage, deadheart and stems damages and produced appreciable grain yield. Several studies have reported antixenosis and antibiosis to *C. partellus* in sorghum (Kumar *et al.*, 2006; Singh *et al.*, 2011).

Reduced larval development was recorded on ICSV 700 implying that the genotype had antibiosis mechanism of resistance thus the reason why reduced larval development was recorded. The least number of larvae recovered alive was observed on resistant and moderately resistant genotypes ICSA 472, ICSA 464, ICSB 464, IS 1044 (R) and IS 2205. Since tillering serve as a component of recover resistance, it was employed to determine tolerance by counting the number of tillers that produced harvestable panicles. Resistant and moderately resistant genotypes ICSA 464, ICSB 473, ICSA 472, ICSB 472, IS 2205 and IS 21879 displayed tolerance through tillering. Plants with ability to tolerate insect damage may produce more yield than the non tolerant susceptible cultivar at the same level of insect infestation. High grain mass was recorded on resistant genotypes Kari Mtama - 1, IESV 93042 SH, ICSB 472, IESV 91131 DL, ICSB 467, Macia and ICSB 472. Bloom waxiness on the stem and leaves of the plant was used to measure antixenosis since bloom waxiness curtails the movement of borer on the plant. Resistant and moderately resistant genotypes ICSA 472, ICSA 473, IS 21879, Macia and IESV 91131 DL were mostly bloomy. It is worth noting that majority of the resistant and moderately resistant genotypes possessed a combination of the three mechanisms of resistance.

Deadheart, leaf feeding, number of exit holes and stem tunnels had a negative correlation with grain yield and all these damage trait have both direct and indirect effects on grain yield. The

significant and positive association between sugar brix and plant height suggested that high sugar levels could indirectly contribute to grain yield since taller plants mature late, and have significantly high yields. Harvesting was done at the same time for all genotypes, in which case and the later maturing varieties showed less senescence, greener leaves and juicier stems than the early maturing varieties. Current results suggest that low sugar brix was associated with susceptibility to leaf damage. Leaf damage observed may not have been severe to disrupt photosynthesis thus the insignificant association between the leaf damage and grain yield. This observation agrees with studies on other stem borers that leaf damage due to *Eldana saccharina* (Walker) and *Sesamia calamistis* (Hampson) did not result in a significant grain yield reduction in maize (Odiyi, 2007). Nibouche and Tibere, (2008) also who found that there was no significant genetic relationship between resistance and sugar content on sugarcane stalk damage by *Chilo sacchariphagus*.

Non pigmented genotypes suffered significantly lower deadheart incidence, stem tunneling and exit holes and had high sugar levels as compared to the pigmented ones. Tanning possibly had an adverse effect (antibiosis) on larvae inside the stem. Interestingly, tanned genotypes suffered higher leaf damage than the pigmented ones. Possibly, resistance displayed by leaves was different from that exhibited by the stems. The significant positive relationship between deadheart and leaf damage indicates a close relationship between the two damage parameters and either of them can be used to predict the other. Selection for deadheart damage would facilitate selection for leaf damage. The association between deadheart and selection index based on the leaf damage, deadheart, exit holes and stem tunneling was significant implying that an increase in damage parameters would lead to susceptibility of genotype under consideration.

Stem tunneling and exit holes correlated negatively with days to 50% flowering respectively indicating poor tolerance to stem tunneling by early flowering plants since the plants only have a relatively short period for growth. This observation agrees with observation made on other stalk borers *Eldana saccharina* and *Sesamia calamistis* on maize (Odiyi, 2007). Schulz et al. (1997) too observed that late silking plants suffered less stem tunneling owing to antibiosis against European corn borer in maize. Information on relationship between stem borer damage and grain yield loss is important in developing an efficient improvement program for resistance to stem

borers. The positive correlation between grain yield and plant height indicated that dwarf genotypes were better grain yielders than late maturing. A positive but weak association was observed between bloom waxiness and grain mass suggesting that completely bloomy genotypes would give significantly higher yields.

Conclusion and recommendation

This study demonstrated that there are genotypic differences in resistance and/or susceptibility to damage by *C. partellus*. Sorghum genotypes resistant to *C. partellus* based on reduced leaf damage, deadheart, exit holes and stem tunneling were identified in this study. The stem borer resistant sorghum genotypes can be used as sources of resistance to improve local farmer preferred varieties which are susceptible to *C. partellus*.

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CHAPTER 5

Inheritance of resistance traits to the African stem borer in sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important traditional cereal crop in Africa (Opole *et al.*, 2007). The African stem borer *Busseola fusca* Fuller (Lepidopteran: Noctuidae) is recognized as an economically important insect pest of sorghum and maize in sub Saharan Africa (Kfir *et al.*, 2002). *Busseola fusca* is associated with significant grain yield loss ranging between 15 % - 80% depending on the pest population, time of infestation, variety and agroecosystem (Wale *et al.*, 2006). *Busseola fusca* inflicts damage through leaf feeding, deadheart, exit holes and stem tunneling (Chabi-Olaye *et al.*, 2005). The *B. fusca* larvae remain inside the stems, and thus, are less vulnerable to insecticides and natural enemies (Muhammad *et al.*, 2009). *Busseola fusca* is endemic in higher altitudes and co-exists in mid-altitude zones of Kenya with *Chilo partellus* Swinhoe, (Lepidopteran: Crambidae) an introduced pest from Asia (Kfir *et al.*, 2002). In Kenya, much research effort has been accorded to *C. partellus*, consequently neglecting indigenous economically important stem borers. Research on management of *B. fusca* in sorghum has mainly focused on cultural control, predominantly intercropping, residue management, fertilizer use, and recently, genetic engineering (Chabi *et al.*, 2005; Markus and Gurling, 2006; Amsalu *et al.*, 2008).

Host plant resistance is an efficient approach in managing insect pests especially in Africa where subsistence farmers practice little or no pest management in cereals (Sharma *et al.*, 2006). Understanding genetic mechanisms underlying *B. fusca* tolerance in sorghum is imperative for an effective breeding strategy. High narrow sense heritability *B. fusca* damage estimates suggests that mass selection would be effective and when low progeny selection is more important (Nyquist and Baker, 1991). Combining ability is the capability of parents to combine amid each other in hybridization so that favourable alleles are transmitted to their progenies (Panhwar *et al.*, 2008). The concept of combining ability has been employed in other crops particularly maize in determining superior combiners for agronomic, disease and insect pest resistance traits. The objective of this study was to investigate the type of gene action conditioning tolerance to *B.*

fusca in sorghum.

Materials and Methods

Experimental site

Experiments to determine inheritance of resistance traits to *B. fusca* were conducted in Embu. Embu lies between longitude 37°42' E and latitude 0°44' S at an elevation of 1,510 metres above sea level (Beyene *et al.*, 2011). The site has maximum and minimum daily temperatures of 25°C and 14°C with an average rainfall of about 1200 mm.

Experimental material

The experimental material consisted of sorghum lines with varying levels of resistance to *B. fusca* and *C. partellus* (Table 5.1). North Calorina mating design 2 was employed where 15 lines (Gadam, ICSA 464, ICSA 467, ICSA 472, ICSA 474, ICSB 464, ICSB 467, ICSB 474, IESV 91131 DL, IESV 93042 SH, IS 21879, IS 21881, IS 8193, Macia and Seredo) were used as females and 2 (Kari Mtama - 1 and ICSB 473) as males.

Table 5.1: Characteristics of sorghum lines used in this study

Genotype	Pedigree	<i>Busseola fusca</i>	<i>Chilo partellus</i>	Plant colour
ICSA 474	(IS 18432 x ICSB 6)11-1-1-2-2	MR	MR	P
Seredo	Seredo	MR	MS	P
ICSB 474	(IS 18432 x ICSB 6)11-1-1-2-2	MR	MS	P
ICSA 464	[(ICSB 11 x ICSV 702) x PS 19349B]5-1-2-2	MR	R	T
ICSA 472	(ICSB 51 x ICSV 702)7-3-1	MR	R	T
IS 21879	IS 21879	MS	MR	P
*Kari Mtama - 1	Kari Mtama - 1	MS	MR	T
ICSA 467	[(ICSB 11 x ICSV 700) x PS 19349B] x ICSB 13]4-1	MS	MR	T
IESV 93042 SH	IESV 93042 SH	MS	R	T
*ICSB 473	(ICSB 102 x ICSV 700)5-2-4-1-2	MS	R	T
Macia	Macia	R	MR	T
IESV 91131 DL	IESV 91131 DL	R	MR	T
ICSB 464	[(ICSB 11 x ICSV 702) x PS 19349B]5-1-2-2	R	MR	T
ICSB 467	[(ICSB 11 x ICSV 700) x PS 19349B] x ICSB 13]4-1	R	MR	T
IS 21881	IS 21881	R	MS	P
Gadam	Gadam	R	MS	P
IS 8193	IS 8193	S	S	P

R = Resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible; T = tan, P = pigmented *male parents

Classification of the sorghum lines into different categories was based on leaf damage, deadheart, exit holes and stem tunneling in 2010 long and short rain seasons. The test material was sown in an α -lattice design, consisting of sixteen plots in three blocks, replicated twice. The rows were 2 m long and 0.75 m apart, and the spacing between plants within rows was 0.25 m.

Stem borer neonates

First instar neonates of *B. fusca* utilized in this study were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. At 30 days after sowing, five plants in each row were tagged and artificially infested on the whorls with five larvae per plant using a camel hairbrush. Data was taken from marked plants.

Data collection

Observations on leaf damage and deadheart were recorded on per plant basis at two and four weeks after artificial infestation. Percentages of plants showing leaf and deadheart were computed by expressing the number of plants damaged as a percentage of the total number of plants sampled. At harvest, the plants were stripped and the numbers of stem borer exit holes on the stem were counted on each sampled plant. The main stem of plants infested with stem borer larvae were split open from the base to the apex, and the cumulative tunnel length measured in centimeters.

Seedling vigor was scored at 30 days after sowing on a scale of 1 – 5, where 1 = low vigor (plants showing minimum growth, less leaf expansion and poor adaptation; 3 = moderate vigor; 5 = high vigor (tall plants with expanded leaves and robustness). Leaf glossiness was recorded at 30 days after sowing on a scale of 1 - 5 where 1 = highly glossy, 3 = moderately glossy, and 5 = non glossy. Waxy bloom was recorded on a scale of 1 – 9; where 1 = no observable bloom, 3 = slightly present, 5 = medium, 7 = mostly bloomy, 9 = completely bloomy at the 50% flowering stage. Agronomic traits monitored included plant height, days to panicle emergence, 50% flowering and panicle length. Total grain yield and hundred-grain mass were recorded for each of the sampled plants using a weighing balance (Mettler PM 6000, CH- 8606 GREIFENSEE-ZURICH, made in Switzerland).

Statistical analyses

Data on percentages was arcsin transformed while that of counts was log transformed before analysis of variance. General analysis of variance was performed for all the traits observed using Genstat version 14 statistical software (Genstat Release 12 Reference Manual, Part 1 Summary, 2009, VSN International, Hemel Hempstead HP1 1ES, United Kingdom). Genetic analyses were performed using line x tester method using the same software (Panhwar *et al.*, 2008).

The analysis facilitated an estimate of the variances from expected mean squares and general combining ability (GCA) effects representing additive gene effects and specific combining ability (SCA), denoting non-additive gene effects. The sums of squares of the crosses were partitioned into GCA and SCA effects, and their interaction with the environment was estimated. Narrow-sense heritability and proportional contribution of females, males, and their interaction were also computed. Narrow-sense heritability = $(V_{gca}) / (V_{gca} + V_{sca} + VE)$ x 100; where, V_{gca} = general combining ability variance, V_{sca} = specific combining ability variance, and VE = error variance (Dhillon *et al.*, 2006). Relative importance of GCA and SCA was estimated according to Baker's (1978) as the ratio $\delta^2 GCA_{(f)} + \delta^2 GCA_{(m)} / \delta^2 GCA_{(f)} + \delta^2 GCA_{(m)} + \delta^2 SCA$ where $\delta^2 GCA_{(f)}$, $\delta^2 GCA_{(m)}$ and $\delta^2 SCA$ are the variance components for GCA and SCA, respectively. Correlations analysis was also performed to understand the association between the morphological and traits linked with sorghum resistance to *B. fusca*.

Results

Plant damage and morphological traits

The results of plant damage and morphological traits are presented in Table 5.2. The mean squares due to GCA female were significant at $P = \leq 0.05$ or $P = \leq 0.01$ for leaf damage, exit holes, stem tunneling and vigor. Significant GCA male were observed on exit holes, leaf glossiness and bollom waxiness. Significant SCA mean squares were observed exit holes and stem tunneling. Baker's ratio estimates for leaf feeding, deadheart, exit holes and stem tunnel damages ranged between 15% - 46% while that of leaf glossiness, seedling vigour and bloom waxiness ranged between 38% - 43%. Narrow sense heritability for leaf damage, deadheart, exit holes and stem tunnels ranged from 4% to 26% while that of leaf glossiness, seedling vigour and

bloom waxiness ranged between 38 % - 56 %.

Table 5.2: Mean squares for general and specific combining ability for various damage and morphological traits and heritability in sorghum

Source of variation	d.f.	DH	LD	EH	ST	GL	VG	BW
Rep	1	913.7	2382.6	0.59ns	640.9	0.8	0.3	0.469
Season	1	2397.3*	2207.3*	0.01ns	396.1ns	0.6ns	4.9**	7.3*
GCA _f	14	323.4ns	726.8*	0.13*	770.5**	0.9ns	0.9**	2.2ns
GCA _m	1	136.9ns	949.2ns	0.73**	9.4ns	2.1*	0.7ns	21.3**
SCA	14	480.8ns	621.9ns	0.30**	842.4**	0.4ns	0.2ns	2.2ns
Residual	59	335.6	368.9	0.07	151.2	0.5	0.234	1.494
Proportional contribution to total variance								
Females		6.10	178.95	0.03	309.65	0.17	0.27	0.37
Males		13.25	38.69	0.04	9.45	0.10	0.03	1.32
Females x Males		145.20	253.00	0.23	691.20	0.07	0.01	0.75
Baker's Ratio		0.15	0.46	0.25	0.30	0.79	0.97	0.69
Narrow sense heritability (%)								
		4	26	21	26	38	56	43

* ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively; ns = non significant

DH = Deadheart %, LD = Leaf damage (%), EH = Exit holes, ST = stem tunneling, GL= Leaf glossiness, VG = Seedling vigour, BW = Bloom waxiness, DF = Degrees of freedom, GCA_f = general combining ability for females, GCA_m = general combining ability for males, SCA = specific combining ability

Agronomic traits

The results of analysis of variance for plant height, panicle emergence, days to 50% flowering, panicle length, total grain weight and hundred grain mass are presented in Table 5.3.

Table 5.3: Mean squares for general and specific combining ability for various agronomic traits and heritability estimates in sorghum

Source of variation	d.f.	PH	PE	FL	PL	TGW	HGM
Rep stratum	1	608	44.4	61.6	58.3	341.1	1.0
Season	1	15790.1**	745.0**	1540.8**	2760.6**	6.6ns	1.1ns
GCA _f	14	8449.9**	33.8*	34.8*	55.7**	447.0**	4.9**
GCA _m	1	3322.9*	99.0*	116.0*	19.8ns	316.3*	2.1*
SCA	14	6020.1**	15.1ns	20.5ns	130.0**	372.8**	2.4**
Residual	539	668.2	17.6	18.1	11.1	76.8	0.5
Proportional contribution to total variance							
Females		3890.85	22.32	8.37	22.32	185.11	2.20
Males		176.98	0.58	6.53	0.58	15.96	0.11
Females x Males		5351.90	118.97	2.39	118.97	295.99	1.95
Baker's Ratio		0.43	0.16	0.86	0.16	0.40	0.54
Narrow sense heritability (%)							
		40	15	42	15	35	49

* ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively; ns= non significant; PH=Plant height, PL=Panicle length, FL=50 % flowering, PL=Panicle length, TGW= Total grain weight, HGM= Hundred grain mass, DF= Degrees of freedom, GCA_f = general combining ability for females, GCA_m = general combining ability for males, SCA = specific combining ability

Mean squares due to GCA_f and GCA_m were significant for plant height, panicle emergence, days to 50 % flowering, total grain weight, hundred grains mass except for GCA_m for panicle

length. SCA mean squares were significant for plant height, panicle length, total grain weight and hundred grain mass. Baker's ratio for agronomic traits ranged between 16% - 86% while narrow sense heritability estimates ranged between 15 % - 49%.

Stem borer damage, agronomic and morphological traits

The results for exit holes, stem tunnels, plant height, panicle length, total grain yield and hundred grain mass were significantly different and are presented in Table 5.4. Based on the combined means for deadheart, leaf feeding, stem tunnels and exit holes, the least damage was observed on crosses ICSB 464 x ICSB 473, ICSB 474 x Kari Mtama - 1, ICSA 464 x ICSB 473, IS 21881 x ICSB 473, IS 8193 x Kari Mtama - 1, Gadam x Kari Mtama - 1, Gadam x ICSB 473, ICSA 472 x ICSB 473, Macia x Kari Mtama - 1 and ICSB 467 x ICSB 473 increasing order. This information is derived from the table by pooling means of leaf damage, deadheart, exit holes and stem tunneling and considering the least means interms of the mentioned damage.

Panicle length ranged from 17 cm to 26 cm on crosses Seredo x Kari Mtama - 1 and ICSB 464 x Kari Mtama - 1 respectively. Cross ICSB 474 x Kari Mtama - 1 was the tallest with 177 cm while Gadam x ICSB 473 was the shortest and measured 110 cm. Days to 50 % flowering ranged from 65 - 90 days on Gadam x Kari Mtama - 1 and IS 21879 x ICSB 473 respectively. Cross ICSA 467 x Kari Mtama - 1 produced about three times more total grain weight than IS 8193 x Kari Mtama - 1 which produced the least. Hundred grain mass ranged from 1.4 gm - 4.0 gm on IS 8193 x Kari Mtama - 1 and ICSB 464 x ICSB 473 respectively. Some of the crosses with high grain yield were ICSA 467 x Kari Mtama - 1, ICSB 474 x Kari Mtama - 1, IESV 91131 DL x Kari Mtama - 1, ICSA 472 x ICSB 473, ICSB 467 x Kari Mtama - 1 in increasing order.

Table 5.4: Means for damage, agronomic and morphological traits for sorghum F1 hybrids

F1 hybrids	DH	LD	EH	ST	PH	PE	FL	PL	TGW	HGM	BW	GL	VG
ICSA 467 x Kari Mtama - 1	35.8	38.9	0.7	14.0	149.2	65	72	21.4	38.3	3.1	6.5	2.8	3.3
ICSB 474 x Kari Mtama - 1	13.3	16.4	0.8	19.3	177.4	61	69	22.6	31.0	2.9	6.8	2.9	3.5
IESV 91131 DL x Kari Mtama - 1	19.6	54.5	0.7	17.8	149.0	60	67	21.2	30.2	3.1	4.5	3.4	3.4
ICSA 472 x ICSB 473	26.0	26.3	0.7	15.6	138.9	66	73	23.8	29.1	3.1	5.5	3.5	3.4
ICSB 467 x Kari Mtama - 1	51.1	45.0	0.7	20.5	138.0	64	72	23.8	26.9	3.1	6.0	2.9	3.1
ICSB 464 x ICSB 473	16.4	15.9	0.4	9.1	175.0	65	73	19.9	25.6	4.0	7.0	2.8	3.3
ICSA 472 x Kari Mtama - 1	32.3	48.8	0.5	12.6	118.8	66	71	18.9	24.4	2.7	6.0	2.7	2.9
ICSA 464 x Kari Mtama - 1	23.1	48.5	0.6	13.3	126.7	65	71	20.5	24.3	2.8	5.8	3.0	2.9
ICSA 474 x ICSB 473	26.3	44.4	0.6	16.7	172.7	69	76	19.0	23.9	2.9	7.0	2.3	3.8
IESV 91131 DL x ICSB 473	26.3	48.2	0.7	27.7	152.3	64	71	22.5	23.6	2.9	7.0	3.0	3.4
ICSB 474 x ICSB 473	38.9	26.3	0.7	19.2	150.1	65	72	21.1	23.1	2.7	6.8	2.9	4.0
ICSA 467 x ICSB 473	35.2	38.7	0.4	10.9	130.0	69	78	20.6	22.9	2.7	7.0	2.3	3.0
IS 21879 x ICSB 473	25.7	32.6	0.6	28.6	130.2	83	90	21.2	22.9	2.3	7.0	2.3	3.4
IS 21881 x Kari Mtama - 1	32.0	64.6	0.8	20.0	161.5	61	67	21.5	22.8	2.6	6.0	3.4	2.4
Gadam x Kari Mtama - 1	22.5	28.1	0.7	13.3	137.7	57	65	21.7	22.2	2.9	5.5	3.3	3.0
ICSA 464 x ICSB 473	24.8	17.9	0.6	9.3	151.6	68	76	20.7	21.9	3.1	6.3	2.3	3.4
IS 21879 x Kari Mtama - 1	32.6	44.4	0.7	19.7	126.7	77	84	19.3	21.5	2.2	6.5	2.9	3.0
IESV 93042 SH x Kari Mtama - 1	32.0	42.4	0.6	14.0	140.9	61	67	21.0	21.5	2.5	6.1	3.6	3.1
Gadam x ICSB 473	16.4	35.5	0.7	15.6	108.2	60	66	17.4	21.3	2.9	7.0	3.5	2.3
IESV 93042 SH x ICSB 473	9.8	54.8	0.7	23.6	122.1	66	73	20.7	21.3	2.8	5.5	3.1	3.3
Seredo x ICSB 473	35.2	26.3	0.7	27.7	163.8	64	74	23.1	20.8	2.4	6.5	3.0	3.1
IS 8193 x ICSB 473	35.8	38.4	0.8	22.3	176.8	65	71	25.0	20.5	2.2	7.0	2.9	3.4
Macia x ICSB 473	42.1	36.1	0.7	24.0	141.1	65	72	19.8	20.4	2.9	6.5	3.1	3.2
IS 21881 x ICSB 473	19.3	19.3	0.6	19.5	131.6	62	71	20.9	19.9	2.4	6.3	3.4	2.7
Seredo x Kari Mtama - 1	47.9	26.0	0.8	22.2	149.2	61	69	16.9	19.7	2.8	4.0	3.1	3.0
ICSB 464 x Kari Mtama - 1	35.2	16.4	0.8	36.9	167.7	61	70	26.3	19.5	2.5	6.5	3.1	3.4
ICSA 474 x Kari Mtama - 1	19.6	41.8	0.8	22.4	146.1	64	70	21.0	19.4	2.9	7.0	2.8	3.5
Macia x Kari Mtama - 1	19.9	32.3	0.7	19.1	122.1	64	71	17.8	19.4	2.7	4.8	3.3	3.1
ICSB 467 x ICSB 473	26.0	23.1	0.6	23.1	164.7	64	72	21.9	17.6	2.7	6.8	2.8	3.1
IS 8193 x Kari Mtama - 1	19.3	19.6	0.8	24.0	127.4	62	68	18.4	15.4	1.4	4.5	3.9	2.8
F value	0.167	0.083	<.001	<.001	<.001	0.184	0.064	<.001	<.001	<.001	0.139	0.736	0.423
LSD	25.92	27.18	0.16	7.64	16.06	3.11	4.15	2.07	5.44	0.42	1.73	1.02	0.68
CV	65.4	54.8	6.6	63.5	17.8	3.4	4.1	15.9	38	24.6	19.8	24	15.3

DH = Percent deadheart, LD = Percent leaf damage, EH = Number of exit holes, ST = Stem tunnel length, PH = Plant height, FL = Days to 50 % flowering, PE = Days to panicle emergence, PL = Panicle length, TGW = Total grain weight, HGM = Hundred grain mass, BW = Bloom waxiness, GL = Leaf glossiness, VG = Seedling vigour

Nature of gene action

The results of leaf damage, deadheart, exit holes and stem tunnels GCA effects are shown shown in Table 5.5. These results suggested that female lines ICSB 464, Gadam and ICSB 473 exhibited maximum negative GCA effect of -17.58, -16.72 and -3.71 respectively for leaf damage, deadheart, exit holes and stem tunneling. GCA values for leaf damage, deadheart, exit holes and stem tunneling were pooled and the average estimated and reported as above. Parents IESV 91131 DL, ICSB 467 and Seredo scored the highest positive GCA in regard to leaf damage, Deadheart, exit holes and stem tunnels. Genotypes such as Seredo, IESV 91131 DL, IS 8193 and IS 21879 showed significant undesirable positive GCA effects to either deadheart, leaf damage, exit holes and stem tunneling. More over, Seredo showed significant positive undesirable effects to Deadheart and stem tunneling.

Maximum GCA effect for plant height was observed on ICSB 464 (26.5) and ICSB 474 (18.8) among females and ICSB 473 (2.37) among males. High GCA effects for days to 50 % flowering among females were recorded on IS 21879 (3.10) and ICSA 467 (2.47) while a low GCA effect was recorded on IESV 91131 DL (-3.53) and Gadam (-3.15). High positive significant GCA effect for total grain yield was observed on ICSA 467 (7.56), ICSA 472 (3.72), IESV 91131 DL (3.83), ICSB 474 (4.03) and Kari- mtama 1 (0.73). The positive significant GCA effects for grain yield are importance since it implies that these parents possess favourable alleles for high grain yield. High GCA effects for bloom waxiness were observed on ICSA 474, ICSA 467 and ICSB 474. Seedlings of ICSA 474 and ICSA 467 were highly vigourous and glossy while IS 8193 and IS 21881 were non glossy the least vigorous.

Table 5.5: General combining ability (GCA) effects of females and males for damage, agronomic and morphological traits in sorghum

Females	DH	LD	EH	ST	PH	FL	PE	PL	TGW	HGM	BW	GL	VG
Gadam	-8.50	-3.30	0.03	-4.95	-21.90	-3.15	-2.73	-1.45	-1.28	0.15	0.07	0.38	-0.56
ICSA 464	-4.10	-1.90	-0.08	-8.13	-5.80	0.47	0.39	-0.37	0.02	0.20	-0.18	-0.36	-0.03
ICSA 467	7.50	3.80	-0.08	-6.98	-5.30	2.47	2.52	-0.02	7.56**	0.14	0.57	-0.45	-0.04
ICSA 472	1.10	2.50	-0.06	-5.28	-16.10	-1.65	-2.11	0.33	3.72*	0.17	-0.43	0.10	-0.03
ICSA 474	-5.10	8.10	0.02	0.15	14.50*	2.22	2.64	-1.00	-1.37	0.15	0.82*	-0.50	0.47*
ICSB 464	-2.20	-18.90	-0.06	3.58	26.50*	-1.90	-1.61	2.10*	-0.46	0.53**	0.57	-0.06	0.16
ICSB 467	10.50	-1.00	-0.04	2.45	6.40	0.47	1.89	1.82*	-0.80	0.13	0.20	-0.18	-0.03
ICSB 474	-1.90	-13.70	0.05	-0.15	18.80*	-2.53	-1.73	0.88	4.03*	0.12	0.57	-0.12	0.60*
IESV 91131 DL	-5.10	16.30*	0.02	3.35	5.80	-3.53	-3.36	0.88	3.83*	0.26*	-0.43	0.19	0.22
IESV 93042 SH	-7.10	13.60	-0.01	-0.60	-13.40	1.85	1.89	-0.15	-1.65	-0.09	-0.37	0.38	0.03
IS 21879	1.10	3.50	-0.02	4.75*	-16.50	3.10	3.02	-0.72	-0.82	-0.49	0.57	-0.43	0.03
IS 21881	-2.30	6.90	0.03	0.36	1.60	0.47	-0.11	0.20	-1.70	-0.26	-0.05	0.38	-0.62
IS 8193	-0.50	-6.10	0.13*	3.75	7.20	0.22	-0.36	0.70	-5.12	-0.92	-0.43	0.38	-0.09
Macia	3.00	-0.90	0.03	2.15	-13.30	0.60	-0.61	-2.20	-3.12	0.07	-0.55	0.23	0.01
Seredo	13.50*	-8.90	0.05	5.55*	11.60*	0.85	0.27	-0.97	-2.83	-0.16	-0.93	0.07	-0.12
Males													
ICSB 473	-1.05	-2.75	-0.03	0.12	2.37	-0.98	-0.91	0.18	-0.72	0.06	0.42*	-0.13	0.08
Kari- mtama 1	1.08	2.85	0.04	-0.13	-2.34	0.98	0.91	-0.19	0.73	-0.06	-0.42	0.13	-0.08

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

DH = Percent deadheart, LD = Percent leaf damage, EH = Number of exit holes, ST = stem tunnels, PH = Plant height, FL = Days to 50 % flowering, PE = Days to panicle emergence, PL= Panicle length, TGW = Total grain weight, HGM = Hundred grain mass, BW = Bloom waxiness, GL = Leaf glosiness, VG = Seedling vigour,

Results on specific combining ability for the different damage, agronomic and morphological characteristics are presented in Table 5.6. There were significant differences in SCA effects among crosses in regard to leaf damage, exit holes, stem tunnels, plant height, panicle length, grain yield and leaf glossiness. Most of the other SCA estimates were generally negative, low or non-significant. Crosses IS 21881 x ICSB 473, ICSB 474 x Kari Mtama - 1, IS 8193 x Kari Mtama - 1 and ICSB 464 x ICSB 473 showed the least negative SCA effects for leaf damage, deadheart, exit holes and stem tunnels. In regard to plant height, high positive SCA values of 22 and 17 were observed on IS 8193 x ICSB 473 and IS 21881 x Kari Mtama - 1 respectively. Highest positive specific combining ability for panicle length was recorded on crosses ICSB 464 x Kari Mtama - 1 and IS 8193 x ICSB 473. Crosses IC5A 467 x Kari Mtama - 1 and ICSB 467 x Kari Mtama - 1 were the best specific combiners for total grain yield.

Table 5.6: Specific combining ability estimates of F1 hybrids from line x tester analysis of various characters in sorghum

F1 hybrids	DH	LD	EH	ST	PH	PE	FL	PL	TGW	HGM	BW	GL	VG
Gadam x ICSB 473	-2	6.5	0.06	1	-17.1	-0.27	-1.32	-2.31	0.28	-0.09	0.33	0.26	-0.43
Gadam x Kari Mtama - 1	2	-6.5	-0.06	-1	17.10**	0.28	1.32	2.31*	-0.28	0.09	-0.33	-0.26	0.43
ICSA 464 x ICSB 473	1.9	-12.5	-0.01	-2.13	10.1	0.1	0.43	-0.08	-0.48	0.09	-0.17	-0.23	0.17
ICSA 464 x Kari Mtama - 1	-1.9	12.5	0.01	2.13	-10.1	-0.1	-0.43	0.08	0.48	-0.09	0.17	0.23	-0.17
ICSA 467 x ICSB 473	0.8	2.7	-0.11	-1.68	-11.9	0.23	0.93	-0.58	-6.97	-0.24	-0.17	-0.09	-0.22
ICSA 467 x Kari Mtama - 1	-0.8	-2.7	0.11*	1.68	11.90*	-0.22	-0.93	0.58	6.97**	0.24*	0.17	0.09	0.22
ICSA 472 x ICSB 473	-2.1	-8.4	0.16*	1.42	7.7	-1.52	-1.44	2.27*	3.05	0.12	-0.67	0.53*	0.17
ICSA 472 x Kari Mtama - 1	2.1	8.4	-0.16	-1.42	-7.7	1.53	1.44	-2.27	-3.05	-0.12	0.67	-0.53	-0.17
ICSA 474 x ICSB 473	4.4	4.1	-0.03	-2.95	10.90*	1.1	0.81	-1.16	2.96	-0.04	-0.42	-0.12	0.05
ICSA 474 x Kari Mtama - 1	-4.4	-4.1	0.03	2.95	-10.9	-1.1	-0.81	1.16	-2.96	0.04	0.42	0.12	-0.05
ICSB 464 x ICSB 473	-8.3	2.5	-0.19	-14.03	1.3	0.6	-0.32	-3.36	3.78*	0.66**	-0.17	-0.06	-0.14
ICSB 464 x Kari Mtama - 1	8.3	-2.5	0.19*	14.03**	-1.3	-0.6	0.32	3.36**	-3.78	-0.66	0.17	0.06	0.14
ICSB 467 x ICSB 473	-11.5	-8.1	-0.01	1.17	11.00*	-1.52	-1.82	-1.13	-3.92	-0.25	-0.05	0.07	-0.08
ICSB 467 x Kari Mtama - 1	11.5	8.1	0.01	-1.17	-11	1.53	1.82	1.13	3.92*	0.25*	0.05	-0.07	0.08
ICSB 474 x ICSB 473	13.9	7.7	0	-0.2	-16	0.35	-0.32	-0.93	-3.24	-0.16	-0.42	0.13	0.17
ICSB 474 x Kari Mtama - 1	-13.9	-7.7	0	0.2	16.00*	-0.35	0.32	0.93	3.24*	0.16	0.42	-0.13	-0.17
IESV 91131 DL x ICSB 473	4.4	-0.4	0.06	4.85*	-0.7	0.35	0.31	0.47	-2.57	-0.13	0.83	-0.06	-0.08
IESV 91131 DL x Kari Mtama - 1	-4.4	0.4	-0.06	-4.85	0.7	-0.35	-0.31	-0.47	2.57	0.13	-0.83	0.06	0.08
IESV 93042 SH x ICSB 473	-10	9	0.11*	4.7	-11.7	0.85	0.81	-0.36	0.58	0.09	-0.73	-0.12	-0.02
IESV 93042 SH x Kari Mtama - 1	10	-9	-0.11	-4.7	11.70*	-0.85	-0.81	0.36	-0.58	-0.09	0.73	0.12	0.02
IS 21879 x ICSB 473	-2.4	-3.1	0.02	4.3	-0.6	1.6	3.81	0.77	1.4	-0.02	-0.17	-0.18	0.11
IS 21879 x Kari Mtama - 1	2.4	3.1	-0.02	-4.3	0.6	-1.6	-3.81	-0.77	-1.4	0.02	0.17	0.18	-0.11
IS 21881 x ICSB 473	-5.3	-19.8	-0.06	-0.41	-17.3	-0.77	-0.07	-0.46	-0.69	-0.18	-0.3	0.13	0.08
IS 21881 x Kari Mtama - 1	5.3	19.80*	0.06	0.41	17.30*	0.78	0.07	0.46	0.69	0.18	0.3	-0.13	-0.08
IS 8193 x ICSB 473	9.3	12.2	-0.01	-1	22.30**	-0.02	-0.82	3.09*	3.30*	0.33*	0.83	-0.37	0.23
IS 8193 x Kari Mtama - 1	-9.3	-12.2	0.01	1	-22.3	0.03	0.82	-3.09	-3.3	-0.33	-0.83	0.37	-0.23
Macia x ICSB 473	12.2	4.7	0.05	2.35	7.1	-1.15	-1.32	0.84	1.23	0.05	0.45	0.03	-0.04
Macia x Kari Mtama - 1	-12.2	-4.7	-0.05	-2.35	-7.1	1.15	1.32	-0.84	-1.23	-0.05	-0.45	-0.03	0.04
Seredo x ICSB 473	-5.3	3	-0.04	2.6	4.9	0.1	0.31	2.92*	1.29	-0.24	0.83	0.07	0.01
Seredo x Kari Mtama - 1	5.3	-3	0.04	-2.6	-4.9	-0.1	-0.31	-2.92	-1.29	0.24*	-0.83	-0.07	-0.01

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

DH = Percent deadheart, LD = Percent leaf damage, EH = Number of exit holes, ST= stem tunnel, PH = Plant height, FL = Days to 50 % flowering, PE = Days to panicle emergence, PL = Panicle length, TGW= Total grain weight, HGM = Hundred grain mass, BW = Bloom waxiness, GL = Leaf glossiness, VG = Seedling vigour,

Association between *B. fusca* damage parameters, agronomic and morphological traits

The results of correlation between damage parameters, agronomic and morphological traits are presented in Table 5.7.

Table 5.7: Correlation matrix between damage parameters, agronomic and morphological characters in sorghum

BW	-													
DH	0.01	-												
DPW	0.03	0.09	-											
EH	-0.31	0.08	-0.06	-										
FL	0.38*	0.1	-0.02	-0.36*	-									
GL	-0.57**	-0.16	-0.22	0.49**	-0.70**	-								
HGM	0.17	-0.09	0.52**	-0.40*	-0.09	-0.29	-							
LD	-0.06	0.09	0.16	0.05	-0.06	0.02	0.01	-						
PE	0.35*	0.08	0.0	-0.3	0.96**	-0.63**	-0.15	0.08	-					
PH	0.32	0.14	0.17	0.06	-0.08	-0.28	0.25	-0.26	-0.15	-				
PL	0.33	0.18	0.15	0.19	0.0	-0.07	0.03	-0.09	-0.07	0.51**	-			
ST	0.03	0.17	-0.36*	0.61**	0.11	0.14	-0.46**	-0.08	0.07	0.18	0.39*	-		
TGW	0.08	0.02	0.94**	-0.08	0.01	-0.18	0.54**	0.18	0.02	0.17	0.22	-0.38*	-	
VG	0.25	0.07	0.25	-0.03	0.32	-0.46**	0.26	-0.18	0.26	0.49**	0.36*	0.15	0.25	-
	BW	DH	DPW	EH	FL	GL	HGM	LD	PE	PH	PL	ST	TGW	VG

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

BW = bloom waxiness, DH = deadheart damage, DPW = dry panicle weight, EH = Number of exit holes, FL = days to 50% flowering, GL = leaf glossiness, HGM = hundred grain mass, LD = leaf feeding damage, PE = days to panicle emergence, PH = plant height, PL = panicle length, ST = stem tunnels, TGW = total grain weight, VG = seedling vigour.

Bloom waxiness was significantly and positively correlated with days to 50 % flowering and days to panicle emergence ($r = 0.38^*$ and $r = 0.35^*$) respectively. Days to 50 % flowering correlated positively and highly significantly with days to panicle emergence ($r = 0.96^{**}$). Plant height significantly and positively correlated with vigour ($r = 0.49^{**}$) and with panicle length ($r = 0.51^{**}$). A positive and significant association was observed between panicle length and vigour ($r = 0.36^*$). Exit holes positively and significantly correlated with stem tunnels ($r = 0.61^{**}$), and with non glossy leaves ($r = 0.49^{**}$). A positive and highly significant relationship was observed between dry panicle weight and total grains ($r = 0.94^{**}$) and with hundred grain mass ($r = 0.52^{**}$). Hundred grain mass positively and significantly correlated with total grain yield ($r = 0.54^{**}$).

Bloom waxiness negatively and significantly correlated with leaf glossiness implying that these traits are inherited in different ways and each one of them cannot be used to predict the other ($r = -0.57^{**}$). Days to 50 % flowering negatively and significantly correlated with leaf glossiness ($r = -0.70^{**}$). Leaf glossiness was negatively and highly significantly associated with days to panicle emergence ($r = -0.63^{**}$) and vigour ($r = -0.46^{**}$). A negative relationship was observed between exit holes and hundred grain mass ($r = -0.40^*$) and exit holes and days to 50 % flowering ($r = -0.36^*$). A negative and highly significant association was observed between dry panicle weight and stem tunnels ($r = -0.36^*$). Negative relationship was observed between hundred grain mass and stem tunnels ($r = -0.46^{**}$). Stem tunneling negatively and significantly correlated with total grain yield ($r = -0.38^*$).

Discussion

General combining ability as well as specific combining abilities were significant suggesting that additive and non-additive gene effects were important in conditioning resistance traits to *B. fusca*. The negative general combining ability effects for damage (leaf damage, deadheart, stem tunnels and exit holes) signified contribution of the genotype towards resistance, while positive combining ability effects on the parents indicated contribution *B. fusca* damage susceptibility in the crosses. The significance of GCAf means squares for leaf damage, exit holes, stem tunneling, seedling vigor and significant GCAM for exit holes, seedling vigor and bloom waxiness implied presence of additive genes in controlling these traits. The significance of GCAf and GCAM in controlling exit holes may imply that both female and males influenced the trait. GCA (female) contribution was predominant over GCA (male) for leaf damage and stem tunneling suggesting that there might be maternal effects. Baker's ratio estimates indicated that leaf damage was conditioned by both additive and non-additive genes since GCA SCA ratio was almost 1:1. Thus, the response of hybrids to leaf damage could be predicted based on the GCA of the parents.

The significance of SCA over GCA as estimated from Baker's ratio for deadheart, exit holes and stem tunnels damage suggested that these characters were controlled largely by non-additive type of gene action. The significance of SCA variance implied that cross combinations would be efficient in breeding for deadheart, exit holes and deadheart. This also indicated that the GCA

alone could not give a complete prediction of hybrids' deadheart and stem tolerance to *B. fusca*. . Studies on inheritance of resistance in maize to *B. fusca* in South Africa reported that estimates of combining ability showed greater SCA than GCA in most crosses (Taylor *et al.*, 2003). Gene effects for *B. fusca* resistance in sorghum are not fixable with ease owing to the presence of significant non-heritable interaction present in F₁.

In order to fix the genes of interest, unconventional methods such as DNA marker technology can be considered to fix the genes. In contrast, a study conducted by Karaya *et al.* (2009) investigating combining ability in maize resistant to *B. fusca* and *C. partellus* observed importance of additive gene action in conditioning condition exit holes, and tunnel length implying that it is possible to fix these genes. Narrow sense heritability estimates for leaf damage, exit holes and stem tunneling were moderate (ranged between 21% – 26% implying that genetic gain is likely to be realized in selection. The moderate heritability estimates for leaf glossiness, seedling vigour and bloom waxiness implied that genetic gain is likely to be realized when selection is done. Genetic gain may not be realized for deadheart as estimated from the narrow sense heritability since the values are too low. There is need to consider other options such as DNA marker technology to fix the desired genes. Further analysis using Baker's ratio equation to estimate the preponderance mode of gene action suggested days to 50% flowering was largely conditioned by additive genes. Both additive and non-additive genes conditioned plant height, panicle weight and grain yield.

Non additive genes mainly influenced panicle emergence and length of the panicle. Parents IESV 91131 DL, ICSA 467 and Kari Mtama - 1 which scored high positive GCA effect in regard to leaf damage, deadheart, exit holes and several parameters is due to the fact that these parents contributed alleles for susceptibility to borer damage in the crosses. The maximum negative GCA effects observed on ICSB 464, and ICSB 473 implied that these parents contributed positive alleles in the crosses in which they were involved with genes that could augment resistance to *B. fusca* damage. The low negative SCA effects observed on ICSB 474 x Kari Mtama - 1, and ICSB 464 x ICSB 473 implied that these crosses were the best specific combinations for leaf feeding, deadheart, exit holes and stem tunnel damages.

The negative relationship observed between stem tunnels, exit holes, total grain yield and hundred grain mass implied that stem damage contributed to significant reduction in grain yield. This observation may imply that selection for reduction in stem tunneling would lead to increased grain yield. The probable explanation could be due to the fact that stem tunneling causes obliteration of vascular tissues important in translocation of nutrients from the soil to the photosynthetic sites and from the source to the sink organs. The reverse relationship observed between hundred grain mass with exit holes and stem tunnels implied that stem damage contributed to significant reduction in grain yield. A similar observation was reported by Munyiri et al. (2013) on response of tropical maize landraces to damage by *C. partellus*. A study conducted by Odiyi et al. (2007) against the effect of *Sesamia calamistis* (Hampson) and *Eldana saccharina* (Walker) in maize observed that stem tunneling was the major trait contributing to yield loss. Sorghum genotypes with highly glossy, non-vigorous seedlings suffered lower damage compared to the non-glossy but highly vigorous ones. Similar observations have earlier been reported for sorghum shootfly, using some other populations where highly glossy lines were resistant to shoot fly attack (Dhillon *et al.*, 2005).

Tall plants were observed to have high vigor and produced long panicles. This implied that vigorous plants had photosynthetic advantage and were efficient in channeling assimilates to the sink organs hence higher grain yields from such genotypes. Songa et al. (2001) reported that maize with good physical characteristics had significantly increased yield. The positive and significant relationship between exit holes and stem tunnels suggested a close and direct relationship between these two damage traits and therefore, one of them could be used to predict the other. This study also showed that stem tunneling and exit hole damages are controlled by non-additive type of gene action thus the direct significant association between the damage two traits.

Conclusion and recommendation

Additive and non-additive gene effects were important in conditioning resistance traits to *B. fusca*. The preponderance of GCA in conditioning leaf damage implied that additive type of gene action conditioned resistance to this character among the F1 hybrids studied. The significance of SCA over GCA for deadheart, exit holes and stem tunnels damage suggested that non-additive

gene effects which are not fixable were predominant in conferring shoot and stem resistance to *B. fusca*. This study indicated that expression of high leaf glossiness, plant vigour and bloom waxiness in F1 hybrids was conditioned by additive genes and these traits can be used as morphological markers to select for resistance to *B. fusca* in sorghum. ICSB 464 x ICSB 473 was the best combiner for stem borer resistance and grain yield. Parents ICSA 464, ICSB 464 and ICSB 474 were good combiners for resistance to *B. fusca*. These parents can be utilized in development of superior *B. fusca* resistant sorghum hybrids.

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CHAPTER 6

Inheritance of resistance traits to the spotted stem borer in sorghum

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is a key food, fodder as well as income generation crop to many households in semi arid tropics. Sorghum grain output ranges between 0.5 – 0.8 t/ha in low resource farming compared to potential yields of 10 t/ha (Singh *et al.*, 2011). Lepidopteran spotted stem borer, *Chilo partellus* Swinhoe (Crambidae) is one of the most damaging insect pests of sorghum and maize in Asia, East and South Africa (Nanqing *et al.*, 2004). The insect pest was introduced from Asia and has replaced some indigenous stem borers in Kenya (Kfir *et al.*, 2002). *Chilo partellus* causes estimated yield losses of over 13% - 49% in maize depending on pest population, variety and phenological stage of the crop at the time of invasion (Kfir *et al.*, 2002). Stem borers cause an estimated loss of US \$266 million annually in sorghum (Sharma *et al.*, 2003). Chemical control is expensive and unsafe to humans and environment thus development of resistant cultivars is the preeminent option. Enhancement of *C. partellus* resistance has been difficult owing to the complex inheritance of the trait and the large genotype by environment interactions (Yueying *et al.*, 2010). This is the reason why the current study was done in Kenya was to assess the levels of resistance of sorghum genotypes reported to be resistant to *C. partellus* in Asia in Kenya. Multiple traits mainly leaf damage, deadheart, exit holes and stem tunnels are considered for efficient identification of resistant genotypes to *C. partellus* (Singh *et al.*, 2011).

The larvae of *C. partellus* infest sorghum seedlings and thrive till maturity, resulting in grain yield ranging between 15% - 80% (Kfir *et al.*, 2002). The larvae remain protected within stems, and thus, not exposed to insecticides and natural enemies (Muhammad *et al.*, 2009). Sources of sorghum resistance to *C. partellus* have been identified, and genetic effects involved in the inheritance of traits related to sorghum damage by this borer have been investigated in different backgrounds (Yueying *et al.*, 2010). Identification and utilization of resistance genes, from varieties that demonstrate high levels of resistance, could be an outstanding contribution in management of this insect pest. An estimate of various genetic parameters suggests a strategy for effective breeding strategies (Panhwar *et al.*, 2008). The objective of this study was to

establish the nature of gene action for components that contribute to resistance or susceptibility to *C. partellus* in grain sorghum.

Materials and Methods

Experiments to investigate inheritance of resistance traits to *C. partellus* were conducted in Kenya Agricultural Research Institute (KARI), Kiboko. Kiboko lies between longitude 37° 75'E and latitude 2° 15' S at an elevation of 975 meters above sea level (Karaya *et al.*, 2009). The area receives average rainfall of about 280 mm and overall annual mean temperature is 24°C (Beyene *et al.*, 2011).

Experimental material

The experimental material consisted of sorghum lines with varying levels of resistance to *C. partellus* as described in Table 5.1 of this thesis.

Stem borer neonates

First instar neonates of *C. partellus* utilized in this study were obtained from the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. At 30 days after sowing, five plants in each row were tagged and artificially infested on the whorls with five larvae per plant using a camel hairbrush. Data was taken from marked plants.

Data collection

Observations on leaf damage and deadheart were recorded on per plant basis at two and four weeks after artificial infestation. Percentages of plants showing leaf and deadheart were computed by expressing the number of plants damaged as a percentage of the total number of plants sampled. At harvest, the plants were stripped and the numbers of stem borer exit holes on the stem were counted on each sampled plant. The main stem of plants infested with stem borer larvae were split open from the base to the apex, and the cumulative tunnel length measured in centimeters. Seedling vigor was scored at 30 days after sowing on a scale of 1 – 5, where 1 = low vigor (plants showing minimum growth, less leaf expansion and poor adaptation; 3 = moderate vigor; 5 = high vigor (tall plants with expanded leaves and robustness). Leaf glossiness was recorded at 30 days after sowing on a scale of 1 - 5 where 1 = highly glossy, 3 = moderately glossy, and 5 = non glossy.

Bloom waxiness was recorded on a scale of 1 – 9; where 1 = no observable bloom, 3 = slightly present, 5 = medium, 7 = mostly bloomy, 9 = completely bloomy at the 50% flowering stage. Agronomic traits monitored included plant height, days to panicle emergence, 50% flowering and panicle length. Total grain yield and hundred-grain mass were recorded for each of the sampled plants using a weighing balance (Mettler PM 6000, CH- 8606 GREIFENSEE-ZURICH, made in Switzerland).

Statistical analyses

Data on percentages was arcsin transformed while that of counts was log transformed before analysis of variance. General analysis of variance was performed for all the traits observed using Genstat version 14 statistical software (Genstat Release 12 Reference Manual, Part 1 Summary, 2009, VSN International, Hemel Hempstead HP1 1ES, United Kingdom). Genetic analyses were performed using line x tester method using the same software (Panhwar *et al.*, 2008). The analysis facilitated an estimate of the variances from expected mean squares and general combining ability (GCA) effects representing additive gene effects and specific combining ability (SCA), denoting non-additive gene effects. The sums of squares of the crosses were partitioned into GCA and SCA effects, and their interaction with the environment was estimated.

Narrow-sense heritability and proportional contribution of females, males, and their interaction were also computed. Narrow-sense heritability = $(V_{gca} / (V_{gca} + V_{sca} + VE)) \times 100$; where, V_{gca} = general combining ability variance, V_{sca} = specific combining ability variance, and VE = error variance (Dhillon *et al.*, 2006). Relative importance of GCA and SCA was estimated according to Baker (1978) as the ratio $\delta^2 GCA_{(f)} + \delta^2 GCA_{(m)} / \delta^2 GCA_{(f)} + \delta^2 GCA_{(m)} + \delta^2 SCA$ where $\delta^2 GCA_{(f)}$, $\delta^2 GCA_{(m)}$ and $\delta^2 SCA$ are the variance components for GCA and SCA, respectively. Correlations analysis was also performed to understand the association between the morphological and traits linked with sorghum resistance to the stem borer.

Results

Leaf feeding, deadheart formation, exit holes and stem tunnel damages

Results of mean squares for deadheart, leaf damage, exit holes and stem tunneling are presented in Table 6.1.

Table 6.1: Mean squares of stem borer damage traits and narrow sense heritability estimates across environment for *C. partellus* in grain sorghum in Kiboko, Kenya in 2010/2011 rain season

Source of variation	d.f.	DH	LD	EH	ST
Rep	1	1511.6	985.2	1.1255	3042
Season	1	4789.3**	2960.2**	0.4177 ns	1476.4*
GCA _f	14	778.2**	284.5*	0.6007**	1309.4**
GCA _m	1	2338.1**	72.7 ns	0.2884ns	173ns
SCA	14	405**	176.6 ns	0.5059**	1481.5**
Residual	59	162.1	151.5	0.1493	238.9
Proportional contribution to total variance					
Females		308.05	66.5	0.23	535.25
Males		145.07	-5.25	0.01	-4.39
Females x Males		242.9	25.1	0.36	1242.6
Baker's Ratio		0.65	0.71	0.40	0.30
Narrow-sense heritability (%)					
		53	26	31	26

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

GCA_f = general combining ability for females, GCA_m = general combining ability for males, SCA = specific combining ability, DF = Degrees of freedom, DH = Deadheart damage; LD = Leaf feeding damage; EH = Number of exit holes; ST = Stem tunnels damage

The mean squares due to GCA_f were significant for deadheart, leaf damage, exit holes and stem tunneling. Among the four damage traits measured, significant mean squares due to GCA_m was significant for deadheart. Significant SCA mean squares were recorded for deadheart, exit holes and stem tunneling. Proportional contribution to total variance for deadheart was highest among the females and the least among males. Proportional contribution to total variance for leaf damage was highest for the females and the lowest from the males. Proportional contribution to total variance for exit holes was highest for female and male interaction and the least from the males. Proportional contribution to total variance for stem tunneling was highest for female and male interactions and lowest due to males. Baker's ratio for deadheart, leaf damage, exit holes and stem tunneling ranged between 30% - 71% while narrow-sense heritability for the same traits was ranged between 26% - 53%.

Agronomic and morphological traits

The results on analysis of variance for agronomic and morphological traits are presented in Table 6. 2. Mean squares due to GCAf were significant for plant height, days to 50 % flowering, days to panicle emergence, panicle length, total grain weight, hundred grain mass and bloom waxiness. Mean square GCAm were significant for plant height, panicle length, total grain weight, hundred grain mass and bloom waxiness. SCA mean squares were significant for all the traits measured. Proportional contribution to total variance was greatest among females for plant height and panicle length. Proportional contribution to total variance was greatest in the interaction between females and males for days to 50% flowering, days to panicle emergence, panicle length, total grain weight, hundred grain mass and bloom waxiness. Baker's ratio ranged between 34% - 89% while narrow sense heritability for the same traits ranged between 31% - 87%.

Table 6. 2: Mean squares from line x tester analysis for agronomic and morphological traits in sorghum in 2010/2011 rain season, Kiboko Kenya

Source	Df	PH	FL	PE	PL	TGW	HSM	BW
Rep	1	33.5	0.13	70.53	1.07	272.4	7.55	0.03
GCA _f	14	12470.4**	208.2**	54.56**	169.52**	5185**	3.12**	8.23**
GCA _m	1	2324.2*	4.8	16.13ns	66.2*	16102.5**	12.13**	2.7*
SCA	14	4687.9**	149.59**	53.99**	170.25**	4853.4**	3.60**	3.2**
Residual	539	518.3	15.57	7.4	12.46	531.3	0.26	0.4
Proportional contribution to total variance								
Females		5976.1	96.3	3276.3	437	2326.9	1.4	47.9
Males		120.4	-0.7	62.5	10.5	1038.1	0.8	-0.1
Females x Males		4169.6	134	8060.4	53.7	4322.1	3.3	133.6
Baker's Ratio		0.59	0.42	0.34	0.89	0.43	0.4	0.59
Narrow sense heritability (%)								
		0.56	0.39	0.31	0.87	0.41	0.38	0.56

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively, ns=non significant

GCA_f = general combining ability for females, GCA_m = general combining ability for males, SCA = specific combining ability, Df = Degrees of freedom, PH = Plant height; FL= Days to 50 % flowering; PE = Days to panicle emergence; PL= Panicle length; TGW = Total grain yield; HSM = Hundred seed mass; BW = Bloom waxiness

Chilo partellus sorghum damage, agronomic performance and morphological traits

The results of the mean performance of the least damages due to leaf feeding, deadheart, exit holes and stem tunneling were observed on crosses ICSA 464 x ICSB 473 and Macia x ICSB 473 as presented in Table 6.3. Hybrid that was highly susceptible to afore mentioned borer damage was IESV 91131 DL x ICSB 473. Panicle length ranged from 17-30 cm with a grand mean of 24 cm. Longest panicles were recorded on ICSB 464 x Kari Mtama - 1 and IS 8193 x ICSB 473, while Seredo x Kari Mtama - 1 produced the smallest panicles. The tallest cross was ICSA 474 x Kari Mtama - 1 while Gadam x ICSB 473 was the shortest. Days to 50 % flowering ranged from 62 to 82 days on Seredo x ICSB 473 and ICSA 464 x Kari Mtama - 1 respectively. ICSA 464 x Kari Mtama - 1 and Seredo x ICSB 473 took the longest and shortest days to panicle emergence respectively.

Cross IESV 91131 DL x Kari Mtama - 1 produced the highest total grain weight while ICSB 464 x Kari Mtama - 1 weighed the least. Highest hundred grain mass was observed on IESV 91131 DL x Kari Mtama - 1, ICSB 474 x Kari Mtama - 1, IESV 93042 SH x ICSB 473 and ICSA 474 x Kari Mtama - 1 while Seredo x Kari Mtama - 1 recorded the least. Leaf toughness ranged between 0.20 to 0.35 in ICSA 472 x Kari Mtama - 1 and ICSA 474 x ICSB 473 and respectively. Bloom waxiness ranged from 3.5 (slightly present) on ICSA 472 x ICSB 473 to 8.0 (mostly bloomy) on Gadam x Kari Mtama - 1, IESV 91131 DL x Kari Mtama - 1 and IESV 91131 DL x ICSB 473 at 50% flowering stage.

Table 6. 3: Means for *Chilo partellus* damage, agronomic and morphological traits for sorghum F₁ crosses

F1 hybrids	DH	LD	EH	ST	PH	PE	FL	PL	TGW	HGM	BW
Gadam x ICSB 473	13	37	7	25	125	62	68	21	40	2.2	6.0
Gadam x Kari Mtama - 1	32	28	3	20	129	64	71	22	53	2.7	8.0
ICSA 464 x ICSB 473	7	40	7	18	154	63	70	23	42	2.5	5.0
ICSA 464 x Kari Mtama - 1	1	35	3	9	175	62	67	24	17	1.6	5.0
ICSA 467 x ICSB 473	7	36	2	9	142	62	70	24	19	1.8	5.0
ICSA 467 x Kari Mtama - 1	7	40	11	25	176	67	73	27	39	2.5	4.0
ICSA 472 x ICSB 473	1	28	4	9	183	68	75	19	16	2.4	3.5
ICSA 472 x Kari Mtama - 1	26	27	5	11	156	61	69	22	20	1.9	5.0
ICSA 474 x ICSB 473	13	40	5	12	194	64	70	23	14	2.1	5.0
ICSA 474 x Kari Mtama - 1	26	33	11	18	204	64	71	24	30	2.9	5.5
ICSB 464 x ICSB 473	1	30	6	17	159	60	70	26	49	2.6	7.0
ICSB 464 x Kari Mtama - 1	20	31	10	37	174	55	62	30	13	2.5	5.5
ICSB 467 x ICSB 473	7	40	10	18	192	62	69	27	17	1.9	5.0
ICSB 467 x Kari Mtama - 1	7	35	8	27	174	63	70	28	45	2.3	5.0
ICSB 474 x ICSB 473	1	32	6	35	186	60	67	27	26	2.4	5.0
ICSB 474 x Kari Mtama - 1	26	15	3	10	191	60	66	24	45	3.0	6.0
IESV 91131 DL x ICSB 473	45	33	10	34	159	64	72	24	33	2.4	8.0
IESV 91131 DL x Kari Mtama - 1	23	33	10	33	140	64	71	23	61	3.0	8.0
IESV 93042 SH x ICSB 473	23	28	6	22	154	60	68	24	56	2.9	4.5
IESV 93042 SH x Kari Mtama - 1	26	32	3	10	157	61	68	25	55	2.7	5.0
IS 21879 x ICSB 473	20	30	6	24	161	63	69	24	52	2.0	5.0
IS 21879 x Kari Mtama - 1	19	36	5	18	139	65	72	24	53	2.5	7.0
IS 21881 x ICSB 473	20	45	5	17	155	62	69	25	34	2.4	5.0
IS 21881 x Kari Mtama - 1	32	46	7	16	167	67	74	23	43	2.4	5.5
IS 8193 x ICSB 473	32	52	6	23	159	59	65	30	43	1.9	5.5
IS 8193 x Kari Mtama - 1	23	30	5	17	145	65	72	21	39	2.0	6.0
Macia x ICSB 473	16	37	4	8	173	69	75	22	17	1.6	5.0
Macia x Kari Mtama - 1	45	32	9	22	180	71	76	25	49	2.7	5.0
Seredo x ICSB 473	25	35	5	26	153	65	73	27	48	2.6	7.5
Seredo x Kari Mtama - 1	36	46	4	14	161	66	72	17	19	1.4	5.0
F value	0.007	0.325	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD	18.01	17.42	0.24	9.60	14.14	3.82	5.54	2.19	14.32	0.32	0.89
CV	26	36	10	16	14	4	5	15	35	22	18

DH = Deadheart damage; LD = leaf feeding damage; EH = exit holes; ST = stem tunnels; PH = plant height; PE = days to panicle emergence; FL = days to 50 % flowering; PL = Panicle length; TGW = total grain mass; HGM = hundred grain mass; BW = bloom waxiness

Nature of gene action

The results of the GCA effects for females and males are presented in Table 6.4. The GCA effects suggested that among female lines, ICSA 472 and ICSA 464 exhibited maximum GCA effect of -24.39 and -19.87 respectively, while between males, ICSB 473 displayed highest significant GCA effect of -4.68 implying their good general combining ability for leaf feeding, deadheart formation, exit holes and stem tunnels damages. Genotype IESV 91131 DL and Kari Mtama - 1 scored high significant positive GCA effects for heart damage and exit holes. Parent IS 21881 scored the highest significant positive GCA effects for leaf damage and exit holes. Genotype ICSA 467, ICSA 474, ICSB 464, ICSB 467, Macia and Kari mtama - 1 scored significant positive GCA effects for exit holes.

Maximum GCA positive significant effects for plant height among females were observed on ICSA 474 (33.89), ICSB 467, and ICSB 474 (23.19) while among males ICSB 473 displayed higher GCA effect of 1.98. On the other hand, low GCA effect of - 38.08 and - 15.91 were observed for plant height on Gadam and IESV 91131 DL respectively suggesting that these parents contributed alleles for dwarfness in the crosses. High significant positive GCA effects for days to 50 % flowering and panicle emergence among females were recorded on ICSA 464, ICSA 472 and Macia while a low GCA effect for the same trait was recorded on IS 21881 and Gadam. Greatest GCA effects among females for panicle length were observed on females ICSB 474, ICSA 467, IS 8193 and male ICSB 473. Significant positive GCA effects were recorded on genotypes Gadam, IS 21879, IESV 93042 SH, IESV 91131 DL, IS 8193, and Kari Mtama - 1 for grain yield. High GCA effects for bloom waxiness was observed on Seredo, ICSB 464, Gadam, IESV 91131 DL while low GCA effects for the same trait were observed on ICSA 472, ICSA 467 and IESV 93042 SH.

Table 6. 4: General combining ability effects of females and males for *Chilo partellus* damage on leaf feeding, deadheart and stem damages, agronomic and other traits in sorghum

Females	DH	LD	EH	ST	PH	FL	PE	PL	TGM	HGM	BW
Gadam	3.52	-1.96	-1.14	2.87	-38.08	-6.03	-2.62	-2.71	10.91**	0.08	1.45**
ICSA 464	-15.85	3.34	-1.24	-6.12	-1.08	12.72**	5.63*	-0.63	-10.61	-0.27	-0.55
ICSA 467	-12.55	3.64	0.34**	-2.68	-6.51	0.72	0.38	1.38*	-11.45	-0.15	-1.05
ICSA 472	-6.15	-6.79	-1.89	-9.56	4.92	6.72**	4.13**	-3.53	-17.26	-0.21	-1.3
ICSA 474	0.65	1.44	1.71**	-1.26	33.89**	2.72*	0.88	-0.88	-13.02	0.18*	-0.8
ICSB 464	-9.35	-7.79	1.94**	7.38**	1.39	-1.03	-0.37	3.74**	-4.49	0.21*	0.7**
ICSB 467	-12.55	2.58	2.76**	2.81	17.79**	-2.03	0.38	3.52**	-4.05	-0.2	-0.55
ICSB 474	-6.15	-10.96	-1.41	2.78	23.19**	-6.53	-4.37	1.12*	0.31	0.35**	-0.05
IESV 91131 DL	14.59**	-1.56	3.44**	13.82**	-15.91	-3.53	-0.62	-0.46	11.65**	0.4**	2.45**
IESV 93042 SH	5.39	-4.49	-1.76	-3.73	-9.54	-0.78	1.38	0.17	20.37**	0.5**	-0.8
IS 21879	0.32	-1.49	-0.96	1.14	-15.31	2.47*	-0.12	-0.16	17.21**	-0.09	0.45*
IS 21881	6.82	11.41*	0.14*	-3.55	-4.61	-5.78	-3.37	-0.11	3.37	0.1	-0.3
IS 8193	8.12*	6.24	-0.51	0.39	6.79*	-2.53	-0.37	1.54*	5.95*	-0.37	0.2
Macia	11.72*	0.04	0.39**	-4.82	11.39**	2.47*	1.13	-0.6	-7.21	-0.21	-0.55
Seredo	11.45*	6.34	-1.76	0.52	-8.31	0.47	-2.12	-2.41	-1.71	-0.31	0.7**
Males											
ICSB 473	-4.42	0.78	-0.5	-0.54	1.98	0.2	0.37	0.33	-5.18	-0.14	-0.15
Kari- mtama 1	4.41**	-0.78	0.51**	0.54	-1.97	-0.2	-0.37	-0.33	5.18	0.14*	0.15
LSD Female	17.9**	15.19ns	0.17**	6.7*	11.7*	7.0**	4.8**	1.7**	10.5**	0.2**	1.1**
Male	6.6**	5.55ns	.06*	0.06*	2.5	4.2*	2.6*	1.8ns	4.8**	3.8**	0.4ns

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively, ns=non significant

DH = deadheart damage; LD = leaf feeding damage; EH =; number exit holes ST = stem tunnel damage; PH = plant height; FL = days to 50 % flowering; PE = days to panicle emergence; PL = panicle length; TGM = total grain mass; HGM = hundred grain mass; BW = bloom waxiness.

Specific combining ability results for different traits are presented in Table 6.5. Crosses ICSB 464 x ICSB 473, Macia x ICSB 473, IESV 91131 DL x Kari Mtama - 1, ICSB 474 x Kari Mtama - 1, ICSA 467 x ICSB 473, ICSA 472 x ICSB 473, ICSA 474 x ICSB 473, IESV 93042 SH x Kari Mtama - 1, ICSA 464 x Kari Mtama - 1, ICSB 467 x Kari Mtama - 1 and Gadam x ICSB 473 were the best specific combiners for reduced leaf feeding, deadheart, exit holes and stem tunneling damages. Results indicated that Seredo x ICSB 473, Macia x Kari Mtama - 1, ICSA 472 x ICSB 473, ICSA 464 x ICSB 473, ICSA 474 x Kari Mtama - 1, IESV 93042 SH x ICSB 473, ICSA 467 x Kari Mtama - 1, ICSB 464 x ICSB 473, IESV 91131 DL x Kari Mtama - 1 and ICSB 474 x Kari Mtama - 1 were the best specific combinations for hundred grains mass.

Highest specific combining ability for dry panicle weight was observed on ICSB 464 x ICSB 473 (36.19) and Macia x Kari Mtama - 1 (21.86). The best specific combiners for panicle length were Seredo x ICSB 473, IS 8193 x ICSB 473, ICSB 464 x Kari Mtama - 1, ICSA 472 x Kari Mtama - 1, ICSA 467 x Kari Mtama - 1, Macia x Kari Mtama - 1, ICSB 474 x ICSB 473 and Gadam x Kari Mtama - 1. Regarding plant height, ICSA 467 x Kari Mtama - 1, ICSA 472 x ICSB 473, ICSB 464 x Kari Mtama - 1, IS 21879 x ICSB 473, ICSA 464 x Kari Mtama - 1, IS 21881 x Kari Mtama - 1 were among the longest while IS 8193 x Kari Mtama - 1, ICSA 467 x ICSB 473, ICSA 472 x Kari Mtama - 1 and ICSB 464 x ICSB 473 were among the dwarfed.

Table 6. 5: Specific combining ability estimates of F1 hybrids from line x tester analyses of various traits in sorghum

F1 hybrids	DH	LD	EH	ST	PH	PL	TGW	HGM	BW	FL	PE
Gadam x Kari Mtama - 1	4.93	4.02	-2.08	-3.04	4.12	1.11	1.19	0.11	0.85**	0.2	-1.12
Gadam x ICSB 473	-4.93	-4.02	2.08**	3.04	-4.12	-1.11	-1.19	-0.11	-0.85	-0.2	1.12
ICSA 464 x ICSB 473	-1.12	3.38	1.27**	3.82	-8.38	-0.32	11.99*	0.32*	-0.15	3.0*	2.87*
ICSA 464 x Kari Mtama - 1	1.12	-3.38	-1.27	-3.82	8.38*	0.32	-11.99	-0.32	0.15	-3.0	-2.87
ICSA 467 x ICSB 473	4.42	-3.8	-3.70	-7.23	-19.07	-1.77	-9.73	-0.22	0.65*	2.6	1.38
ICSA 467 x Kari Mtama - 1	-4.42	3.08	3.70**	7.23*	19.07**	1.77	9.73*	0.22*	-0.65	-2.6	-1.38
ICSA 472 x Kari Mtama - 1	8.58	0.23	-0.33	0.29	-10.48	2.08*	-3.36	-0.37	0.6*	6.5**	0.87**
ICSA 472 x ICSB 473	-8.58	-0.23	0.33**	-0.29	10.48*	-2.08	-3.36	0.37*	-0.60	-6.5	-0.87
ICSA 474 x Kari Mtama - 1	2.08	-2.22	2.62**	5.99*	6.87	0.53	2.61	0.29*	0.60*	-2.6	-0.12
ICSA 474 x ICSB 473	-2.08	2.22	-2.62	-5.99	-6.87	-0.53	-2.61	-0.29	-0.60	2.6	0.12
ICSB 464 x Kari Mtama - 1	5.38	5.53	1.8**	9.46**	9.27*	2.16*	-23.18	-0.20	-0.90	-0.8	-2.13
ICSB 464 x ICSB 473	-5.38	-5.53	-1.80	-9.46	-9.27	-2.16	23.18**	0.2*	0.90**	0.8	2.13
ICSB 467 x Kari Mtama - 1	-4.42	-1.67	-1.98	3.69	-6.83	0.88	9.15*	0.06	-0.15	0.2	0.62
ICSB 467 x ICSB 473	4.42	1.67	1.98**	-3.69	6.83	-0.88	-9.15	-0.06	0.15	-0.2	-0.62
ICSB 474 x ICSB 473	-8.58	7.82	1.71**	12.65**	-4.67	1.47	-4.45	-0.14	-0.35	3.3*	3.63*
ICSB 474 x Kari Mtama - 1	8.58	-7.82	-1.71	-12.65	4.67	-1.47	4.45	0.14	0.35	-3.3	-3.63
IESV 91131 DL x ICSB 473	15.67*	-0.78	0.41**	0.59	7.23	0.04	-8.91	-0.15	0.15	1.3	0.38
IESV 91131 DL x Kari Mtama - 1	-15.67	0.78	-0.41	-0.59	-7.23	-0.04	8.91*	0.15	-0.15	-1.3	-0.38
IESV 93042 SH x ICSB 473	2.97	-3.03	2.01**	6.34*	-3.42	-0.63	5.27	0.26*	-0.10	0.5	0.38
IESV 93042 SH x Kari Mtama - 1	-2.97	3.03	-2.01	-6.34	3.42	0.63	-5.27	-0.26	0.1	-0.5	-0.38
IS 21879 x ICSB 473	4.57	-4.03	0.96**	3.46	9.13*	-0.36	4.65	-0.11	-0.85	5.8*	0.88*
IS 21879 x Kari Mtama - 1	-4.57	4.03	-0.96	-3.46	-9.13	0.36	-4.65	0.11	0.85**	-5.8	-0.88
IS 21881 x ICSB 473	-1.93	-1.33	-0.40	1.02	-7.87	0.39	0.48	0.14	-0.10	-1.5	-1.37
IS 21881 x Kari Mtama - 1	1.93	1.33	0.40**	-1.02	7.87	-0.39	-0.480	-0.14	-0.15	1.5	1.37
IS 8193 x ICSB 473	9.17	10.22*	1.06**	3.21	24.63**	4.09**	6.8	0.09	-0.10	0.8	0.63
IS 8193 x Kari Mtama - 1	-9.17	-10.22	-1.06	-3.21	-24.63	-4.09	-6.80	-0.09	-0.10	-0.8	-0.63
Macia x ICSB 473	-10.03	1.62	-1.80	-6.22	-5.47	-1.59	-15.38	-0.40	0.15	4.8*	4.13
Macia x Kari Mtama - 1	10.03*	-1.62	1.80**	6.22*	5.47	1.59	15.38*	0.40*	-0.15	-4.8	-4.13
Seredo x ICSB 473	-0.78	6.18	1.06**	6.39*	-5.87	4.79**	19.69*	0.72**	1.40**	-11.2	-6.62
Seredo x Kari Mtama - 1	0.78	-6.18	-1.06	-6.39	5.87	-4.79	-19.69	-0.72	-1.40	11.2**	6.62**

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively

DH = deadheart damage; LD = leaf feeding damage; EH = Number of exit holes; ST = stem tunnel damage; PH = plant height; PL = panicle length; TGW = total grain yield; HGM = hundred grain mass; BW = bloom waxiness; FL = days to 50 % flowering; PE = days to panicle emergence

Association between *C. partellus* damage parameters, agronomic and morphological traits

Results of correlation between *C. partellus* damage, agronomic and morphological traits are presented in Table 6.6. Bloom waxiness was significantly and positively associated with deadheart ($r = 0.38$) ($P = 0.04$) and stem tunnels damages ($r = 0.38$, $P = 0.04$). A positive and significant correlation between bloom waxiness and total grain yield was observed ($r = 0.46$, $P = 0.01$) while a negative and highly significant correlation was observed between bloom waxiness and plant height ($r = -0.49$, $P = 0.006$). A positive and highly significant correlation was observed between exit holes and stem tunnels ($r = 0.67$, $P = <0.001$) and exit holes and panicle length ($r = 0.39$, $P = 0.03$). A positive and significant correlation was observed between days to 50 % flowering and days to panicle emergence ($r = 0.83$, $P = <0.001$).

Negative relationship between days to 50 % flowering and panicle length was observed ($r = -0.53$, $P = 0.003$). Significant and negative correlation was observed between total grain yield and plant height ($r = -0.47$, $P = 0.009$). A significant and negative correlation was observed between days to panicle emergence and panicle length ($r = -0.47$, $P = 0.009$). A positive and significant association was observed between panicle length and stem tunnel ($r = 0.49$, $P = 0.005$).

Table 6. 6: Correlation matrix between morphological and *C. partellus* damage parameters

BW	-									
DH	0.38*	-								
EH	0.08	0.13	-							
FL	0.09	0.25	-0.16	-						
TGM	0.46*	0.31	0.08	0.06	-					
LD	-0.16	0.03	0.16	0.02	-0.17	-				
PE	-0.09	0.16	0.04	0.83**	0.02	0.15	-			
PH	-0.49**	-0.17	0.28	-0.07	-0.47**	-0.07	0.11	-		
PL	0.04	-0.12	0.39*	-0.53**	0.14	0.12	-0.47**	0.24	-	
ST	0.38*	0.15	0.67**	-0.18	0.27	0.03	-0.20	-0.12	0.49**	-
	BW	DH	EH	FL	TGM	LD	PE	PH	PL	ST

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively. Ns=not significant

BW = bloom waxiness, DH = deadheart damage, EH = number of exit holes, FL= days to 50 % flowering, TGM = total grain mass, LD = leaf feeding damage, PE = Days to panicle emergence, PH = plant height, PL = panicle length, ST = stem tunneling damage

Discussion

This study found out that from Baker's ratio, general combining ability (GCA) and specific combining ability (SCA) for deadheart damage, leaf damage, exit holes and stem tunnel damages were important. This observation implied that these damage traits are conditioned by both additive and non-additive types of gene actions. Additive type of gene action was predominant for leaf feeding and deadheart damages while both additive and non-additive types of gene action were important for number of stem borer exit holes and stem tunnels. The negative combining ability effects for plant damage mainly leaf feeding, deadheart formation, stem tunnels and exit holes damages suggested contribution of the genotype towards resistance, while positive combining ability effects implied contribution to susceptibility in the crosses. Selection of resistant parents with high negative GCA effects to these traits could be effective for development of *C. partellus* resistant sorghum. The moderate values for narrow sense heritability in the present study suggested that conventional pedigree and early generation selection methods can be effective for initial improvement in sorghum resistance against *C. partellus*. The reason for considering leaf damage, dead heart formation, exit holes and stem tunnel damages is due to the fact that selecting for resistance based on a single parameter is not effective and reliable (Singh *et al.*, 2011).

Several authors have reported similar observations with the current study in different backgrounds. Pathak (1990) reported that both additive and non additive genes are important for inheritance of stem borer resistance but, additive gene effect is more important. A study conducted by Butron *et al.* (2009) on genetics of resistance to the pink stem borer (*Sesamia nonagrioides*) in maize showed that genetic effects of traits related to stem and ear damage by the pink stem borer fitted an additive– dominant model. In this study, the relative significance of GCA to SCA variances as depicted from Baker's ratio implied that the additive type of gene action conditioned deadheart and leaf damage. Similar observation was reported by Singh and Verma (1988) who observed that additive gene effects were predominant for deadheart and leaf injury in sorghum against *C. partellus*. Selection of resistant parents to these traits could thus be effective for development of *C. partellus* resistant sorghum cultivars.

The comparative significance of SCA to GCA for exit holes and stem tunneling implied that non additive mode of gene action was predominant over additive gene action. Singh and Verma

(1988) also observed that GCA effects were predominant in sorghum for leaf damage, whereas specific combining ability SCA effects were important for stem damage. The significance of GCA as well as SCA for agronomic and morphological traits studied suggested the presence of both additive and non-additive gene actions. Sharma et al. (2007) observed that leaf feeding damage, overall resistance, panicle initiation and plant height were conditioned by dominance type of gene action. Specific combining ability was greater than GCA males for maturity dates and grain components implying that female parents might have been influential in determining days to maturity and grain yield.

Females for example ICSA 472 and ICSA 464 and male ICSB 473 that exhibited highest negative GCA effects in regard to leaf feeding, deadheart formation, exit hole and stem tunnels implying their good general combining ability to these damage traits. These parents could be utilized in development of *C. partellus* resistant sorghum cultivars for cultivation by farmers in *C. partellus* endemic regions. Genotype IESV 91131 DL that scored the highest GCA in regard to leaf damage, deadheart, exit holes and stem tunnels possibly contributed tolerance to stem borer damage since crosses involving this line had relatively high total grain yield. Genotypes that were completely bloomy suffered low leaf feeding, deadheart and stem damages as a result of *C. partellus* attack implying that bloom waxiness possibly contributed to resistance mechanism.

Bloom wax may have interfered with stem borer larvae movement and leaf feeding. The positive and highly significant association between exit holes and stem tunnel imply a positive and direct relationship between the two damage parameters and so either of the two traits can be used to predict the other. In addition, selecting for exit holes would also ensure selecting for stem tunneling thus possibility of selecting for more than one trait. The negative significant correlation between panicle length and days to flowering implied that late maturing sorghum genotypes were high grain yielders. The highly significant and positive correlation between panicle length and stem tunnel implied that plants with a long panicle would suffer intense damage since a large surface area was available for *C. partellus* feeding hence grain yield would be negatively affected. In addition the positive correlation between panicle length and stem tunnel may imply tolerance mechanism of resistance where by the panicle size would not be affected by stem borer damage.

Conclusion and recommendation

This study suggests that spotted stem borer leaf feeding and deadheart damages are governed by additive type of gene action while exit holes and stem tunneling are conditioned by both additive and non-additive types of gene action. Genetic gain is likely to be realized through conventional breeding for these traits since the narrow sense heritability estimates were moderate. The significance of GCA and SCA mean squares suggests the importance of both additive and non-additive types of gene action respectively for all the traits studied. The SCA effects revealed that, for hybrid development, cross ICSB 464 x ICSB 473 could be the best option for *C. partellus* damage resistance.

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CHAPTER 7

QTL mapping for trait associated with resistance to the African and spotted stem borers in sorghum

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important food crop in arid and semi arid tropics where it is cultivated by more than 500 million small scale farmers. Sorghum has become a key industrial crop in alcohol production and has untapped potential in bio-energy production in East Africa (Taylor, 2010). African stem borer, *Busseola fusca* Fuller (Noctuidae) and the spotted stem borer *Chilo partellus* Swinhoe (Crambidae) lepidopterans are among the economically important insect pests of sorghum and maize Eastern and South Africa (Beyene *et al.*, 2011). Stem borers are associated with grain yield loss of 15 % - 80 % depending on borer population and variety phenological stage at the time of attack (Karaya *et al.*, 2009). Utilization of sorghum resistance through genetic enhancement could address these constraints. Breeding for stem borer resistance has been slow partly due to their quantitative nature and the strong genotype by environment interactions. Phenotypic evaluations for resistance to *B. fusca* and *C. partellus* suggest that both additive and non-additive gene effects condition resistance to these borers (Chapters 5 and 6 of this thesis). Identification of QTLs associated with sorghum resistance to stem borers could enhance the pace of development of resistant cultivars for sustainable production. There is no information about genomic areas associated with dual resistance to *B. fusca* and *C. partellus* in sorghum.

Single nucleotide polymorphisms (SNPs) markers represent the finest resolution (a single nucleotide) of a DNA sequence. There are generally abundant in populations with a low mutation rate (Divya *et al.*, 2011). SNPs markers have become widely accepted as a tool for understanding complex genetic traits and evolution (Bernado, 2008). SNPs have been found to be the most efficient genetic marker for gene identification since they are codominant, highly polymorphic and have good reproducibility (Divya *et al.*, 2011). Currently, genotyping-by-sequencing approach has been employed in whole genome sequencing to discover SNPs for mapping studies in maize and barley (Metzker, 2010; Elshire *et al.*, 2011). Quantitative trait loci mapping using recombinant inbred lines increases power of QTL detection compared to F_{2:3}

population because of complete homozygosity at QTLs and marker loci. The objective of this study was to identify and characterize QTLs associated with resistance traits for *B. fusca* and *C. partellus* in sorghum.

Materials and Methods

Phenotyping

Development of mapping population

Two hundred and forty three F_{9:10} recombinant inbred lines (RILs) derived from a cross between stem borer susceptible cultivar ICSV 745 and stem borer resistant PB 15520-1 were selected for the mapping study. The progenies and their parents were developed following single seed descent approach in ICRISAT- Patancheru, India (Vinayan, 2010). The two parents were crossed and the F₁ seeds obtained were advanced to F₂ by selfing of single F₁ plant. The F₂ seeds were selfed and the resulting F₃ population grown in progeny rows. Single plants in each of the progeny row were selfed and the process continued up to F₉ generation. Seeds from the F₉ plants of each row were bulked to produce the 243 F_{9:10} recombinant inbred line population employed in this study.

Experimental design, site and source of stem borer larvae

The 243 RILs along with their parents were imported to Kenya and phenotyped in Embu and Kabete for *B. fusca* resistance and in Kiboko for resistance against *C. partellus* in 2011 and 2012 rain seasons. Information on sites, source of first instar stem borers neonates and infestation is described in Chapters 3 and 4 of this thesis. Each experiment was laid out in 25 × 10 alpha-lattice design consisting of twenty five plots in ten blocks, replicated two times. Each plot consisted of 2m rows with plants spaced at 0.75m x 0.25m between within rows respectively

Parameters measured and phenotypic data analysis

Damage parameters monitored included leaf damage, deadheart, number of exit holes and stem tunnel length. Morphological traits measured included leaf toughness, seedling vigour, bloom waxiness, leaf glossiness and total grain yield. Procedure on how each trait was measured is described in Chapters 3 and 4 of this thesis. Data were subjected to analysis of variance using

residual maximum likelihood model (ReML) in Genstat Version 14 statistical package. The predicted means for each genotype were estimated with genotypes as fixed and reps as random effects in individual and across environment analyses. Phenotypic and genetic correlation coefficients were calculated from adjusted entry means across environments for each parameter. Estimates of variance components, including genotypic variance (σ^2_g), genotype \times environment interaction ($\sigma^2_{g \times e}$) and residual (σ^2_e) were calculated by equating the mean squares to their expected values. Broad-sense heritability (H) was estimated using $H = (\hat{\sigma}^2_g) / (\hat{\sigma}^2_g + \hat{\sigma}^2_e)$ where $\hat{\sigma}^2_g$ = Genotypic variance, $\hat{\sigma}^2_e$ = error variance (Kassa *et al.*, 2007). Direct and indirect effects analysis was conducted using path analysis to study interrelationship among resistance parameters and their relationship to grain yield reduction (Garson, 2012). Correlations were calculated to understand the relationship between stem borers resistance and agronomic traits.

DNA extraction, quantification and normalization

Leaf tissue from the parental lines and the 243 RILs were harvested from 10 days old sorghum seedlings. DNA was extracted according to Mace *et al.* (2003). The quality of DNA in each sample was checked using 0.8% agarose gels stained with ethidium bromide (Vinayan, 2010). Each well of the agarose gel was loaded with 5 μ l of sample and the gel was allowed to run at 100 V for 5 minutes. After electrophoresis, DNA banding patterns on the gel were visualized under UV light. A smear of DNA indicated poor quality and the DNA was re-extracted whereas a clear band indicated good quality DNA. The quantity of DNA in each sample was assessed using a fluorescence spectrophotometer (Switzerland) by staining the DNA with pico greenTM (1/200 dilution). DNA concentration of each sample was calculated and then normalized to 2.5ng/ μ l for the PCR.

PCR amplification of SNPs

PCR reactions were conducted in 96-384 well plate using the PE9700 Perkin Elmer DNA thermal cycler (USA). The PCR program consisted of an initial denaturation for 15 minutes at 94⁰C and then 10 cycles of denaturation for 10 seconds at 94⁰C, 35 cycles of annealing for 20 seconds at 61⁰C-52⁰C, and extension for 30 seconds at 72⁰C and 35 cycles of denaturation for 10 s at 94⁰C, annealing for 20 s at 54⁰C and extension for 30 s at 72⁰C. The last PCR cycle was followed by a 20 min extension at 72⁰C to ensure amplification of the same lengths of both DNA strands. PCR products were then separated in 6% non-denaturing polyacrylamide gels and silver

stained.

Scoring of sequenced products

The SNPs markers screened on the RILs were scored as; A = Homozygote carrying allele from female parent (ICSV 745), B = Homozygote carrying allele from male parent (PB 15520-1), and - = Missing data for individual at a locus. The data were saved in a Microsoft excel sheet for linkage map analysis using Join Map 4.0 software package to develop linkage maps from molecular data obtained from the crosses between the two parents (Ooijen, 2006).

Construction of linkage map

The two hundred and forty three RILs were used for linkage mapping using JoinMap 4.0 (Ooijen, 2006). The genotypic data was used to construct a genetic linkage map, which spanned 4692.4 cM with a total of 4955 SNP markers distributed into the 10 sorghum linkage groups (Figure 7.1).

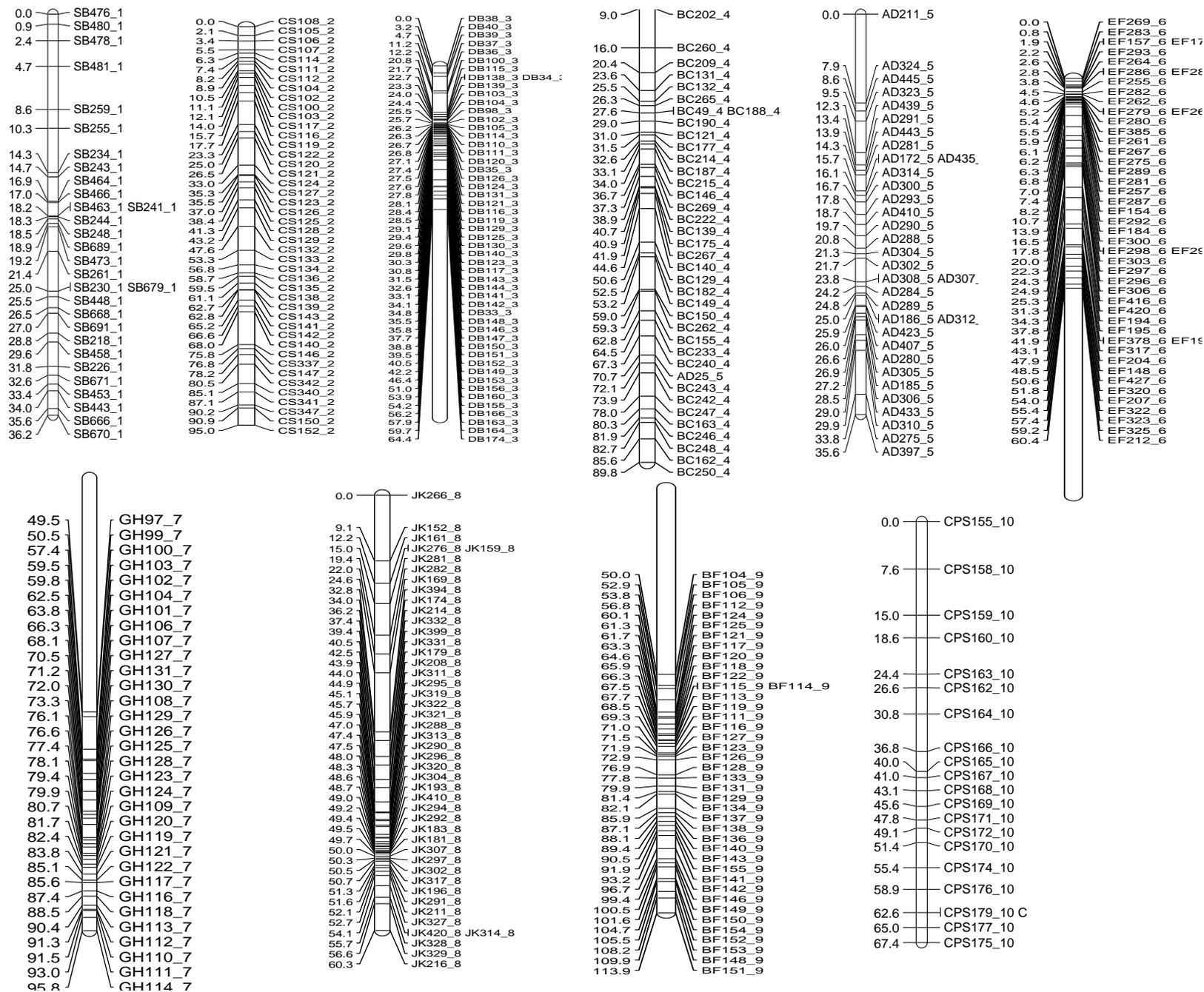


Figure7. 1: Genetic map of ICSV 745 x PB 15520 recombinant inbred lines of sorghum generated using 4955 polymorphic SNP markers

The segregation of each marker was tested with a chi-square goodness of fit test to the expected Mendelian segregation ratio (1:1) of parental configuration. The markers that did not conform to the expected segregation ratio were excluded from the analysis. Three-point linkage analysis was performed for each linkage group and the most saturated linkage was adopted (Ooijen, 2006). After the addition of each loci, a ripple was used to verify local locus orders (Ooijen, 2006).

QTL analysis

QTL analyses were performed on stem borer leaf damage, deadheart damage, exit holes and stem tunneling. Composite interval mapping (CIM) was performed on the data using PLABQTL software, version 1.2 (Utz and Melchinger, 2003). Whole-genome scan with CIM was conducted using automatic cofactor selection, model to determine additive effects at individual QTL and F value of ten options were selected. Cofactors were chosen by step-wise regression and Akaike's information criterion (AIC) of 3.0 with the "cov" statement in PLABQTL. Dependence of QTL estimation on sampling effects was estimated by a five-fold cross validation by dividing the genotypes into five subsets. The LOD threshold for declaring a putative QTL was set to 3.0 after performing 1000 permutation tests (type I error level $\alpha = 10\%$). All QTLs identified in this study explained $\geq 10\%$ of the total phenotypic variation thus classified as major QTLs (Kassa *et al.*, 2007). Genetic maps and QTL graphs were drawn using MapChart program, version 2.1 (Voorrips 2002, Wageningen, Netherlands).

Results

Phenotypic analysis

The results of mean squares for leaf feeding, deadheart, exit holes and agronomic and morphological traits in Kiboko are presented in Table 7.1.

Table 7.1: Mean squares for *Chilo partellus* damage, agronomic and morphological traits

Source	df	DH	LD	EH	ST	PH	PE	FL	PL	VG	GL	BW	TGY
Rep	1	9957.3	1276.3	360.2	8265.5	1920	5.87	0.7	41.35	96.60	5.24	27.4	3.4
Genotype	242	260.5	459.6	80.58**	591.3**	28927**	31.4	39.5	184.1**	0.98**	0.25**	3.085	3.033**
Residual	5052	734.6	427.6	33.8	266.9	23343	32.8	34.8	17.5	0.7	0.16	2.5	1.682
BSH (<i>H</i>)		0.47	0.04	0.40	0.38	0.11	0.49	0.53	0.83	0.17	0.22	0.10	0.29

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

DF = Degrees of freedom, DH = Deadheart damage, LD = Leaf feeding damage, EH = Number of exit holes, ST = Stem tunnel damage, PH = Plant height, PE = Days to panicle emergence, FL = days to 50% flowering, PL = Panicle length, VG = Seedling vigour, GL = Leaf glossiness, BW = Bloom waxiness, TGW= Total grain weight, BSH (*H*) = Broad sense heritability estimates

There were significant differences observed for all the traits measured except for deadheart, leaf feeding damage, days to panicle emergence and days to 50% flowering. The heritability estimates for damage parameters namely deadheart, leaf feeding, exit holes and stem tunnel length ranged from low to moderate between 0.04% - 47 %. Heritability estimates for plant height, days to panicle emergence, days to 50% flowering, panicle length, seedling vigor, leaf glossiness, bloom waxiness and total grain yield ranged between 10% - 83%.

The results of mean squares for leaf feeding, deadheart, exit holes, agronomic and morphological traits in Embu are presented in Table 7.2.

Table 7.2: Mean squares for *Busseola fusca* damage, agronomic and morphological traits

Source	df	DH	LD	EH	ST	PH	PE	FL	PL	VG	GL	BW	TGY
Rep	1	18491	5552.1	0.8	594.3	682.1	5.9	1.4	41.8	36.6	0.1	11.3	0.9
Genotype	242	981.6**	945**	0.34**	377.5**	2622.4**	54.61**	53.47**	81.93**	1.69**	0.50**	3.94**	0.561**
Residual	2393	440	329.5	0.12	161	439	6.227	5.468	21.85	0.9548	0.3253	1.828	0.1207
BSH (<i>H</i>)		0.38	0.48	0.47	0.4	0.71	0.79	0.81	0.58	0.28	0.22	0.37	0.65

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

DF = Degrees of freedom, DH = Deadheart damage, LD = Leaf feeding damage, EH = Number of exit holes, ST = Stem tunnel damage, PH = Plant height, PE = Days to panicle emergence, FL = days to 50% flowering, PL = Panicle length, VG = Seedling vigour, GL = Leaf glossiness, BW = Bloom waxiness, TGW = Total grain weight, BSH (*H*) = Broad sense heritability estimates

There were significant differences observed for the damage, agronomic and morphological traits measured. Heritability estimates for damage traits namely dead eart formation, Deadheart fotmation, exit holes and stem tunneling was moderate and ranged between 38% - 48%. Narrow sense heritability for plant height, days to panicle emergence, days to 50% flowering, panicle length, seedling vigor, leaf glossiness, bloom waxiness and total grain yield ranged form 22% - 81%.

The results of mean squares for leaf feeding, deadheart, exit holes and agronomic and morphological traits in Kabete are presented in Table 7.3.

Table 7.3: Mean squares for *Busseola fusca* damage, agronomic and morphological traits

Source	df	DH	LD	EH	ST	PH	PE	FL	PL	VG	GL	BW	TGY
Rep	1	427.1	37498.3	0.0012	33	3607.7	2.57	0.01	29.43	0.5305	3.9774	16.9286	1.5414
Genotype	242	521.1*	243.1	0.356**	487.5**	3381**	72.8**	71.61**	134.89**	0.60**	0.34**	1.63**	2.78**
Residual	2393	412.3	366.4	0.1382	212	368.5	18.58	22.13	27.64	0.33	0.2054	0.622	0.73
BSH (<i>H</i>)		0.12	0.20	0.44	0.39	0.80	0.59	0.53	0.66	0.28	0.25	0.45	0.58

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively; DF = Degrees of freedom, DH = Deadheart damage, LD = Leaf feeding damage, EH = Number of exit holes, ST = Stem tunnel damage, PH = Plant height, PE = Days to panicle emergence, FL = days to 50% flowering, PL = Panicle length, VG = Seedling vigour, GL = Leaf glossiness, BW = Bloom waxiness, TGW = Total grain weight, BSH (*H*) = Broad sense heritability estimates

There were significant differences observed for all the traits measured except for leaf feeding damage. Broad sense heritability estimates for damage parameters mainly deadheart, leaf damage, exit holes and stem tunneling ranged between low and moderate with 12% - 44%. Heritability estimates for plant height, days to panicle emergence, days to 50% flowering, panicle length, seedling vigor, leaf glossiness, bloom waxiness and total grain yield ranged between 25% - 80%.

Morphological traits characterizing the RILs during *B. fusca* and *C. partellus* infestation at Embu, Kabete and Kiboko, Kenya

Results of damage and morphological traits of the materials in individual locations are presented in Table 7.4.

Table 7.4: Damage and morphological traits (means \pm SE, n=8) linked to *B. fusca* and *C. partellus* infestation

Trait	Environments		
	Embu (<i>B. fusca</i>)	Kabete (<i>B. fusca</i>)	Kiboko (<i>C. partellus</i>)
Deadheart (%)	47.9 \pm 14.3	64.0 \pm 14.4	41.5 \pm 14.3
Leaf damage (%)	43.6 \pm 12.8	74.1 \pm 13.5	60.0 \pm 36.5
Number of exit holes	6.2 \pm 1.5	5.8 \pm 0.6	6.5 \pm 1.7
Stem tunneling (cm)	21.5 \pm 4.0	21.4 \pm 4.6	22.9 \pm 4.8
Plant height (cm)	112.6 \pm 19.5	122.3 \pm 8.6	128.1 \pm 6.9
Days to panicle emergence	60.1 \pm 2.5	71.5 \pm 4.3	58.1 \pm 4.1
Days to 50% flowering	70.8 \pm 1.7	78.9 \pm 3.3	69.3 \pm 4.2
Panicle length (cm)	15.8 \pm 4.4	16.6 \pm 2.4	18.4 \pm 1.3
Seedling vigour (score)	2.3 \pm 0.7	2.2 \pm 0.4	2.4 \pm 0.6
Bloom waxiness (score)	4.2 \pm 1.0	7.0 \pm 0.6	3.1 \pm 0.9
Total grain weight (t/ha)	0.6 \pm 0.11	1.0 \pm 0.3	1.4 \pm 0.3

Deadheart and leaf feeding damages due to *B. fusca* were higher in Kabete than Embu. In Embu, the top 5 genotypes that showed no dead heart formation included RILs 83, 217, 225, 86 and 222 and RILs 83, 84, 211, 185 and 244 had low incidence of leaf feeding damage. Genotypes with the least number of exit holes were RILs 91, 180, 38, 77 and 29 while RILs 27, 26, 16, 52 and 77 had the least stem tunnels. The highest yielding RILs in Embu were 112, 108, 118, 34 and 67. In Kabete, genotypes with the least deadheart damage were RILs 191, 86, 236, 68 and 34 while the least leaf feeding damage were RILs 112, 93, 20, 49 and 15. Genotypes with the least number of exit holes were RILs 26, 28, 2, 41 and 91 and the least number of stem tunnels were recorded on

RILs, 2, 27, 4, 5, 123 and 26. The highest grain yielding genotypes were RILs 200, 246, 249, 61 and 251. Genotypes with the least number of deadheart in Kiboko were RILs 85, 71, 229, 193 and 245 while the least number of leaf feeding damage were recorded on RILs 247, 229, 180, 138 and 94. Genotypes with the least number of exit holes were RILs 131, 77, 168, 109 and 228 while RILs 137, 168, 59, 78 and 109 had the least number of stem tunnels. The highest yielding genotypes in Kiboko were RILS 214, 12, 223, 182 and 45 in that order. Stem tunneling damage for the same borer was comparable at Embu and Kabete. Days to panicle emergence and days to 50% flowering were comparable at Embu and Kiboko and took the longest days at Kabete. Total higher grain yield was realized in Kiboko than Embu and Kabete.

Correlations of means among stem borer damage and morphological parameters

Results for correlations of mean for *B. fusca* damage and agronomic traits in Embu are presented in Table 7.5.

Table 7.5: Correlation co-efficients between *B. fusca* damage and agronomic traits in Embu

PH	-												
DH	-0.03	-											
EH	0.12	0.15*	-										
FL	0.07	-0.03	0.01	-									
GL	0.05	-0.08	0.01	-0.07	-								
LD	0.06	0.25**	-0.07	-0.01	0.12	-							
PL	0.45**	0.03	0.06	0.10	0.07	0.12	-						
ST	0.18**	0.11	0.71**	0.03	0.01	-0.07	0.12	-					
TR	0.01	0.02	-0.06	0.00	0.18**	0.06	-0.01	-0.09	-				
VG	0.21**	-0.40**	-0.20**	-0.04	0.14*	-0.01	0.13*	-0.06	-0.02	-			
BW	0.11	-0.39**	-0.33**	-0.01	0.17**	-0.09	0.07	-0.20**	0.02	0.60**	-		
YLD	0.09	0.08	0.15*	0.06	-0.07	0.09	0.17**	0.10	0.05	-0.09	-0.06	-	
	PH	DH	EH	FL	GL	LD	PL	ST	TR	VG	BW	YLD	

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

PH = plant height, DH = deadheart damage, EH = number of exit holes, FL = days to 50% flowering, GL = leaf glossiness, LD = leaf damage, PL = panicle length, ST = stem tunnel damage, TR = trichome density, VG = seedling vigor, BW = bloom waxiness, YLD = total grain yield

Significant positive correlation was observed between panicle length and plant height ($r = 0.45$, $P = <0.001$). Stem tunneling had a positive significant association with plant height ($r = 0.18$, $P = 0.0057$) and with exit holes ($r = 0.71$, $P = <0.001$). Seedling vigor had a positive and significant relationship with plant height ($r = 0.21$, $P = 0.0011$). Exit holes had a positive significant relationship with deadheart damage ($r = 0.15$, $P = 0.02$). Leaf damage had a significant positive relationship with deadheart formation ($r = 0.25$, $P = <0.001$). Positive significant correlation was observed between trichome density and leaf glossiness ($r = 0.18$, $P = 0.0039$), with seedling vigor ($r = 0.14$, $P = 0.033$), and with bloom waxiness ($r = 0.17$, $P =$

0.0075). Positive significant correlation was recorded between seedling vigor and panicle length ($r = 0.13$, $P = 0.03$). Bloom waxiness had a significant positive correlation with seedling vigor ($r = 0.60$, $P = <0.001$). Negative significant relationship was recorded between seedling vigour and deadheart formation ($r = -0.40$, $P = <0.001$) and with exit holes ($r = -0.20$, $P = 0.002$). Bloom waxiness had a negative significant correlation with deadheart damage $r = -0.39$, $P = <0.001$), with exit holes ($r = -0.33$, $P = <0.001$), and with stem tunneling ($r = -0.20$, $P = 0.0016$) respectively.

Results for correlations of mean for *B. fusca* damage and agronomic traits in Kabete are presented in Table 7.6.

Table 7.6: Correlation co-efficients between *B. fusca* damage and agronomic traits in Kabete

DH	-												
EH	0.05	-											
FL	0.07	-0.13*	-										
GL	-0.09	-0.09	0.11	-									
LD	0.31**	0.02	0.05	0.01	-								
PH	-0.04	0.10	-0.05	-0.07	-0.01	-							
PL	0.02	0.14*	-0.03	-0.05	0.11	0.16*	-						
ST	0.05	0.70**	-0.16*	-0.11	0.03	0.12	0.15*	-					
TR	-0.04	0.12	0.02	0.01	-0.05	0.01	-0.05	0.05	-				
VG	-0.38**	-0.15*	-0.03	0.13*	-0.01	0.08	-0.14*	-0.17*	0.04	-			
BW	-0.09	0.03	0.07	0.20**	0.12	-0.03	0.05	-0.01	0.02	0.15*	-		
YLD	0.09	0.32**	-0.14*	-0.09	0.14*	0.15*	0.30**	0.27**	-0.07	-0.12	-0.00	-	
	DH	EH	FL	GL	LD	PH	PL	ST	TR	VG	BW	YLD	

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

DH = deadheart damage, EH = number of exit holes, FL = days to 50% flowering, GL = leaf glossiness, LD = leaf feeding damage, PH = plant height, PL = panicle length, ST = stem tunnel damage, TR = trichome density, VG = seedling vigor, BW = bloom waxiness, YLD = total grain yield

Leaf feeding had a positive significant correlation with deadheart damage ($r = 0.31$, $P = <0.001$). Stem tunneling had a positive significant relationship with exit holes ($r = 0.70$, $P = <0.001$). Bloom waxiness had a positive significant correlation with leaf glossiness ($r = 0.20$, $P = 0.002$), and with seedling vigor ($r = 0.15$, $P = 0.02$). Yield had a positive significant correlation with plant height ($r = 0.15$, $P = 0.021$), and with panicle length ($r = 0.30$, $P = <0.001$). Seedling vigour had a negative significant correlation with deadheart damage ($r = -0.38$, $P = <0.001$), with exit holes ($r = -0.15$, $P = 0.02$), and with stem tunneling ($r = -0.17$, $P = 0.008$). Days to 50% flowering had a significant negative correlation with number of exit holes ($r = -0.13$, $P = 0.04$).

Results for correlations of mean for *C. partellus* damage and agronomic traits in Kiboko are presented in Table 7.7.

Table 7.7: Correlation co-efficients between *C. partellus* damage and agronomic traits in

Kiboko, Kenya

DH	-										
EH	-0.10	-									
FL	-0.09	0.01	-								
GL	0.01	0.00	0.02	-							
LD	0.09	-0.01	0.17**	0.05	-						
PH	0.03	0.12	0.13*	0.04	0.07	-					
PL	-0.02	0.07	0.12	-0.09	-0.05	0.27**	-				
ST	0.02	0.68**	-0.13*	-0.03	-0.04	0.00	0.03	-			
VG	-0.06	-0.03	-0.33**	-0.03	-0.13*	-0.00	-0.01	0.13*	-		
BW	-0.05	-0.09	0.12	0.03	0.04	-0.03	0.13*	-0.26**	0.13*	-	
YLD	0.07	0.06	-0.08	-0.01	-0.16*	0.12	0.34**	0.15*	0.02	-0.02	-
	DH	EH	FL	GL	LD	PH	PL	ST	VG	BW	YLD

*, ** Data significant at ≤ 0.05 and ≤ 0.01 probability level respectively;

DH = deadheart damage, EH = number of exit holes, FL = days to 50% flowering, GL = leaf glossiness, LD = leaf feeding damage, PH = plant height, PL = panicle length, ST = stem tunnel damage, VG = seedling vigor, BW = bloom waxiness, YLD = total grain yield

Stem tunneling had a positive significant correlation with exit holes ($r = 0.68$, $P = <0.001$). Days to 50% flowering had a positive correlation with leaf damage ($r = 0.17$, $P = 0.008$) and with plant height ($r = 0.13$, $P = 0.04$). Panicle length had a positive significant relationship with plant height ($r = 0.27$, $P = <0.001$), with bloom waxiness ($r = 0.13$, $P = 0.04$), and with total grain yield ($r = 0.34$, $P = <0.001$). Seedling vigor had a positive significant correlation with bloom waxiness ($r = 0.13$, $P = 0.04$). Days to 50% flowering had a significant negative correlation with stem tunneling ($r = -0.13$, $P = 0.04$), and with seedling vigor ($r = -0.33$, $P = <0.001$). Stem tunneling had a significant negative relationship with bloom waxiness ($r = -0.26$, $P = <0.001$). Leaf feeding damage had a significant negative correlation with seedling vigor ($r = -0.13$, $P = 0.04$), and with total grain yield ($r = -0.16$, $P = 0.01$).

Contribution of *B. fusca* damage parameters to total grain yield through leaf feeding, deadheart, stem tunnels and number of exit holes damages in Embu are presented in Table 7.8.

Table 7.8: Direct and indirect effects of *B. fusca* damage traits on total grain yield in Embu

character	correlation with grain yield	direct effect	indirect effect via			
			DH	EH	LD	ST
DH	-0.96	42.62**	-	-0.0012	-0.0007	0.004
EH	-0.96	4.927**	0.0026	-	0.0132	-0.0325
LD	-0.96	37.62**	0.0005	0.0031	-	-0.0001
ST	-0.96	19.32**	0.0001	0.001	0.0057	-

** Data significant at $P \leq 0.01$ probability level

DH = deadheart damage, EH = number of exit holes, LD = leaf feeding damage, ST = stem tunnel damage,

Damage effects were partitioned into direct and indirect associations by path coefficient analysis and grain yield was used as the resultant variable. Deadheart, exit holes, leaf damage and stem tunneling damages had a negative correlation with grain yield. Deadheart had a significant positive direct effect on grain yield supported by positive indirect positive effect through stem tunneling and negative indirect effects through number of exit holes and leaf feeding damages. Number of exit holes had a positive direct effect on grain yield supported by positive indirect effects through deadheart and leaf feeding damages and indirect negative effect through stem tunneling. Leaf damage had a positive direct effect on grain yield supported by positive indirect effects through deadheart and number of exit holes and negative indirect effect through stem tunneling. Stem tunneling damage had a positive direct effect on grain yield supported by positive indirect effects through deadheart, number of exit holes and leaf feeding damages.

Contribution of *B. fusca* damage parameters to total grain yield through leaf feeding, deadheart, stem tunnels and number of exit holes damages in Kabete are presented in Table 7.9.

Table 7.9: Direct and indirect effects of *B. fusca* damage traits on total grain yield in Kabete

character	correlation with grain yield	direct effect	indirect effect via			
			DH	EH	LD	ST
DH	-0.87	60.37**	-	0.0009	0.0008	0.004
EH	-0.87	4.078**	0.0164	-	0.0012	-0.0066
LD	-0.87	71.24**	0.0018	-0.0006	-	-0.0005
ST	-0.87	18.10**	-0.0053	-0.0071	-0.0039	-

** Data significant at $P \leq 0.01$ probability level

DH = deadheart damage, EH = number of exit holes, LD = leaf feeding damage, ST = stem tunnel damage,

Deadheart formation, number of exit holes, leaf feeding and stem tunneling damages had a

negative correlation with grain yield. Deadheart had a positive direct effect on grain yield supported by positive indirect effects on number of exit holes, leaf feeding and stem tunneling damages. Number of exit holes had a positive direct effect on grain yield supported by positive indirect effects through deadheart, leaf feeding and positive indirect effects through stem tunneling damages. Leaf feeding damage had positive direct effect on grain yield supported by positive indirect effect through deadheart damage and indirect negative effects through exit holes and stem tunneling damages. Stem tunneling had positive direct effects on grain yield supported by negative indirect effects through deadheart, number of exit holes and leaf feeding damages. Contribution of *C. partellus* damage parameters to total grain yield through leaf feeding, deadheart, stem tunnels, number of exit holes damages in Kiboko are presented in Table 7.10.

Table 7.10: Direct and indirect effects of *C. partellus* damage traits on total grain yield in Kiboko

character	correlation with grain yield	direct effect	indirect effect via			
			DH	EH	LD	ST
DH	-0.97	68.11**	-	-0.0006	-0.0061	0.0056
EH	-0.97	6.003**	-0.0015	-	0.0055	0.0161
LD	-0.97	36.93**	-0.0013	-0.0060	-	-0.0003
ST	-0.97	16.69**	0.00149	0.0016	0.0065	-

** Data significant at $P \leq 0.01$ probability level; DH = deadheart damage, EH = number of exit holes, LD = leaf feeding damage, ST = stem tunnel damage

Deadheart, number of exit holes, leaf feeding and stem tunneling damages had a negative correlation with grain yield. Deadheart had a direct positive effect on grain yield supported by positive indirect effect through stem tunneling and indirect negative effect through number of exit holes and leaf feeding damages. Number of exit holes had positive direct effects on grain yield supported by positive indirect effect through leaf feeding and stem tunneling damages and indirect positive effect through deadheart damage. Leaf feeding damage had a positive direct effect on grain yield supported by negative indirect effects through deadheart, exit holes and stem tunneling damages. Stem tunneling had a direct positive effect on grain yield supported by positive indirect effects through deadheart, number of exit holes and leaf feeding damages.

QTL Mapping

Results of the QTL analyses with composite interval mapping are summarized in Table 7.11. Findings presented in this study were common to both *B. fusca* and *C. partellus*. In summary, a total of twenty major QTLS for deadheart formation, leaf feeding, exit holes and stem tunnel damages were detected on all sorghum chromosomes.

Table 7.11. Summary of quantitative trait loci identified for *B. fusca* and *C. partellus* sorghum damage traits from RIL population derived from ICSV 745 x PB 15520-1 cross

Damage trait	QTL	Chromosome	Position (cM)	Marker and loci interval	Supp interval	LOD	R ²	Additive effects	SE	Adj σ^2_g
Deadheart	1	4	74	BC242-4 2342-2347	72- 78	3.81	9	16.6	1.587*	
	2	5	24	m05/023.7 2703-2679	22- 26	3.31	7.6	3.3	0.981**	24.8
	3	10	22	CPS160-10 4619-4622	18- 28	3.16	8.2	5.4	1.336*	
	4	8	40	JK399-8 36- 42	36- 42	3.41	7.8	-9.5	2.758*	
Leaf damage	1	1	14	SB255-1 245- 224	12- 16	6.28	15.4	3.8	0.822**	
	2	3	54	DB160-3 1584-1579	52- 56	7.88	17.4	5.1	0.657	56
	3	3	74	DB169-3 1593-1592	72- 84	3.48	8.1	-2	0.646	
	4	6	76	EF222-6 3083-3203	72- 78	3.26	7.6	3.9	1.077	
	5	8	48	JK290-8 3938-3944	46- 52	3.24	7.6	2.2	0.705	
	6	7	24	GH70-7 3361-3371	22- 26	3.61	8.4	-3.1	0.681*	
Exit holes	1	2	88	CS195-2 1016- 971	86- 90	3.94	9	-0.5	0.142**	
	2	4	58	BC149-4 2249-2250	56- 60	3.92	9	-0.7	0.167**	47.3
	3	6	4	EF255-6 3116-3143	2- 6	5.14	11.7	-0.7	0.154**	
	4	6	26	EF416-6 3277-3281	24- 28	4.64	10.5	3.2	0.743**	
	5	9	88	BF138-9 4205-4203	84- 90	3	7	1.2	0.361**	
Stem tunneling	1	2	16	CS116-2 937- 940	14- 18	5.18	11.8	1.6	0.365**	
	2	2	52	CS132-2 953- 954	46- 56	4.62	10.5	-1.5	0.391**	
	3	3	20	CS132-2 1460-1524	16- 24	3.34	10.1	4.4	0.618**	57.4
	4	4	46	DB36-3 2240-2229	42- 48	6.57	14.7	3	0.631**	
	5	9	90	BF140-9 4207-4210	88- 92	4.44	10.3	-3.4	0.458	

Position = Maximum peak in cM, relative to the first locus on each chromosome; Additive effect = regression coefficient of the QTL at the specific position from the multiple regression analysis. Positive additive effects indicate that PB 15520-1 (resistant parent) allele increased the value of the trait; SE = Standard error; R² = Coefficient of determination between the respective QTL and the phenotypic observations from whole data set

The phenotypic variances (Adj σ^2_g) of the seventeen QTLs on the-afore mentioned damage parameter ranged between 24.8% and 57.3% on deadheart and stem tunneling, respectively. There was evidence of more than one QTL associated with the four traits on chromosomes 2, 3, 4, 6 and 9. The alleles for reduced deadheart, leaf damage and stem tunneling were inherited

from the resistant parent PB 15520-1. Five-fold cross validation analysis performed on deadheart (chromosomes 4, 5 and 10), leaf damage (chromosomes 1, 3, 6 and 8), exit holes (chromosome 2) and stem tunneling (chromosomes 2 and 4) revealed that the QTLs mapped on these chromosomes were consistently detected in each of the five validation splits.

Sorghum morphological traits associated with *B. fusca* and *C. partellus* resistance

The results of a summary of morphological traits associated with *B. fusca* and *C. partellus* are presented in Table 7.12.

Table 7.12: Summary of QTL associated with resistance to *B. fusca* and *C. partellus* for ICSV 745 x PB 15520-1 sorghum population

Trait	QTL	Chromosome	Position (cM)	Marker and loci interval	Supp interval	LOD	R ²	Additive effects	SE	Adj σ^2 g
Bloom waxiness	1	3	10	DB39-3 1463-1461	2-12	4.88	19.1	0.5	0.105**	27.3
	2	9	42	BF97-9 4164-4168	40-46	3.52	8.2	1.2	0.201	
Leaf glossiness	1	5	14	AD443-5 2838-2676	41624	3.32	8.1	-0.7	0.220**	15.2
	2	7	82	GH120-7 3411-3410	80-84	3.01	7.1	-0.5	0.162*	
Trichome density	1	1	10	SB259-1 249- 245	8 - 12	3.03	7.7	-6.6	1.752	40.5
	2	2	122	CS380-2 1201-1205	120 - 124	4.66	10.4	-4.1	1.007**	
	3	3	108	DB390-3 1814-1619	102 - 110	3.19	7.3	4.6	1.428*	
	4	6	10	EF154-6 3015-3153	8 - 12	6.90	15.0	-7.8	1.435*	
Leaf toughness	1	2	56	CS133-2 954- 955	54 -58	3.01	7.1	0.01	0.006*	41.1
	2	2	138	CS402-2 1223-1224	136 -140	3.23	7.7	0.03	0.011	
	3	4	24	BC131-4 2231-2232	22 -26	3.51	8.8	-0.04	0.012**	
	4	6	48	EF204-6 3065-3009	46 -50	3.82	8.9	0.1	0.029*	
	5	9	72	BF123-9 4190-4193	70 -74	3.67	8.6	-0.1	0.025*	
	6	10	64	m10/062.5 4638-4636	60 - 66	3.22	10.6	0.1	0.011**	
Seedling Vigour	1	8	12	JK152-8 3800-3809	8 - 16	3.05	8.7	0.3	0.099**	19.5
	2	9	20	BF84-9 4151-4149	18 - 22	4.59	10.8	-0.9	0.076	

Position = Maximum peak in cM, relative to the first locus on each chromosome; Additive effect = regression coefficient of the QTL at the specific position from the multiple regression analysis. Positive additive effects indicate that PB 15520-1 (resistant parent) allele increased the value of the trait; SE = Standard error; R² = Coefficient of determination between the respective QTL and the phenotypic observations from whole data set

The analysis revealed associations of 16 common genomic regions conditioning morphological traits associated with resistance to *B. fusca* and *C. partellus*. Bloom waxiness was controlled by chromosomes 3 and 9. Leaf glossiness was controlled by chromosomes 5 and 7. Trichome density was regulated by chromosomes 1, 2, 3 and 6. Leaf toughness was conditioned by chromosomes 2, 4, 6, 9 and 10 and seedling vigour was conditioned by chromosomes 8 and 9.

The LOD score values ranged between 3.01 and 6.90 with phenotypic variation explained ranging from 15.2% - 40.5%. Five-fold cross validation analysis performed on bloom waxiness, leaf glossiness and seedling vigour confirmed that the QTLs mapped on these chromosomes were consistently detected in each of the five cross validation splits in the analysis. The frequency of QTL detection gives an estimation of the precision of QTL localization. Additional genomic regions conditioning leaf toughness and trichome density were identified on chromosomes 5, 10 and 8 respectively.

The molecular linkage maps and the location of QTLs detected in the RILs population are presented in Figure 7.2. Supplementary information to these traits in each environment is presented in Appendixes 1 and 2.

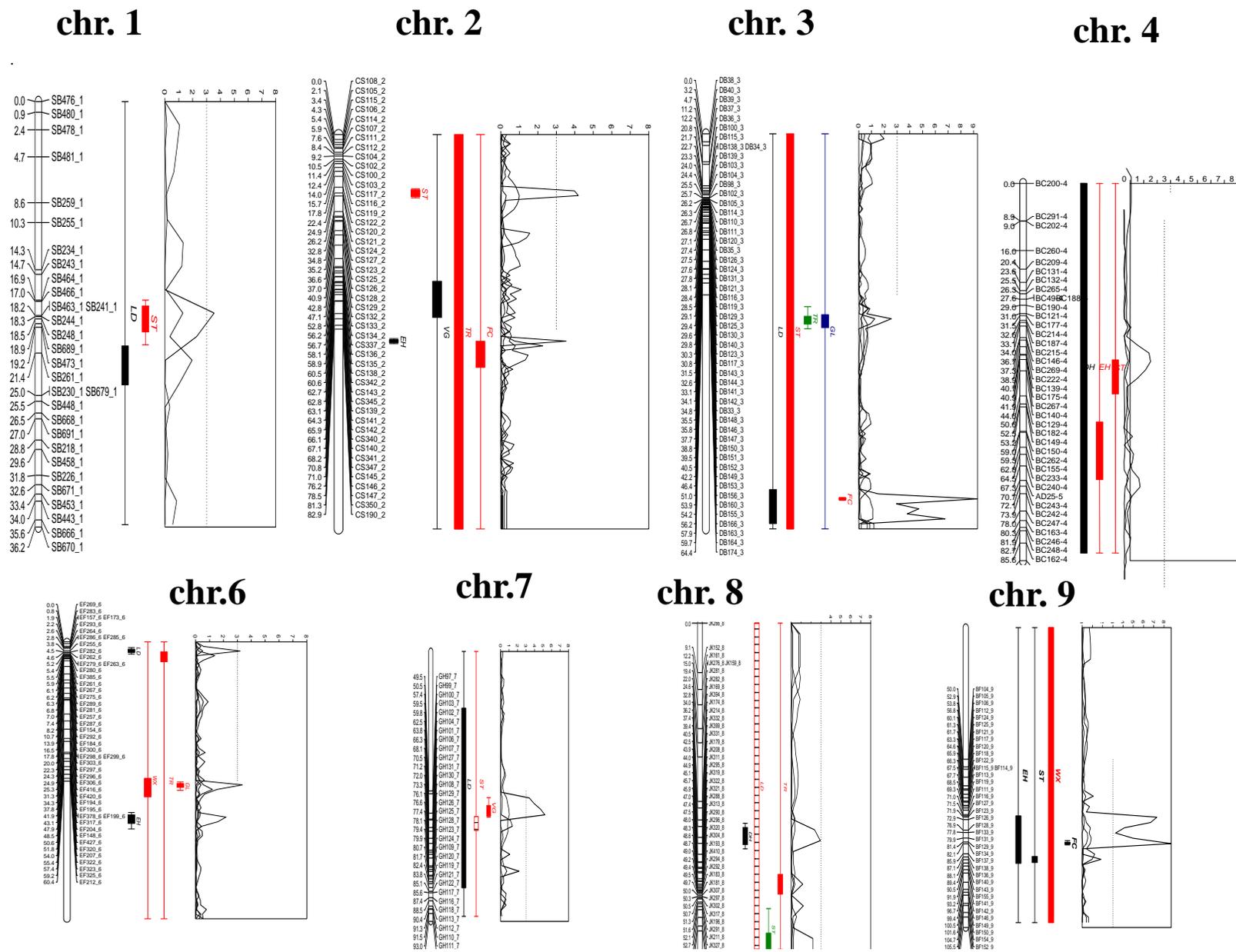


Figure 7.2: Molecular linkage map and the location of QTLs detected in the RILs population derived from ICSV 745 x PB 15520-1

Key: LD=leaf damage; ST= stemtunneling, EH= exit holes, VG= seedling vigour, TR= trichome density, FC= leaf toughness; GL= leaf glossiness; DH= dead heart formation; WX= Bloom waxiness;

Discussion

Large number of RILs and the high density of evenly spaced genetic markers scored ensured high power and precision in QTL mapping (Matthieu *et al.*, 2005). The reason for running a five-fold cross validation analysis is to confirm the frequency of QTL detection that gives an estimation of the precision of QTL localization. The identification of QTLs that influence resistance to *B. fusca* and *C. partellus* resistance traits would increase the efficiency of selection for resistance of these two stem borers. Path coefficient analysis revealed that leaf feeding, deadheart, exit holes had stem tunnel had a negative correlation with grain yield for both borers. All the damage traits (leaf feeding, deadheart, exit holes and stem tunnel) had negative indirect effect through each other on grain yield. This would be attributed to the fact that leaf feeding reduces the surface area and tissues available for photosynthesis thus reducing photosynthetic rate. The significant positive correlations observed between leaf damage and deadheart implied that there is a direct relationship between these two traits and either of the traits can be used to predict the other. Chapters 5 and 6 of this thesis suggested that leaf feeding and deadheart damage due to *B. fusca* and *C. partellus* infestation are governed by additive type of gene action. The positive significant association between exit holes and stem tunneling implied a direct link between the two traits and either of the traits can be used to predict the other.

Majority of the identified loci were highly significant and they accounted for substantial amount of the phenotypic variation for *B. fusca* and *C. partellus*. This implies that these QTLs govern leaf damage, deadheart, exit holes and stem tunnel damages on sorghum and fixing these traits would lead to development of resistant cultivars. This study identified common QTL as were detected in the previous study. The QTLs identified in the present study on chromosomes 1, 3, 4, 5, 6, 8, 9 and 10 are novel and were not reported by Vinayan (2010). These genomic regions identified are important and add to those identified by Vinayan (2010). This also suggests that conducting mapping studies in more than one experimental site and employing numerous markers is important and avoid possibilities of missing important genomic regions containing QTLs. Findings of the present study are novel as QTLs governing more than one resistance trait were identified (pleiotropic QTLs). For example, chromosome 3 controlled stem tunneling and

leaf damage; chromosome 4 conditioned deadheart, exit holes and stem tunneling and chromosome 6 conditioned exit holes and leaf damage.

In maize, chromosome 1 has been associated with multiple resistance to lepidopteran insect pests mainly European corn borer *Ostrinia nubilalis* (Hubner), southwestern corn borer *Diatrea grandiosella* (Dyar)], sugarcane borer [*Diatraea saccharalis* (Fabricius)] and maize weevil resistance (Silverio *et al.*, 2009). Chromosomes 1, 3 and 8 have been associated with resistance to Mediterranean corn borer *Sesamia nonagrioides* stem tunneling using EP39 × EP42 maize recombinant inbred lines (Bernardo *et al.*, 2010). These observations support the current finding that chromosomes 2, 3, 7 and 9 are involved in conditioning resistance against cereal borers suggesting that these genomic areas are conserved in cereals chromosomes. Chromosomes 1 and 3 have been associated with resistance to sorghum shoot fly, *Atherigona soccata* Rondani using IS18551 x 296B RILs (Apotikar *et al.*, 2011). Chromosomes 4, 5, 6, 7 and 9 have been observed to condition sorghum grain mold resistance using RTx430 x Sureno RILs population (Rooney and Klein, 2000).

This study detected 2 QTLs for bloom waxiness, two for leaf glossiness, four for trichome density, five for leaf toughness and two for seedling vigour. Genomic regions governing trichome density detected on sorghum chromosomes 2, 3 and 6 are novel and have not been reported. The QTL identified for trichome density on chromosome 1 has also been reported in sorghum against shoot fly (Satish *et al.*, 2009; Apotikar *et al.*, 2011). Chromosome 9 was observed to possess pleiotropic effects and controlled seedling vigour, leaf toughness and bloom waxiness. The strong positive significant association observed in this study between exit holes and stem tunneling could be attributed to the fact that both damage traits are controlled by chromosomes 2, 4 and 9. The positive significant relationship between deadheart and leaf feeding damage could be explained by the fact that chromosome 8 controlled both traits.

Conclusion and future prospects

The detection of more than one locus for different traits supports the hypothesis that several genomic regions control the expression of resistance to *B. fusca* and *C. partellus* in sorghum.

Eight QTLs identified in this study and observed to condition resistance to *B. fusca* and *C. partellus* are novel and had not been reported before. These QTLs can be used in marker-assisted selection and the breeding of stem borer resistant sorghum cultivars. Pleiotropic QTLs identified in this study are valuable since more than one trait can be improved with the help of the same linked markers. The QTLs identified in the present study are novel and can be useful in marker assisted selection and the breeding of stem borer resistant sorghum. There is need for further studies to identify gene(s) underlying the mapped QTLs. Discovery of high through-put strategies that allow greater power and precision in utilization of QTLs conditioning sorghum resistance to stem borers will enhance sorghum improvement against these insect pests.

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Appendix 1: Quantitative trait loci identified for *B. fusca* and *C. partellus* damage traits from RIL population derived from ICSV 745 x PB 15520-1 cross

Pest species	Site	Damage trait	QTL	Chromosome	Position (cM)	Marker and loci interval	Supp interval	LOD	R ²	Additive effects	SE	Adj σ ² g
<i>B. fusca</i>	Embu	Deadheart	1	1	22	SB261-1 251- 220	20- 26	3.27	7.5	35.13	10.154**	28.3
			2	3	24	DB103-3 1527-1528	22- 26	4.11	9.6	-7.79	1.680**	
			3	5	10	AD323-5 2718-2834	8-12	3.97	11.2	8.86	1.731**	
		Leaf damage	1	2	4	CS115-2 936- 927	2-6	3.11	9.1	-6.48	2.335	
			2	2	84	CS190-2 1011-1172	82- 86	3.55	8.1	8.13	1.821**	
			3	2	134	CS389-2 1210-1041	132- 136	3.09	7.1	5.27	1.888	
			4	2	148	CS414-2 1235-1083	146- 150	3.67	8.7	-8.01	1.687**	
			5	10	30	CPS162-104621-4623	26- 36	3.26	7.7	7.04	2.323**	
			1	7	26	GH89-7 3380-3379	24- 28	3.98	9	-1.42	0.337*	
			1	7	22	GH87-7 3378-3376	20- 24	3.51	8.2	-2.39	0.584**	
Exit holes	2	9	72	BF123-9 4190-4193	70- 74	3.48	8.1	-7.33	1.910**			
	2	9	72	SB691-1 681- 208	26- 30	3.53	8.1	12.95	3.602**			
<i>B. fusca</i>	Kabete	Deadheart	1	1	28	CS402-2 1223-1224	136- 140	3.34	7.6	-7.2	2.177**	16.3
			2	2	138	CS259-2 1080-1099	152- 168	4.54	14.6	8.1	1.615*	
			3	2	154	CS350-2 1171-1011	80- 84	3.19	7.3	-8.52	2.359*	
		Leaf damage	4	2	82	DB172-3 1596-1603	68- 72	8.16	17.5	-6.08	1.140**	
			5	3	70	DB169-3 1593-1592	72- 78	3.91	9	-5.32	1.229**	
			6	3	74	BC149-4 2249-2250	52- 60	3.29	7.7	5.77	1.590**	
			7	4	58	JK399-8 4047-3979	38- 42	4.38	9.9	-20.68	5.332**	
			8	8	40	BF97-9 4164-4168	38- 46	4.96	11.2	9.67	2.373**	
			9	9	42	BF106-9 4173-4179	52- 58	5.19	11.7	-14.34	3.455**	
			10	9	56	CS403-2 1224-1064	138- 142	3.01	7	-9.54	2.926**	
Exit holes	1	2	140	CS111-2 932- 933	6-10	5.05	12.6	6.56	0.863**			
	2	2	8	CS397-2 1218-1219	130- 134	5.41	12.1	4.15	0.902**			
	3	3	132	DB164-3 1588-1598	62- 66	4.53	10.2	-3.37	0.834**			
	4	4	64	EF334-6 3195-3079	70- 74	3.3	7.6	-14.42	4.207**			
	5	6	72	m08/014.93924-3929	14- 22	3.17	7.7	-4.3	3.193**			
	6	8	18	CPS158-104617-4618	6-16	3.24	9.6	4.07	1.032*			
	7	10	12	CS342-2 1163- 964	60- 64	4.26	9.6	1.17	0.266**			
	1	2	62	CS190-2 1011-1172	82-86	4.89	10.9	-1.32	0.284**			
	2	4	84	AD25-5 2420-2343	70- 74	7.38	16.5	-2.29	0.434**			
	3	4	72	AD185-5 2580-2701	26- 30	3.27	7.8	1.05	0.323**			
<i>C. partellus</i>	Kiboko	Deadheart	4	5	28	EF255-6 3116-3143	2-6	3.67	8.4	-0.89	0.250**	84.7
			5	6	4	BF138-9 4205-4203	86- 90	3.25	7.5	-1.75	0.568**	
			6	9	88	JK208-8 3856-3959	42- 46	3.7	8.4	-7.28	0.96	
		Leaf damage	7	8	44	BF137-9 4204-4205	84- 88	7.13	15.6	-6.49	0.878**	
			8	9	86	SB466-1 456- 453	16- 20	3.48	8.1	2.7	0.736**	
			1	1	18	CS132-2 953- 954	48- 58	3.27	7.5	-1.9	0.531**	
			2	2	52	CS133-2 954- 955	48- 58	3.2	7.4	-2.01	0.563**	
			3	2	54	CS150-2 971-1177	88- 92	3.99	9.1	-2.3	0.597**	
			4	2	90	DB140-3 1564-1547	28- 32	3.97	4.49	2.68	0.550**	
			5	3	30	GH87-7 3378-3376	20- 24	3.04	7.1	-2.1	0.644*	
6	7	22	DB143-3 1567-1568	30- 34	6.17	13.9	3.98	0.515				
Exit holes	7	3	32	BC140-4 2240-2229	42- 50	3.64	8.5	2.98	0.868**			
	8	4	46	GH108-7 3399-3420	72- 76	3.48	8.2	-4.53	1.351*			
	9	7	74	CS369-2 1190-1197	114- 118	3.62	8.6	3.32	0.916**			
	1	2	116	CS389-2 1210-1041	132- 136	4.99	11.7	-2.71	0.622**			
	2	2	134	EF322-6 3183-3184	54- 58	3.49	8.3	-3.17	0.762*			
	3	6	56	BF152-9 4219-4220	104- 108	3.13	12.6	-8.46	.815*			
	4	9	106	CS133-2 954- 955	52- 58	3.84	8.7	-3.07	0.758**			
	Stem tunnel	1	2	56	CS397-2 1218-1219	130- 134	3.78	8.6	-3.06	0.804**		
		2	2	132	EF184-6 3045-3161	14- 18	3.14	7.2	2.76	0.801**		
		3	6	16	DB153-3 1577-1580	42- 52	3.99	9.1	0.59	0.153*		
1		3	50	DB208-3 1632-1635	130- 136	3.67	8.5	-1.01	0.287**			
2		3	134	BC222-4 2322-2239	38- 42	3.12	7.2	-0.89	0.255*			
3		4	40	m05/015.62567-2709	14- 18	3.6	8.4	-8.04	2.229**			
4		5	16	CS414-2 1235-1083	146- 150	3.04	7.4	0.61	0.183			
Stem tunnel	5	2	148	EF255-6 3116-3143	2- 6	3.21	7.6	-0.55	0.172**			
	6	6	4	GH66-7 3357-3367	14- 18	4.05	10.3	-0.51	0.148*			
	7	7	16	DB152-3 1576-1573	40- 46	5.08	11.4	2.64	0.391*			
	1	3	42	GH70-7 3361-3371	22- 26	3.4	7.8	-1.83	0.471*			
2	7	24	GH118-7 3409-3404	88- 94	3.99	9.5	-3.56	0.837				
3	7	90							28.7			

Appendix 2: Quantitative trait loci associated with resistance to *B. fusca* and *C. partellus* for ICSV 745 x PB 15520-1 sorghum population

Pest species	Site	Trait	QTL	Chromosome	Position (cM)	Marker and loci interval	Supp interval	LOD	R ²	Additive effects	SE	Adj σ^2_g		
<i>B. fusca</i>	Embu	Bloom waxiness	1	3	56	DB155-3 1579-1590	54- 58	3.17	7.3	0.57	0.112**	44.3		
			2	3	144	DB214-3 1638-1637	140- 146	3.35	8.1	-2.18	0.469*			
			3	6	24	EF297-6 3158-3157	22- 26	3.26	7.4	-3.88	1.085*			
			4	3	10	DB39-3 1463-1461	2-12	3.65	14.5	0.47	0.105**			
			5	9	46	BF101-9 4168-4169	40- 48	3.03	7	0.84	0.258			
		Leaf glossiness	1	5	14	AD443-5 2838-2676	12-16	3.08	7.5	-0.69	0.225**		14.9	
			2	6	62	EF211-6 3072-3194	60- 64	3.24	7.4	-0.84	0.221**			
		Trichome density	1	2	64	CS139-2 960- 962	62- 66	3.21	7.5	-3.73	0.862*		23.8	
			2	2	44	CS129-2 950- 953	40- 48	3.27	7.5	3	0.810*			
		Seedling Vigour	3	6	52	EF320-6 3181-3068	50- 54	3.83	8.8	-14.97	3.786**		23.8	
			1	2	70	CS341-2 1162-1168	68- 72	3.51	8	-0.39	0.101**			
			2	2	140	CS403-2 1224-1064	138- 142	3.07	7.1	0.96	0.277*			
		Leaf glossiness	3	5	10	AD323-5 2718-2834	8-12	3.04	8.7	0.45	0.077*		54.8	
			4	6	60	EF325-6 3186-3073	58- 62	3.06	7	-1.57	0.502*			
			5	7	58	GH100-7 3391-3394	52- 60	5.18	11.6	-1.86	0.414**			
			6	7	56	GH99-7 3390-3391	52- 60	5.53	12.4	-1.58	0.323**			
			2	7	52	GH99-7 3390-3391	50- 58	5.43	12.3	0.92	0.219**			
			3	5	20	AD290-5 2685-2683	18- 22	4.01	9.4	-0.33	0.078			33.6
			1	2	104	CS156-2 977-1188	102- 106	3.51	8	-0.24	0.070*			
			2	3	4	DB40-3 1464-1463	2-6	4.17	15.9	0.13	0.029*			
3	3		76	DB169-3 1593-1592	74- 82	3.2	7.3	0.09	0.028					
4	6		60	EF325-6 3186-3073	58- 62	5.62	12.5	0.87	0.196**					
5	9	60	BF112-9 4179-4191	58- 62	4.19	9.6	-0.19	0.052**						
6	5	28	AD185-5 2580-2701	26- 30	3.15	7.6	-0.16	0.046						
7	7	22	GH87-7 3378-3376	18- 24	3.55	8.3	0.15	0.038*						
8	7	36	GH90-7 3381-3382	30- 40	3.24	7.5	-0.32	0.110*						
Trichome density	9	9	48	BF102-9 4169-4171	44- 50	4.08	9.4	-0.28	0.083*	96.1				
	1	3	70	DB172-3 1596-1603	68- 72	3.16	7.3	2.17	0.66					
	2	6	16	EF184-6 3045-3161	12- 18	3.06	7.1	-2.3	0.721**					
<i>C. partellus</i>	Kiboko	Seedling vigour	3	3	58	DB163-3 1587-1588	56- 60	3.03	6.9	0.14	0.040**	6.9		
			3	3	58	DB163-3 1587-1588	56- 60	3.03	6.9	0.14	0.040**			
		Bloom waxiness	2	2	154	CS259-2 1080-1099	152- 168	3.88	13.5	0.63	0.126	13.5		
			2	2	154	CS259-2 1080-1099	152- 168	3.88	13.5	0.63	0.126			
		Leaf glossiness	1	5	14	AD443-5 2838-2676	12-16	3.69	9.1	-0.54	0.141*	57.3		
			2	6	78	EF342-6 3203-3085	76- 80	3.83	9	-0.12	0.031**			
			3	6	20	EF303-6 3164-3158	18- 22	3.76	8.6	-4.73	1.265**			
			4	7	32	GH90-7 3381-3382	28- 38	3.05	7.3	-0.23	0.066**			
			5	9	20	BF84-9 4151-4149	16- 22	3.98	9.8	-0.19	0.025*			
		Seedling vigour	1	2	122	CS380-2 1201-1205	120- 126	3.84	8.7	0.216	0.055**	57.3		
2	6		22	EF303-6 3164-3158	20- 24	3.04	7.1	1.54	0.490*					
3	7		76	GH108-7 3399-3420	74- 78	4.18	9.8	-1.03	0.264*					
4	8		20	JK281-8 3929-3930	16- 22	4	9.6	-0.266	0.073**	35.2				

