Inheritance of Post-Harvest Pest Resistance and Genetic Analysis of Combining Drought, Maize Lethal Necrosis and Maize Weevil Resistance in Tropical Maize Germplasm

BY

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General Abstract

Drought stress, maize lethal necrosis (MLN) and storage pests, mainly maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (*Prostephanus truncatus* Horn), are among the most important maize production constraints and storage problems in tropical and subtropical environments. Drought stress and MLN can cause grain yield losses of up to 90% depending on the severity and stage of growth when they affect the crop, while post-harvest pests can cause 10-60% grain loss. There are no practical agronomic practices that can control these stresses under small-scale farming conditions since investments in irrigation and pesticides are unaffordable for the majority of small-scale farmers in developing African countries. The study was, therefore undertaken to: a) estimate the heritability and gene effects controlling maize weevil and larger grain borer resistance in tropical maize germplasm; b) determine whether resistance to maize lethal necrosis and tolerance to drought can be combined in F1 hybrids developed from tropical maize inbred lines; c) determine the combining ability of tropical maize inbred lines for drought tolerance and resistance to maize weevil and assess the possibility of combining the two traits in one genotype and d) determine gene action controlling the morpho-physiological and agronomic traits of tropical maize under maize lethal necrosis virus infected conditions and maize weevil infestation.

Populations involving six generations; two parents (P1 and P2), F1, F2 and backcrosses (BCP1 and BCP2) were developed from cross one, CKDHL120731 (resistant) × CKDHL120918 (susceptible) and cross two, CKDHL120517 (resistant) × CKDHL120918 (susceptible). The generations were evaluated under artificial infestation of *Sitophilus zeamais* Motschulsky and *Prostephanus truncatus* Horn in separate experiments in a post-harvest laboratory at Kiboko, Kenya. Data was recorded for percentage kernel weight loss, kernel damage and the final number of living insects and data were analysed using generation mean analysis. Results revealed that resistance traits for both crosses did not fit a simple additive-dominance model for *S. zeamais*, suggesting the existence of epistasis effects. However, for *P. truncatus* resistance, cross one fitted a simple additive-dominance model, but cross two did not, suggesting both simple additive-dominance model and digenic interaction model were important in the inheritance of *P. truncatus* resistance. Additive, dominance and epistasis gene effects played a role in the inheritance of resistance to both insects in the selected maize genotypes. This was further confirmed by the moderate

narrow-sense heritability estimates which suggested the involvement of additive and non-additive gene effects in the expression of resistance to both insect pests.

Three separate half-diallel analyses involving eight inbred lines each were conducted involving (1) lines with varying reactions to drought and maize lethal necrosis (MLN), (2) lines with varying drought tolerance and post-harvest pest resistance backgrounds, and (3) inbred lines with varying reactions to maize lethal necrosis (MLN) resistance and maize weevil resistance. The F1 hybrids from these diallel crosses were evaluated in different locations under optimum conditions and managed drought stress, artificial MLN infestation or artificial infestation with maize weevil depending on the objective. For artificial weevil infestation, grain samples were obtained from sites with optimum conditions.

Hybrids differed significantly ($p<0.001$) for MLN resistance and drought tolerance traits, including MLN scores, senescence, days to anthesis and anthesis-silking interval. The yield reduction due to MLND was 93% of the optimum mean grain yield of 6.04 t/ha, while reduction due to drought stress was 67% of the same. Genetic analysis detected highly significant mean squares ($p< 0.001$) due to both general combining ability (GCA) and specific combining ability (SCA) for most of the recorded traits, including grain yield under all environments, suggesting the importance of both additive and non-additive gene effects. However, additive gene action was generally predominant across all evaluation conditions. The results suggest that it is possible to improve tropical maize for combined drought and MLN tolerance and it can be faster when the evaluation is conducted under combined drought and MLN conditions.

Highly significant genotype and genotype × environment interaction mean squares ($p<0.001$) for grain yield and days to anthesis were observed under drought and optimum conditions for the drought tolerance and weevil resistance. Highly significant genotypic effects ($p<0.001$) were also observed on the key parameters for maize weevil resistance; Dobie's Susceptibility Index (SI), living insects, weight loss (WL) and seed damage (SD) revealing different reactions of the tested hybrids. In addition, highly significant mean squares ($p<0.001$) due to both GCA and SCA for grain yield under drought and significant ($p<0.001$) under optimum conditions were detected, suggesting the importance of both additive and non-additive effects. Under maize weevil infestation, highly significant mean squares ($p<0.001$) due to both GCA and SCA for the key parameters were observed except for GCA mean squares for weight loss which was significant ($p<0.05$). Additive gene action

was predominant over non-additive for grain yield under drought, SI, SD and living insects. The cross CKDHL120731 × CKDHL120517 showed tolerance to drought and resistance to maize weevil, while 24 hybrids showed tolerance to drought only.

The maize lethal necrosis (MLN) resistance and maize weevil resistance F1 hybrids showed highly significant ($p<0.001$) genotype differences for field weight and grain yield under MLN infestation. Highly significant ($p<0.001$) genotype and genotype × environment interaction effects were also observed for MLN scores at the early and late-stages under artificial MLN infestation, grain yield under optimum conditions, SI, WL and SD under maize weevil infestation. Significant mean squares ($p<0.01$) due to only GCA for grain yield under MLN and weight loss under maize weevil infestation were detected, while highly significant mean squares ($p<0.001$) due to both GCA and SCA for MLN scores under MLN infestation and grain yield under optimum growing conditions, SI and SD under weevil infestation were observed suggesting the importance of both additive and non-additive effects.

However, for most of the traits under the three evaluation conditions, additive gene action was predominant. Three hybrids CKDHL120918 × CKSBL10060, CKSBL10060 × CKDHL120731 and CML494 × CKDHL120731 showed good performance under the three evaluation conditions. The observed importance of both additive and non-additive gene action, with predominance of additive gene action, especially under the stressed environments, is an indicator of the feasibility of breeding for resistance to combined stresses, and suggests that recurrent selection can be applied for rapid breeding progress. Furthermore, the improvement of tropical maize for combined stress resistance can be faster when the inbred lines and hybrids are developed and evaluated under the combined stress environments, than under a single stress. The identified superior genotypes across environments in this study can be used immediately in breeding programs, especially in sub-Saharan Africa.

Declaration

I, **Egas Jeremias Nhamucho**, declare that:

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

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

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

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

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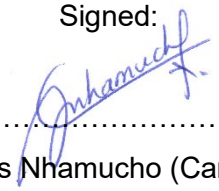
5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference sections.

Signed:



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Egas Nhamucho (Candidate)

As the candidate's supervisors we agree to the submission of this thesis

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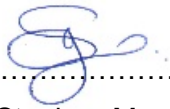
Prof. Julia Sibiya (Supervisor)



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Dr. Stephen Mugo (Co-Supervisor)

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Thank you so much

Muito Obrigado

Dedication

This research work is dedicated to:

GOD:

You are never far from me when I seek you!

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

My parents, **Jeremias Luis and Albertina Capane Nhamuanzo** who raised me under poverty condition but never gave up in supporting my education showing me the way to school as a solution in facing the world;

My beloved wife, **Nértia Urnília Filipe Boca Nhamucho** and my children **Láysha Melizha Egas Nhamucho and Egner Louis Egas Nhamucho**, who were always close to me even with the long distance away from home;

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"Nothing is impossible if you believe and put more effort into it" By: 804

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List of Abbreviations

AD	Days to 50% anthesis
AGRA	Alliance for a Green Revolution in Africa
ANOVA	Analysis of variance
APPSA	Agricultural Productivity Program for Southern Africa
ASI	Anthesis-silking interval
CIMMYT	International Maize and Wheat Improvement Center
dSI	Dobie's susceptibility index
EA	Ear aspect
EPO	Ear position = relative height of ear placement on the plant
EPP	Number of ears per plant
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistics
G x E	Genotype-by-environment interactions
GCA	General combining ability
GMA	Generation mean analysis
GY	Grain yield
IIAM	Acronym for Mozambican Agrarian Research Institute: Instituto de Investigação Agrária de Moçambique
IITA	International Institute of Tropical Agriculture
INIA	Acronym of the National Institute of Agrarian Research: Instituto Nacional de Investigação Agrária
LGB	Larger grain borer
m.a.s.l.	Altitude measured in metres above sea level
MLN	Maize lethal necrosis
MSgca	Mean of square due to general combining ability
MSsca	Mean of square due to specific combining ability
MW	Maize weevil
OPV	Open-pollinated variety
PH	Plant height
SADC	Southern African Development Community
SCA	Specific combining ability
SD	seed damage given in percentage
SI	Selection index
SSA	Sub-Saharan Africa
WL	Grain weight loss given in percentage

1. CHAPTER ONE

General introduction

1.1. Maize origin, production, and importance

Maize (*Zea mays* L.) is one of the most important food sources in the world and originated from wild grass (teosinte) in Mexico around 7000 years ago. It is believed that the native Americans domesticated and improved teosinte into a better source of food for human consumption (Ranum *et al.*, 2014). An improved version of maize was later distributed to the rest of the world. Maize is classified as a grass and therefore a monocot belonging to the family, *Poaceae*, subfamily *Panicoideae*, tribe *Andropogoneae*, and genus *Zea*. It is a monoecious plant with both sexes on the same plant but in different positions and different inflorescences. It is also diploid with a chromosome number of 2n = 2x = 20 (Acquaah, 2007).

Maize currently ranks first in global cereal production followed by wheat and rice (Statista, 2019) and is the second most traded cereal after wheat and followed by rice (FAOSTAT, 2019). Approximately 165 countries cultivate 190-200 million hectares of maize on a wide range of climate, soil, biodiversity and management practices, contributing to 39% of global grain production. The world's largest producer is the United States of America (USA) contributing roughly 36% of the total world maize production (Macauley and Ramadjita, 2015; APEDA, 2019), while Africa produces around 6.5% of the total maize production, with Nigeria as the largest producer contributing around 8 million tons followed by South Africa (IITA, 2019). In Africa, maize is the most produced cereal (Figure 1-1) and is one of the major staple food crops in many African countries, especially in southern, eastern and central Africa (Setimela *et al.*, 2007; Langyintuo *et al.*, 2010; Tiba, 2011; Macauley and Ramadjita, 2015). Sixteen countries out of the 22 where maize is the major source of calorie intake are located in sub-Saharan Africa (SSA). More than 33 million ha of maize are produced in SSA, providing food security and income to around 208 million people (Macauley and Ramadjita, 2015).

Maize is a multipurpose crop that has high nutritive value and can be used as raw material for the production of many industrial products such as food and medicinal products, biofuels for humans and feed for livestock (Dowswell *et al.*, 1996; Zhou *et al.*, 2009). The importance

of maize in Africa as a staple food equals that of rice or wheat in Asia. The highest consumption of maize is in eastern and southern Africa, where it provides 30% and 50% of the calories and protein in diets, respectively, and 15% of the calories and proteins in western and central African population (Pswarayi and Vivek, 2008; Macauley and Ramadjita, 2015). Cutts and Hassan (2003) pointed out that in southern Africa maize dominates as a staple food and South Africa is the only country within this region that has a higher amount of maize for animal consumption.

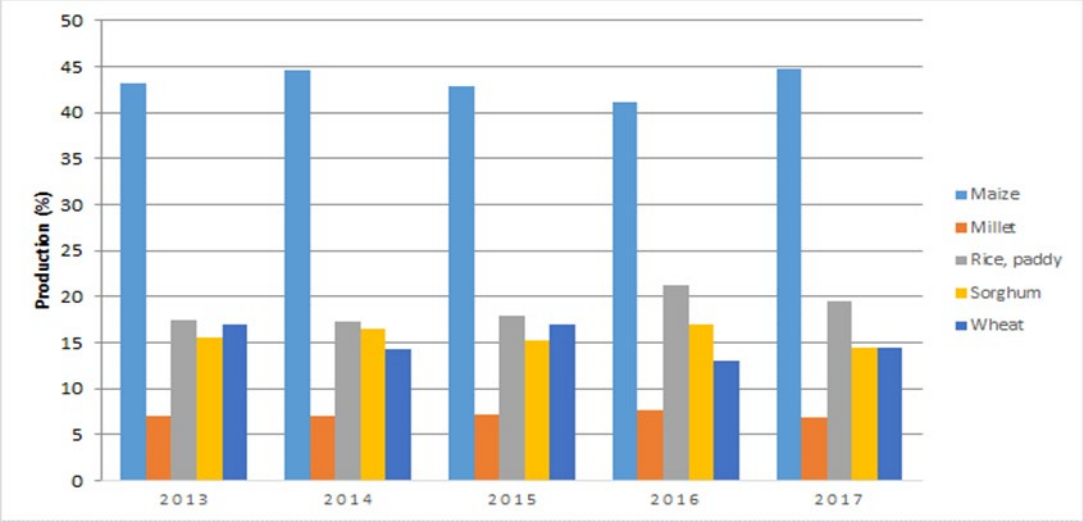

Figure 1-1. Major cereals crop production in Africa

Source of data: FAOSTAT (2019)

1.1.1. Maize production in Mozambique

In Mozambique, maize is one of the most important food crops and it is the main staple food in seven out of the eleven provinces, where 52.8% of the families consider it as their primary food (TIA, 2007). Maize contributes 40% of the total calorie intake in the human diets in Mozambique (FAOSTAT, 2007) which translates to a minimum consumption of 57 kg per capita or 315 kg per household (Tschirley and Abdula, 2007).

As a food crop, the maize share in the total household expenditure varies from place to place in the country. In Maputo (the capital city), maize share is about 2.4% compared to 7.4% of rice. Outside the city and in other provinces the maize share is higher, ranging between 15 – 40% (Tschirley and Abdula, 2007). The importance of maize grain in Mozambique also extends to livestock feeds with some reports indicating large quantities of maize being imported from South Africa for livestock feed, especially for chickens (TIA,

2007). This is due to the national maize production not being adequate to satisfy the demand for both human and animal consumption (SETSAN, 2010).

In Mozambique, maize occupies more than 44% of the total annual cultivated area allocated to the basic food crops and constitutes more than 25% of the annual food crops in all the farming systems (TIA, 2007; INE, 2011). It is grown throughout the country under diverse agro-ecological conditions and farming systems. Grain yields vary from region to region and from environment to environment within a region in the country (Figure 1-2) but the national average is 0.73 t/ha. Maize production in Mozambique is dominated by small scale farmers who account for about 99% of the maize area and 90% of the national maize production (TIA, 2007; Denic *et al.*, 2007; INE, 2011).

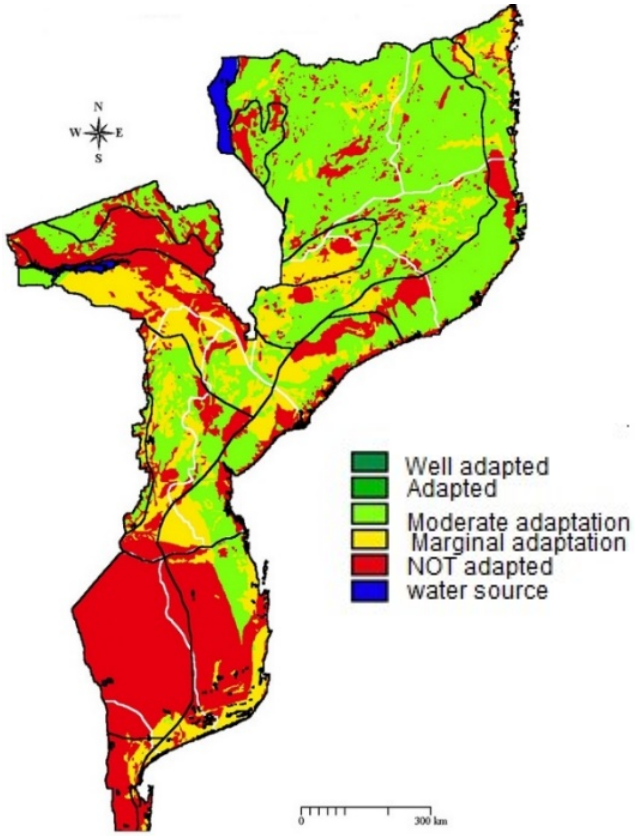

Figure 1-2. Suitable regions for maize production in Mozambique
Source: Adapted from INIA (2000)

The number of holdings, the area and the volume of maize production are higher in the northern and central regions than in the southern region. The northern and central regions account for more than 80% of the total maize land planted and around 90% of the total maize production in the country (Dias, 2013). This is because there are better soil types

and climatic conditions in the northern and central regions than in the southern region (Chaúque, 2009; Cunguara, 2012; Dias, 2013). The annual grain production in million metric tonnes in the past 5 years from 2013 to 2017 (FAOSTAT, 2019), varied from approximately 1.2 to 1.4 with low yields ranging from 0.7 to 0.8 t/ha (FAOSTAT, 2019). The average yield during this period was 0.8 t/ha which is below the SADC average yield of 1.53 t/ha and also below all neighbouring countries except Zimbabwe (Figure 1-3), suggesting that there is a vast potential to increase national maize production by improving yield levels (FAOSTAT, 2019). The most preferred maize grain types for growers and consumers are white flints (Cumbane and Baúque, 2008) because they are harder and easier to make maize mealie meal using a pestle, which is mostly used in rural areas.

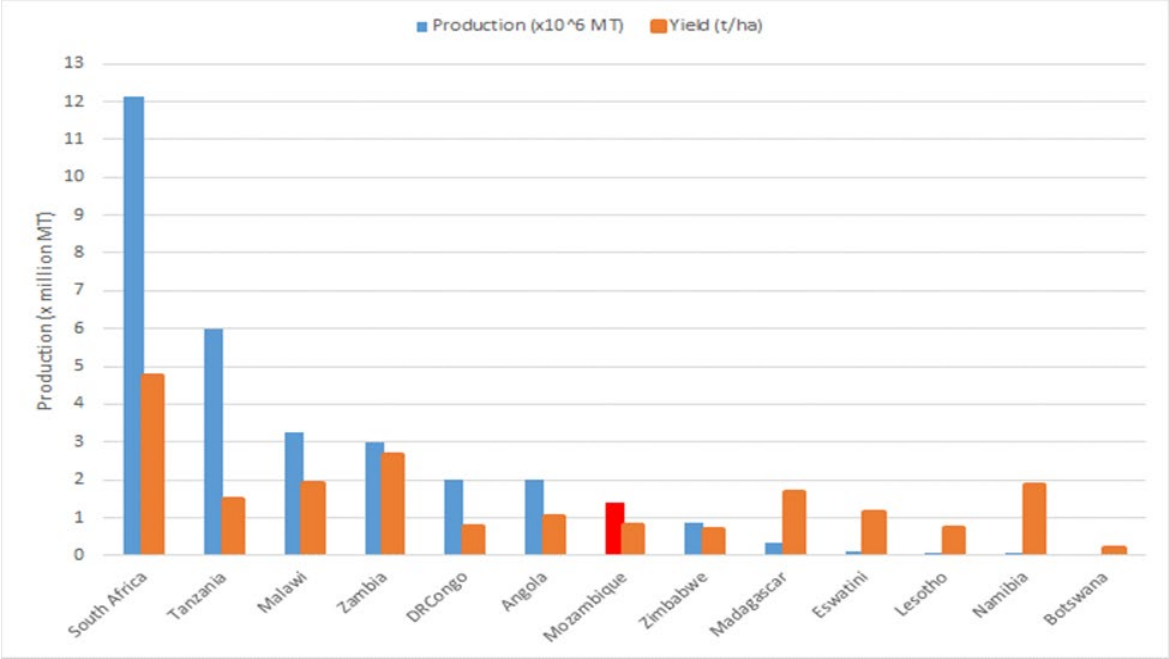

Figure 1-3. Maize production and yields in SADC countries (2013 - 2017)
Source of data: FAOSTAT (2019)

1.1.2. Maize production constraints in Mozambique

Since most of the maize production in Mozambique is dominated by small-scale farmers, it is characterized by lack of mechanization and minimum farm inputs. Production of the crop is constrained by several biotic, abiotic and socio-economic factors. Despite maize having the highest genetic yield potential among all cereals (Chaudhary *et al.,* 2014), in Mozambique the use of unimproved seeds with very low genetic potential for grain yield (Langyintuo *et al.*, 2010; Chaúque, 2009) still dominates. This is largely due to the

weakness of the national seed system and extension services (Chaúque, 2009; Fato, 2010; Chaúque, 2016).

Droughts and low soil fertility are the two major abiotic constraints that affect crop production in Mozambique (Denic *et al.*, 2001; Bänziger *et al.*, 2006; Setimela *et al.*, 2007; SETSAN, 2010). Reynolds *et al.* (2016) defines drought stress as a water deficit at any plant growth stage which results in yield losses ≥ 10% compared to an adequately watered control. In Mozambique, drought is more important and common than low soil fertility. Even in years with good precipitation, drought occurrence during the cropping season varies from region to region across the country, leading to pockets of rainfall shortage and loss of maize production. INGC (2010) reported that drought alone affected more than 16 million people from 1965 to 2008 and more than 100,000 people have died because of drought in Mozambique.

Foliar diseases, field, and post-harvest pests are the most important biotic constraints in Mozambique. Among field pests, stem borers are the most important and the damage is more severe in the southern part of the country, where the spotted stem borer (*Chilo partellus* (Swinhoe)) is the predominant species (Fato *et al.*, 2008). However, from 2017, fall armyworm (FAW; *Spodoptera frugiperda* Smith. Lepidoptera: Noctuidae), became one of the most important field pests across the country, with crop damage reaching 100% (Zacarias, 2018; Prasanna *et al.*, 2018). Among foliar diseases, downy mildew (DM) (*Perenosclerospora sorghi*), maize streak virus (MSV), leaf blight (*Helminthosporium turcicum*), and grey leaf spot (*Cercospora zea-maydis*) are the most important (Denic *et al.*, 2008; Fato, 2010). Downy mildew is predominant in the lowland hot areas, although it has also been reported in the mid-altitude lands of the central provinces, covering five of the 11 provinces in the country, which represents about 45% of the total country maize area. Leaf blight occurs mainly in the mid to high altitude lands of Manica, Tete, Zambezia, and Niassa Provinces where temperatures are relatively low (Denic *et al.*, 2007).

Breeding for drought tolerance (DT) and downy mildew (DM) resistance in maize are among the main breeding objectives of the Mozambique Agriculture Research Institute (IIAM). Some DT and downy mildew resistant open-pollinated varieties (OPVs) have been released in Mozambique (Bueno *et al.*, 1989; Bueno, 1991; Chaúque *et al.*, 2004). However, these are still not enough to satisfy the farmers and the emerging national seed companies (Chaúque, 2009) and are in addition, single trait resistance or tolerance. Therefore,

research addressing drought tolerance and disease resistance in Mozambique continues under the DTMA (Drought Tolerant Maize for Africa), STMA (Stress Resistant Maize for Africa), WEMA (Water Efficient Maize for Africa) and most recently TELA *maize Project* (Drought and Insect protection using transgenic products) projects and IIAM research activities supported by the Alliance for a Green Revolution in Africa (AGRA) and the Agricultural Productivity Program for Southern Africa (APPSA).

The maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (*Prostephanus truncatus* Horn) are the major storage pests in the country (Denic *et al.*, 2007). Both insects can cause losses of around 60% under small scale storage systems annually (Fato *et al.*, 2008). These losses have increased immensely since 1999 after the introduction of *P. truncatus*, where prior losses ranged from 6 to 12% (Cugala *et al.*, 2007a,b). Damage caused by the two pests leads directly to food losses, reduced future maize production for the many farmers who use farm-saved seed and also due to consumption of the infected grain, which is a health risk (Cugala *et al.*, 2007b; Smalley, 1998)

Chemical control against *S. zeamais* and *P. truncatus* has been extensively promoted by extension services but the adoption rate by the smallholder farmers is very low due to the high costs of purchasing these chemicals (INE, 2003; Mariquele, 2006). Consequently, smallholder farmers use cultural methods to control these pests including leaves, smoke and ash from plant species with insecticidal properties. The most used plant species are tobacco (*Nicotiana tabacum L)*, eucalyptus (*Eucalyptus macrorhyncha* F. Muell) and lantana (*Lantana camara* L.) but the efficacy of such control methods is very low (Mariquele, 2006), thus alternative technologies have to be sought.

The interest in developing improved post-harvest technologies in African countries is very high (CIMMYT, 2010). Locally available, ecologically safe, socio-friendly and effective methods for reducing post-harvest losses are therefore needed (Danho *et al.*, 2002; Ogendo *et al.*, 2006; Talukder, 2006; Isman, 2007). It is possible to improve maize varieties for storage pests' resistance through maize breeding (Dhliwayo and Pixley, 2001). Under the Insect Resistant Maize for Africa (IRMA) project and Agricultural Productivity Program for Southern Africa (APPSA) in collaboration with CIMMYT some resistant sources for the two insect pests have been identified and one OPV has been released in the country. However, no breeding activity for combined traits has been done in Mozambique. The aim of this research was, therefore to investigate the feasibility of simultaneous improvement

through breeding of maize germplasm for tolerance to two major constraints in the same genotype.

1.2. Research problem

In Mozambique, small-scale farmers grow maize mainly in the main season from October to March. These farmers use limited external inputs including improved seeds, irrigation, fertilizers and pesticides. Although maize is not well adapted in most parts of Mozambique (Figure 1-2), it is grown throughout the country (TIA, 2007) where it is affected by several constraints. Among those, drought and foliar diseases are the most important in the field and maize weevil in storage, thus worsening the food security of the population (Denic *et al.*, 2007). Maize lethal necrosis (MLN) disease is a new threat to maize production in Mozambique. It has been reported in the neighbouring country, Tanzania where it has devastated maize fields (Wangai *et al.*, 2012; GAIN, 2014; CGIAR, 2015; Mahuku *et al.*, 2015a; Mahuku *et al.*, 2015b), therefore, special attention to this disease should be considered in Mozambique. Breeding of "super maize varieties" which have resistance or tolerance to many stresses has been the biggest challenge faced by maize breeders in Africa. Despite this awareness, combining different traits in breeding has not been done in Mozambique. Most of the developed technologies in the region have only one trait, either tolerance to drought, resistance to post-harvest or resistance to MLN, but not a combination of two traits in a single genotype. This study, therefore, aimed at understanding the genetic factors for resistance to storage pests, maize lethal necrosis, tolerance to drought; and assessing the possibility of combining two traits in the same genotype in order to increase grain yield under stress conditions and reduce post-harvest losses. The findings of this research will ultimately act as a baseline for breeding for combined stress tolerance in maize, thus contribute towards increased food security and productivity by reduction of losses in the field and storage.

1.3. Objectives

1.3.1. Overall goal

To contribute to increased maize productivity in Mozambique through the development of suitable maize germplasm tolerant to MLN and drought stress and resistance to postharvest insect pests (maize weevil and larger grain borer).

1.3.2. Specific objectives

The specific objectives of the study were to:

1. Estimate the heritability and gene effects controlling maize weevil and larger grain borer resistance in tropical maize germplasm
2. Determine whether resistance to maize lethal necrosis and tolerance to drought can be achieved in F1 hybrid combinations using lines resistant to the respective stress in tropical maize germplasm
3. Determine the type of gene action controlling the morpho-physiological and agronomic traits under maize lethal necrosis virus-infected conditions and under maize weevil (*Sitophilus zeamais*) infestation
4. Determine whether tolerance to drought and resistance to maize weevil (*Sitophilus zeamais*) can be achieved in F1 hybrid combinations using lines resistant to the respective stress and the type of gene action controlling drought traits and maize weevil resistance traits.

1.3.3. Research hypotheses

a. Different breeding generations will show different reactions to storage pests' resistance, and maternal effects, additive and non-additive gene action are important in the inheritance of grain resistance to maize weevil and larger grain borer

b. Both additive and non-additive gene action are important in controlling the inheritance of morpho-physiological and agronomic traits in single cross maize hybrids developed from inbred lines with different genetic backgrounds and evaluated under optimum, drought, maize lethal necrosis and maize weevil infestation conditions.

1.4. Thesis outline

The thesis is structured according to the specific objectives, where, each objective is presented as an independent chapter that is a potential manuscript for publication; therefore there may be repetition of content and references across the chapters. The thesis outline is as follows**:**

Chapter 1: General Introduction

Chapter 2: Literature review

Chapter 3: Heritability and gene effects controlling post-harvest, maize weevil and larger grain borer resistance in tropical maize germplasm

Chapter 4: Genetic analyses and potential of combining drought tolerance and lethal maize necrosis resistance in a tropical maize germplasm

Chapter 5: Combining ability for drought tolerance and maize weevil resistance inbred lines in tropical maize

Chapter 6: Gene action controlling grain yield and other traits under maize lethal necrosis and maize weevil infestation and

Chapter 7: General overview of the study- Inheritance of Post-Harvest Pest Resistance and Genetic Analysis of Combining Drought, Maize Lethal Necrosis and Maize Weevil Resistance in Tropical Maize

References

Acquaah, G. 2007. Principles of plant genetics and breeding, United Kingdom, Oxford, Blackwell Publishing, pp 485 - 497.

APEDA. 2019. AgriXchange the changing face of agri-business: Maize [Online]. Available: http://agriexchange.apeda.gov.in/product_profile/prodintro/Maize.aspx [Accessed 16 June 2019].

Bänziger, M., Setimela, P., Hodson, D. and Vivek, B. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agricultural Water Management 80, 212-224.

Bueno, A. 1991. Avaliação e Selecção de Variedades de Milho em Moçambique. Série Investigação No.1. INIA. Maputo.

Bueno, A., Pereira, M. and Mariote, D. 1989. Situação Actual e Programa de Investigação de Milho em Moçambique. Relatório anual. INIA. Maputo.

CGIAR. 2015. Maize Lethal Necrosis: Building a comprehensive response [Online]. Available: http://maize.org/maize-lethal-necrosis-building-a-comprehensive-response/ [Accessed 16 - March - 2019].

Chaudhary, H., Kaila, V. and Rather, S. 2014. Chapter 2: Maize. In: Pratap, A. & Kumar, J. (eds.) Alien gene transfer in crop plants: Archievements and Impacts. India: Springer Science and Business Media, LLC 2014.

Chaúque, P. 2009. Combining Ability for grain yield and related traits of early, flint and drought-tolerant maize inbred lines in Mozambique. MPhil Thesis. MOI University, Kenya.

Chaúque, P. 2016. Genetic and path coefficient analyses and heterotic orientation of maize germplasm under combined heat and drought stress in sub-tropical lowland environments. Ph.D Thesis., University of KwaZulu-Natal, Pietermaritzburg, SA.

Chaúque, P., Fato, P. and Denic, M. 2004. Improvement of maize populations for drought stress tolerance in Mozambique. In: Poland, D., Sawkins M., Ribaut, J.M. & Hoisington, D. (eds.) Resilient crops for water-limited environments. 24 - 28 May, 2004, Cuernavaca, Mexico. Mexico D.F.: CIMMYT: CIMMYT.

CIMMYT. 2010. Effective grain storage for better livelihoods for African farmers- Grain Storage Project [Online]. Available: http://www.cimmyt.org/en/programs-and-units/global-maize-program/projects/effective-grain-storage-project [Accessed 10 October 2010].

Cugala, D., Sidumo, A., Santos, L. and Givá, N. 2007a. Uso do método de controlo biológico contra a broca maior do grão do milho armazenado, *Prostephanus truncatus* (horn) (*Coleoptera*: *Bostrichidae*) nos celeiros das famílias rurais em Moçambique. Moçambique- Maputo: Universidade Eduardo Mondlane.

Cugala, D., Sidumo, A., Santos, L., Mariquele, B., Cumba, V. and Bulha, M. 2007b. Assessment of status, distribuition and weight lost due to *Prostephanus trancutus* (Horn) (*Coleoptera*: *Bostrichidae*) in Mozambique. African Crop Science Journal 8: 975-979.

Cumbane, I. and Baúque, F. 2008. Comportamento de Variedades de Polinização Aberta (OPVs) de Milho (*Zea mays* L.) na Estação Agrária do Umbelúzi, Relatório de Estágio Final do Curso Médio Agro-Pecuário. Instituto Agrário de Boane (IAB)

Cunguara, B. 2012. An exposition of development failures in Mozambique. African Political Economy 39: 161-170.

Cutts, M. and Hassan, R. M. 2003. An econometric model of the SADC maize sector. 41st Annual Conference of the Agricultural Economics Association of South Africa (AEASA). Pretoria, South Africa.

Danho, M., Gaspar, C. and Haubruge, E. 2002. The impact of grain quality on the biology of *Sitophilus zeamais* Motsch. (*Coleoptera*:Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio. Journal of Stored Products Research 38: 259-266.

Denic, M., Chaúque, P., Fato, P., Haag, W., Mariote, D., Langa, M. and Carlos, J. 2001. Maize Screening for multiple stress tolerance and agronomic traits. In: Frisen, D. & Palmer, A. (eds.) Seventh Eastern and Southern Africa Regional Maize Conference. 5- 11 February 2001, Nairobi- Kenya.

Denic, M., Chaúque, P., Fato, P., Senete, C., Mariote, D. and Haag, W. 2007. Breeding approaches in simultaneous selection for multiple stress tolerance of maize in tropical environments. Genetika 39: 113 - 124.

Denic, M., Chaúque, P., Fato, P., Senete, C., Mariote, D. and Haag, W. 2008. Approaches in breeding for high-quality protein maize. Genetika, 40: 237-247.

Dhliwayo, T. and Pixley, K. 2001. Breeding for resistance to maize weevil (*Sitophilus zeamais* Motsch.): Is it feasible? Seventh Eastern and Southern Africa Regional Maize Conference, 11- 15th February, pp. 134-138.

Dias, P. 2013. Analysis of incentives and disincentives for maize in Mozambique. Technical notes series, MAFAP, FAO, Rome, 35pp. In: Technical notes series, M., FAO, Rome.

Dowswell, C., Paliwal, R. and Cantrell, R. 1996. Maize in the Third World, New York, NY 10017, 282pp, Westview Press.

FAOSTAT. 2007. Statistical Yearbook Database of Food and Agriculture Organization of the United Nations 2005/6. FAO, Rome- Italy.

FAOSTAT. 2019. FAOSTAT: Statistical databases and data sets of the Food and Agriculture Organization of the United Nations: FAOSTAT Metadata/Production/Crops. 23 March 2019 Rome: FAO, Rome.

Fato, P. 2010. Investigation of heterotic patterns and genetic analysis of downy mildew resistance in Mozambican lowland maize (*Zea mays* L.) germplasm. Ph.D Thesis., University of KwaZulu-Natal, Pietermaritzburg, SA.

Fato, P., Chaúque, P., Ecole, C. and Cugala, D. 2008. The Status of Development of Maize Resistant to Field and Storage Pests in Mozambique. In: Mugo, S., Gethi, J., Ouma, J., Murenga, G., Mulaa, M., Likhayo, P., Gichuki, V., Kega, V., De Groote, H. & Chavangi, A. (eds.) Book of abstract of the Insect Resistant Maize for Africa (IRMA). (2008) "Consolidating Experiences from IRMA I and II: Achievements, Prospects and Lessons", IRMA project End-of-Phase II Conference, 28-30 October. Nairobi-Kenya KARI and CIMMYT.

GAIN. 2014. Global Agricultural Information Network- Maize Lethal Necrosis- the Growing challenge in Eastern Africa. [Online]. Available: https://gain.fas.usda.gov/Recent GAIN Publications/Maize Lethal Necrosis: The growing challenge in Eastern Africa Nairobi Kenya 12-10-2014.pdf [Accessed 10 October 2019].

INE. 2003. Censo Agro-pecuário 1999-2000. Resultados temáticos: Direcção Nacional de Estatísticas Sectoriais e de Empresas, Maputo, Moçambique.

INE. 2011. Censo Agro-Pecuario CAP 2009-2010: Resultados preliminares - Moçambique: Direcção Nacional de Estatísticas Sectoriais e de Empresas, Maputo, Moçambique.

INGC. 2010. Análise das mudanças climáticas: Alterações climáticas. Relatório [Online]. Maputo: INGC.

INIA. 2000. Mapas de Uso de Terra- INIA-DTA- Dezembro.

Isman, M. 2007. Botanical insecticides: for richer, for poorer. Pest Management. Crop Science 64: 8-11.

IITA. 2019. Maize [Online]. Available: https://www.iita.org/cropsnew/maize/ [Accessed 20 November 2019].

Langyintuo, A. S., Mwangi, W., Diallo, A. O., Macrobert, J., Dixon, J. and Bänziger, M. 2010. Challenges of the maize seed industry in eastern and southern Africa: a compelling case for private-public intervention to promote growth. Food Policy 35: 323-331.

Macauley, H. and Ramadjita, T. 2015. Cereal Crops: Rice, Maize, Millet, Sorghum, Wheat. Feeding Africa 21- 23 October, 2015. Abdou Diuof International Conference Center-Dakar, Senegal: UN Economic Commission for Africa: African Development Bank group.

Mahuku, G., Lockhart, B., Wanjala, B., Jones, M., Kimunye, J., Stewart, L., Cassone, B., Sevgan, S., Nyasani, J., Kusia, E., Kumar, P., Niblett, C., Kiggundu, A., Asea, G., Pappu, H., Wangai, A., Prasanna, B. and Redinbaugh, M. 2015a. Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-Saharan Africa. Phytopathology 105: 956-965.

Mahuku, G., Wangai, A., Sadessa, K., Teklewold, A., Wegary, D., Ayalneh, D., Adams, I., Smith, J., Bottomley, E., Bryce, S., Braidwood, L., Feyissa, B., B. Regassa, B., Wanjala, B., Kimunye, J., Mugambi, C., Monjero, K. and Prasanna, B. 2015b. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis on Maize in Ethiopia. Plant Disease 99: 1870-1870.

Mariquele, B. 2006. Ocorrência da broca maior do grão *Prostephanus truncatus*: Horn (*Coleoptera*: *Bostrichidae*) em Moçambique: O caso do distrito de Manica. Lincenciatura Projecto final, Universidade Eduardo Mondlane.

Ogendo, J., Omolo, E., Deng, A., Matasyoh, J. and Tabu, I. 2006. Field grains losses and insect pest management practices in subsistence agriculture: Farmers' perceptions. Journal of Agriculture, Science and Tecnology, 8: 24- 42.

Prasanna, B. M., Huesing, J. E., Eddy, R. and Peschke, V. M. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management, First Edition. Mexico, CDMX: CIMMYT.

Pswarayi, A. and Vivek, B. 2008. Combining ability amongst CIMMYT's early maturing maize (*Zea mays* L.) germplasm under stress and non-stress conditions and identication of testers. Euphytica 162: 353-362.

Ranum, P., Peña-Rosas, J. P. and Garcia-Casal, M. N. 2014. Global maize production, utilization, and consumption. Annals of the New York Academy of Science 1312: 105-112.

Reynolds, M. P., Quilligan, E., Aggarwal, P. K., Bansal, K. C., Cavalieri, A. J., Chapman, S. C., Chapotin, S. M., Datta, S. K., Duveiller, E., Gill, K. S., Jagadish, K. S. V., Joshi, A. K., Koehler, A.-K., Kosina, P., Krishnan, S., Lafitte, R., Mahala, R. S., Muthurajan, R., Paterson, A. H., Prasanna, B. M., Rakshit, S., Rosegrant, M. W., Sharma, I., Singh, R. P., Sivasankar, S., Vadez, V., Valluru, R., Vara Prasad, P. V. and Yadav, O. P. 2016. An integrated approach to maintaining cereal productivity under climate change. Global Food Security 8: 9-18.

Setimela, P. S., Vivek, B., Bänziger, M., Crossa, J. and Maideni, F. 2007. Evaluation of early to medium maturing open-pollinated maize varieties in the SADC region using GGE biplot based on the SREG model. Field Crops Research 103: 161-169.

SETSAN. 2010. Relatório da Monitoria da Situação de Segurança Alimentar e Nutricional em Moçambique. Secretariado Tecnico para Seguranca alimentar e Nutricional (SETSAN). Maputo.

Smalley, E. 1998. Identification of mycotoxin producing fungi and conditions leading to aflatoxin contamination of stored foodgrains. In: Semple, R., *et al.* (ed.) Mycotoxin

prevention and control in foodgrains. United Nations Development Programme and Food and Agriculture Organization. Bankok- Thailand.

Statista. 2019. Worldwide production of grain in 2018/19, by type (in million metric tons) [Online]. Available: https://www.statista.com/statistics/263977/world-grain-production-by-type/ [Accessed 10 October 2019].

Talukder, F. 2006. Plant Products as potential stored product insect pest management agents-A mini-review. Emirates Journal of Agriculture Science 18: 17- 32.

TIA 2007. Trabalho de Inquerito Agricola (2007). Ministerio de Agricultura. Direcção Nacional de Economia Agraria. Maputo.

Tiba, Z. 2011. 'Maize is Life, but Rice is Money!' A Village Case Study of the2001/02 Famine in Malawi. Journal of Agrarian Change 11: 3-28.

Tschirley, D. and Abdula, D. 2007. Toward Improved Marketing and Trade Policies to Promote Household Food Security in Central and Southern Mozambique 2007 Update. Research Report No. 62E. Economics Directorate, MADER.

Wangai, A., Redinbaugh, G., Kinyua, Z., Miano, D., Leley, P., Kasina, M., Mahuku, J., Scheets, J. and Jeffers, D. 2012. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Diseases 96: 1582- 1586.

Zacarias, A. 2018. USDA Global Agricultural Information Network- Mozambique Fall Armyworm

Zhou, S., Wei, F., Nguyen, J., Bechner, M., Potamousis, K., Goldstein, S., Pape, L., Mehan, M. R., Churas, C., Pasternak, S., Forrest, D. K., Wise, R., Ware, D., Wing, R. A., Waterman, M. S., Livny, M. and Schwartz, D. C. 2009. A Single Molecule Scaffold for the Maize Genome. PLOS Genetics 5: e1000711.

2. CHAPTER TWO

Literature review

2.1. Introduction

This chapter outlines information on the general status of agriculture in Mozambique, focusing on maize, the importance and main constraints for its production including diseases, drought and postharvest pests, control strategies and the role of host resistance in reducing their negative effects. The chapter further describes in detail drought and its effects, maize lethal necrosis, maize weevil and larger grain borer, their importance, biology, ecology, distribution, control methods and breeding methods and strategies. In the process of reviewing the literature, information gaps were identified, some of which have been addressed in the current study.

2.2. Agriculture production constraints in Mozambique

In Mozambique, agriculture is the main economic activity, although, done on less than 10% of the arable land, mainly by small-scale farmers accounting for around 3.2 million households. The small-scale farmers contribute towards 95% of total national agricultural production while 5% is by the commercial sector (FAO, 2019). However, most of the arable land is in biotic and abiotic stress-prone areas and, in addition, the agricultural sector is vulnerable to shocks due to low use of improved inputs, limited access to credit and markets and the dominance of rain-fed production (FAO, 2019). Nevertheless, agriculture sustains almost 80% of the population and provides about 24% of the GDP (Trading Economics, 2019; Nations Encycloedia, 2019).

The major cash crops in Mozambique are cashew nuts, cotton, coconut, sugarcane and tea while the important food crops are maize, cassava, rice, sorghum and legumes, mainly beans, groundnuts and cowpea (NationsEncycloedia, 2019). Maize and cassava are the principal staple food crops. Maize is produced throughout the country and is a staple food for most of the people. Maize production is done mainly by small-scale farmers, using poor technologies, resulting in low yields (Fato, 2010; SETSAN, 2010). Langyintuo *et al.* (2010) reported that the use of local varieties (not improved ones) with low genetic potential for yield is the most common socio-economic constraint in the majority of the small scale farming system. Although the agricultural NGOs, seed companies and the national public

extension services, educate farmers on best agricultural practices, through field days and farmer field schools, the adoption of improved varieties and use of chemicals is still very low due to limited financial resources and the weakness of the national seed system and extension services (Chaúque, 2009; Langyintuo *et al.*, 2010, Fato, 2010; Chaúque, 2016).

Field pests especially stem borers and foliar diseases are the main biotic constraints limiting production of maize in Mozambique (Segeren *et al.*, 1994), while in storage, pests are also important constraints. Stem borers are more severe in the southern part of Mozambique, where *Chilo partellus* (Swinhoe) is the most significant species, followed by *Busseola fusca* and *Sesamia calamistis* (Cugala *et al.*, 2003; Fato *et al.*, 2008; Cugala *et al.*, 2009). *Chilo partellus* is predominant in lowland warm environments while in the mid-altitudes and cool environments *Busseola fusca* and *Sesamia calamistis* are the important species (Segeren *et al.*, 1994; Cugala *et al.*, 2003). From 2017, fall armyworm (*Spodoptera frugiperda* Smith. Lepidoptera: Noctuidae), became one of the most important field pests across the country, causing up to 100% destruction of the crop (Zacarias, 2018; Prasanna *et al.*, 2018).

Among foliar diseases, downy mildew (*Perenosclerospora sorghi*), maize streak virus (MSV), leaf blight (*Helminthosporium turcicum*), and grey leaf spot (*Cercospora zeae-maydis*) are the most important, although common rust (*Puccinia sorghi*), ear rot (*Diplodia maydis*) and common smut (*Ustilago maydis*) (Denic *et al.*, 2008; CIMMYT, 2008; Fato, 2010) are also important. MSV is prevalent throughout the country, while downy mildew is predominant in the lowland hot areas, with minor reports from the mid-altitude lands and leaf blights are common in the mid to high altitude lands where temperatures are relatively low (Denic *et al.*, 2007).

Drought and low soil fertility are the two important abiotic stresses in agricultural production (Denic *et al.*, 2001; Bänziger *et al.*, 2006; Setimela *et al.*, 2007; SETSAN, 2010). Even in years with good precipitation, drought occurrence during the cropping season varies from region to region across the country, leading to pockets of rainfall shortage and loss of maize production.

2.3. Drought

2.3.1. Concept and economic importance

Drought is a shortage in water supply, either from precipitation or from surface water or even groundwater. Drought has a negative influence on the ecosystem, agriculture and economy. In agriculture, drought occurs when rain ends or its distribution is irregular throughout the growing season of the crops affecting negatively the crop production (Toker *et al.*, 2007; Mir *et al.*, 2012).

In sub-Saharan Africa (SSA), majority of small-scale farmers rely on maize (*Zea mays* L.), however, its production is substantially reduced by drought (Derera *et al.*, 2008; Meseka *et al.*, 2011). Drought severity can be classified according to the yield reduction over its potential in the same site and same season. If the yield reduction is about 50%, it is considered moderate drought stress and it is severe when the reduction is around 80 to 85% (Bolaños and Edmeades, 1996; Bänziger *et al.*, 2000; Betrán *et al.*, 2003c). Although drought can affect maize at all growth stages, it is more severe when it occurs at reproduction stage causing yield reduction from 40% to 90% (NeSmith and Ritchie, 1992; Menkir and Akintunde, 2001; Cakir, 2004). During the reproduction stage, it disturbs the synchronization between male and female flowers in the field, causing poor pollination and consequently significant yield reduction (Grime and Campbell, 1991). Whereas when it occurs at grain filling stage, it decreases cellular events before storage product synthesis in the plant, including endoreduplication, endosperm and other cellular events, causing reduced grain filling and grain weight (Bänziger *et al.*, 2002). Campos *et al.* (2006) observed grain yield reduction from 45 to 60% when drought occurred at silk emergence.

Most of the small-scale farmers have limited or no access to irrigation facilities but grow local varieties, majority of which are susceptible to drought. Dryland production in southern Africa accounts for almost 95% of the total agricultural crop production (Banziger and Diallo, 2001, Campos *et al.*, 2004) and it is challenging to reduce the negative drought effects without irrigation. The low production of maize in the region has an adverse effect on food security and economy of the region as revealed by a low annual GDP in most of the countries (Richardson, 2005).

The current global warming and increasingly erratic rainfall caused mainly by climate change may aggravate drought frequency and intensity in the near future, subjecting maize and other crops to more unfavourable production conditions (Bolaños and Edmeades,

1996; Campos *et al.*, 2004; Messmer *et al.*, 2009; Hao *et al.*, 2011). Therefore, developing high yielding maize varieties under optimum and drought conditions is critical in increasing the maize production globally (Campos *et al.*, 2004; Xiong *et al.*, 2006), and thus ensuring less hunger (Derera *et al.*, 2008; Mir *et al.*, 2012).

Development and deployment of hybrids tolerant to drought has been the main activity at International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) in the past fifteen years to support achievement of food security in sub-Saharan Africa (Monneveux *et al.*, 2006). The introduction and use of drought-tolerant exotic germplasm allow the expansion of the genes for drought to adapted germplasm and breeding of new drought-tolerant maize (Kim *et al.*, 1987; Eberhart *et al.*, 1995; Dhliwayo *et al.*, 2009). Elite drought tolerant germplasm and high yielding lines have been used as sources to develop new hybrids with drought tolerance (Hallauer *et al.*, 1988; Dhliwayo *et al.*, 2009). Drought-tolerance is the capacity to produce economic yield under drought stress (Ribaut *et al.*, 2009).

2.3.2. Breeding methods for drought

Drought causes crop yield reduction than any other abiotic stresses (Wang *et al.*, 2019). Drought tolerance is a complex quantitative trait controlled by many genes, affecting the crop at any growth stage over space and time. It is one of the most difficult traits to study and characterize because, the physiological responses to it are also complex and often unpredictable, making breeding of drought tolerant maize genotypes a complex task (Maazou *et al.*, 2016). Breeding for abiotic constraints in the field can be achieved through selection under optimum conditions or through selection under target stress environment (Baum *et al.*, 2007; Van Gioi *et al.*, 2017). The selection under target stress can be divided into two; “empirical breeding” and “analytical (physiological) breeding”. It is considered “empirical breeding” when the selection is done directly for grain yield *per se* under stressed conditions and “analytical (physiological) breeding” when the selection is done using traits which are associated with higher yield potential simultaneously under both optimum and stressed environments (Bänziger *et al.*, 2006; Baum *et al.*, 2007; Araus *et al.*, 2008; Lopes *et al.*, 2011; Van Gioi *et al.*, 2017).

Breeding for grain yield potential under optimum environment has resulted in incredible advances in field crops, using phenotypic selection (Reynolds and Trethowan, 2007; Araus

et al., 2008; Lopes *et al.*, 2011; Maazou *et al.*, 2016). In the past, breeders believed that a genotype exhibiting increased yield potential under optimum conditions would always show relatively better performance under stressed conditions (Bolaños, 1995; Bänziger *et al.*, 2000; Gill and Raj, 2009). However, when selecting specifically for abiotic stress tolerance, the efficiency of phenotypic selection decreases dramatically (Araus *et al.*, 2008; Maazou *et al.*, 2016). It has been reported that high-yielding varieties selected under optimum environmental conditions significantly decrease their performance under moderate to severe stress growing conditions (Maestri *et al.*, 2002; Bänziger *et al.*, 2006; Takeda and Matsuoka, 2008; Vaezi *et al.*, 2010). This is mainly due to the significant genotype-by-environment (GE) interactions and high field variation within a single environment leading to reduced heritability of grain yield and other quantitatively inherited traits under stressed conditions (Bolaños and Edmeades, 1996; Bänziger *et al.*, 2006; Chimenti *et al.*, 2006; Lopes *et al.*, 2011). High yield potential genotypes require high inputs, including water, fertilizer and other resources, so the absence of those and lack of genetic tolerance or resistance under stress will result in failure. Therefore, application of the approaches that combine both empirical and analytical selection methods, complemented by multi local evaluation would be more efficient (Chaúque, 2016; Van Gioi *et al.*, 2017).

In general, selection for secondary traits correlated with grain yield under managed drought in an open field environment is the most popular procedure used by many breeders. Many researchers have published the importance of physiological approaches in breeding for abiotic stresses, including drought. The mechanisms of tolerance to drought, which include, increased osmotic adjustment through accumulation of solutes at the cellular level, proline accumulation, increased net photosynthetic activity (Pn), especially photosystem II, reduced production of abscic acid (ABA), reduced interval between anthesis and silking (ASI), reduced ear barrenness, and slow leaf senescence (prolonged stay-green) have been well reported by several researchers including, Bolaños and Edmeades, 1996; Bänziger *et al.*, 2002, 2006; Xin-Hai *et al.*, 2003; Tollenaar and Lee, 2006; Chen *et al.*, 2010; Shuja *et al.*, 2011; Maazou *et al.*, 2016; Hussain *et al.* 2018; Kondwakwenda *et al.*, 2019 and Wang *et al.*, 2019. However, selection for most of these traits and their influence on drought traits under drought conditions is not an easy task due to the great variability in the field (Lopes *et al.*, 2011; Maazou *et al.*, 2016; Van Gioi *et al.*, 2017).

Currently, new tools have been developed that allow identification and observation of the genetic variation at the molecular level and thereby avoiding the complications due to year-

to-year variability in the frequency, duration and intensity of abiotic stresses within location (Xu, 2010; Lopes *et al.*, 2011; Maazou *et al.*, 2016). Compared to conventional approaches, observation at the molecular level offers extraordinary opportunities for dissecting quantitative traits into their single genetic determinants, quantitative trait loci (QTL), thus paving the way to marker-assisted selection (MAS) and, eventually, cloning of QTLs and their direct manipulation via genetic engineering (Tuberosa and Salvi, 2006; Maazou *et al.*, 2016; Van Gioi *et al.*, 2017).

Althought it has been difficult to identify QTLs associated with drought tolerance by fact that the chance to allocate all genes associated with a complex trait decreases with the number of loci and the magnitude of their effect (Reynolds and Trethowan., 2007; Gill and Raj, 2009). However, Zhu *et al.* 2011, reported that it is possible to identify major QTLs regulating specific drought responses and it can provide an efficient way to improve drought tolerance in maize germplasm. Characterization of molecular markers associated with drought tolerance has been done in quantitative trait loci (QTLs) mapping (Xoconostle-Cazares *et al.*, 2011).

An exhaustive review of the breeding strategies using molecular markers for abiotic stress tolerance and achievements in terms of cultivars developed using this approach has been done by Baum *et al.* (2007). In maize, molecular approaches have been used in identifying QTLs, which explain the genetic variability for most of the morpho-physiological traits highly correlated with yield under abiotic stress. This includes tassel size, ASI, duration of stay-green period, root architecture, ABA induction and photosynthesis II (Bolaños, 1995; Hossain *et al.*, 2016; Wang *et al.*, 2019), cell membrane stability (Dwyer *et al.*, 2007; Hossain *et al.*, 2016) and production of heat-shock proteins (HSPs) (Feder and Hofmann, 1999; Hossain *et al.*, 2016). The increasing number of research reporting QTLs for traits associated with drought tolerance and yield under drought stressed crops indicates increasing interest in this approach (Sari-Gorla *et al.*, 1999; Ribaut *et al.*, 2000; Fu *et al.*, 2008; Li *et al.*, 2010; Messmer *et al.*, 2011; Zhu *et al.*, 2011, Semagn *et al.*, 2013; Almeida *et al.*, 2014; Beyene *et al.*, 2015; Beyene *et al.*, 2016). Ribaut *et al.* (2009) using combined modeling and field measurements, observed common QTLs for both ASI and leaf growth in a recombinant inbred line population under drought stress and also, that QTL associated with drought tolerance are dispersed throughout on the maize genome.

Breeding programs improve drought tolerance by the use of diverse approaches such as recurrent selection and evaluation of segregating population under managed and multi-

location drought-stress environment, use of secondary traits for selection under drought condition, genomic-based approach and transgenic technology (Van Gioi *et al.*, 2017). The rapid progress in biotechnology and genomic sequencing, allows a wide choice of tools for the identification of candidate genes associated with specific processes, including response to drought stress. A lot of drought stress tolerance candidate genes have been reported by various researchers (Ribaut *et al.*, 2000; Bohnert *et al.*, 2006; Fu *et al.*, 2008; Li *et al.*, 2010; Messmer *et al.*, 2011), although few have been validated via different plant improvement methods, including conventional and transgenic approaches (Ribaut *et al.*, 2009).

Some good promising results have been reported by several researchers (Quan *et al.*, 2004; Habben *et al.*, 2014; Kikuchi *et al.*, 2015; Maazou *et al.*, 2016). For example, glycine betaine content has been reported as an important organic osmolyte that accumulates in some plant species, including maize under drought stress. A transformation of DH4866 with betA from *Escherichia coli*, showed that the transgenic plants accumulated higher levels of glycine betaine and showed high drought tolerance and yielded higher than non-transformed genotypes during germination and seedling stages. This suggested that enhanced glycine betaine accumulation in maize provides better protection of the integrity of the cell membrane and superior activity of enzymes compared to non transgenic plants under drought stress conditions (Quan *et al.*, 2004; Hossain *et al.*, 2016; Maazou *et al.*, 2016).

2.3.3. Gene action for grain yield and important traits under drought conditions

Breeding for drought tolerance needs a good understanding of its inheritance. Available information on the inheritance of drought tolerance in maize is still limited, with some contradictory results for grain yield, although the majority of the researchers agree. Guei and Wassom (1992) and Shiri *et al.* (2010) observed that non-additive gene effects were responsible for controlling grain yield of tropical maize germplasm under drought stress, whereas, Betrán *et al.* (2003c), Derera *et al.* (2008), Erdal *et al.*, (2015), Chauque, (2016), and Ertino *et al.* (2017) reported additive gene effects being responsible for grain yield in tropical and temperate maize germplasm. Betrán *et al.* (2003c) and Derera *et al.* (2008) also observed that the ratio of general combining ability (GCA) over specific combining ability (SCA) [(GCA/SCA)] increased with the level of stress, indicating the presence of

additive effects, which requires the two parents to be tolerant to drought to achieve satisfactory performance.

The same conclusions, were reported by several researchers, including Williams *et al.* (1969) and Desai and Singh (2001) who observed that inbred lines with positive and high GCA effects produced the highest number of hybrids tolerant to drought, when they analyzed combining ability effects from a diallel mating design of six sweet corn inbred lines with differing reactions to drought. According to Meseka *et al.* (2011) and Adebayo *et al.* (2014), GCA accounted for 55% to 87% and 49 to 85%, respectively of total observed variation among hybrids for most of the traits under drought. The SCA effects for grain yield were not significant under drought stress conditions, although positive in most hybrids, supporting the significance of additive genetic effects in governing main traits under drought, including grain yield. The predominance of additive gene effects for other important drought associated traits, including anthesis and silk interval (ASI), number of ears per plant, leaf senescence and leaf rolling has been reported unanimously by several researchers including Derera *et al.* (2008); Shiri *et al.* (2010); Chauque, 2016 and Ertino *et al.* (2017).

2.4. Maize lethal necrosis

2.4.1. Biology of maize lethal necrosis

Maize lethal necrosis (MLN) is a severe maize foliar disease caused by infection of two viruses (synergistic interaction) on the plants; *maize chlorotic mottle virus* (*Machlomovirus: Tombusviridae*) in co-infection with one or more other cereal viruses from the Potyviridae group, including, *Sugarcane mosaic virus* (*Potyvirus: Potyviridae*) (most common), *Maize dwarf mosaic virus* or *Wheat streak mosaic virus* (Niblett and Claflin, 1978; Doupnik Jr, 1979; Louie, 1980; Xie *et al.*, 2011; Wangai *et al.*, 2012a; Mahuku *et al.*, 2015a). Thrips *(Frankliniella williamsi Hood)* and *beetles (Cabanas et al., 2013)* mainly transmit *maize chlorotic mottle virus (MCMV*) but some transmission and spread through seeds from infected plants has been reported at a rate of 0.0003% which is enough to cause an epidemic (Jensen *et al.*, 1991). *Sugarcane mosaic virus* is mainly transmitted by aphids (Brandes, 1920) and it is linked with MLN in all countries where MLN is reported in Africa to date. *Maize chlorotic mottle virus* represents a threat on its own and can cause significant economic yield loss even in the absence of the other viruses.

2.4.2. Maize lethal necrosis symptoms

Maize lethal necrosis causes various symptoms subject to the germplasm, period of attack and environmental conditions. The symptoms vary from slight chlorotic mottling to severe stunting, reduced male flower production with reduced and few spikes, partially filled or deformed ears, leaf necrosis and premature death of the whole plant (Castillo and Hebert, 1974; Niblett and Claflin, 1978; Uyemoto *et al.*, 1981). When *maize chlorotic mottle virus* co-infects maize with one of the potyviruses, the infected plants develop a wide range of symptoms. These can be observed as chlorotic mottling of the green leaves, which normally starts from the bottom of the younger leaves and extend up to the leaf tips. Some necrosis can be observed at the margins of the leaf progressing to the mid rib causing the whole leaf to dry. Severely affected plants produce small ears with few or no single grain and the whole plant commonly dies before flowering (Niblett and Claflin, 1978; Uyemoto *et al.*, 1980, 1981; Wangai *et al.*, 2012a).

2.4.3. Distribution and ecology of maize lethal necrosis

The first outbreak of maize lethal necrosis in Africa was from Kenya in September 2011 (Wangai *et al.*, 2012a; Wangai *et al.*, 2012b). From there, it has been observed in Tanzania and Uganda (Wangai *et al.*, 2012b; Makumbi and Wangai, 2013), Democratic Republic of Congo (DRC) (Lukanda *et al.*, 2014), Rwanda (Adams *et al.*, 2014), and more recently in South Sudan and Ethiopia (Mahuku *et al.*, 2015b, Flett and Mashingaidze, 2016). Worldwide, MLN has been reported in Kansas, USA in 1977 as corn lethal necrosis (CLN) disease (Niblett and Claflin, 1978), Nebraska, USA (Doupnik Jr, 1979), Hawaii, USA (Jiang *et al.*, 1992) and China (Xie *et al.*, 2011), Peru, Argentina, Mexico, Brazil and Thailand (Flett and Mashingaidze, 2016). Currently, the environmental conditions favourable for emergence of MLN are still not well understood, creating difficulties in designing good strategies for mitigation (Isabirye and Rwomushana, 2016).

2.4.4. Economic importance of maize lethal necrosis

The MLN virus causes significant economic grain yield reduction even without the presence of the other viruses and they are considered significant in terms of occurrence and negative impact in numerous regions producing maize (Tilahun *et al.*, 2001; Redinbaugh and Pratt, 2008; Wangai *et al.*, 2012b). Losses estimated from 50 to 90% have been reported in Kansas and 10 to 15% in Peru subject on the genotype and the year (Niblett and Claflin,

1978; Uyemoto *et al.*, 1980). In Africa limited information on the economic losses due to MLN is available as the disease is still relatively new.

In Kenya, field observations suggested that MLN affected practically all maize varieties that had been planted in various fields, causing yield reduction or loss ranging from 30 to 100% depending on the disease severity (Wangai *et al.*, 2012a; DeGroote *et al.*, 2016). In 2012, MLN affected 77,000 ha of maize in Kenya, which can be estimated as yield loss of 126 MMT equivalent of USD 52 million (Wangai *et al.*, 2012b; MDRAT, 2012). In 2013, in Tanzania, eight of the twenty main maize-growing areas were affected and in 2014, in Uganda, eight districts where maize is largely produced were affected by MLN disease (IPPC, 2014).

2.4.5. Maize lethal necrosis control

Various methods can be used to control the viruses responsible for MLN infestation. The most common are cultural and chemical methods. On the cultural, crop rotation or green bridge (an interruption in maize planting periods) are the most recommended and can effectively control MCMV. It is recommended to do a rotation of the crops for at least two planting seasons with non-cereal crops, such as potatoes, cassava, beans, and vegetables or leave the farm with no maize crop for 3-4 months (Uyemoto, 1983). Using these methods, the field should be free of weeds to reduce all possible alternative hosts for the vectors (Wangai *et al.*, 2012a).

To decrease the pathogen and the vector, infected leaves must be removed and destroyed. They can be used to feed cattle but rotten cobs and the grains must be burned (CABI, 2019). Restrictions on seed movement is another method, which can be used to reduce the spread of the disease. However, this requires governments' intervention, which includes enforcing quarantine and raising awareness in the farming community on the existence of this disease (CABI, 2019). On the chemical method, there is need to control soil-borne and early season vectors using specific pesticides. In Hawaii, Imidacloprid applied as a seed dressing followed by foliar sprays regularly after planting showed great results in controlling the disease (Nelson *et al.*, 2011).

2.4.6. Host-plant resistance

Host plant resistance (HPR) is an attractive solution to reducing maize yield losses due to infestation by maize lethal necrosis (MLN) in the small-scale farming community. Tropical

germplasm resistant to MCMV is widely available, especially in Hawaii and provides good control of MLN, although no complete immunity has been observed (Nelson *et al.*, 2011). Available information on the inheritance of the resistance to MLN proposes a polygenic control of MLN, with resistance being partially dominant (Nelson *et al.*, 2011) and very little information on the genetics for resistance is available. Beyene *et al.* (2017) reported that combining ability estimates for MLN resistance suggest the predominance of additive gene effects over non-additive gene effects and thus suggesting that quick development can be achieved using recurrent selection.

2.5. Economic importance of maize weevil and larger grain borer

Larger grain borer (*Prostephanus truncatus* Horn) and maize weevil (*Sitophilus zeamais* Motschulsky) and are currently among the economically important maize storage pests in the tropics. The two pests cause the most damage in the low and mid-altitude hot and humid regions when maize is stored without proper moisture content and chemical or botanic protection (Dhliwayo and Pixley, 2003; Tefera *et al.*, 2010).

Grain weight losses due to maize weevil are about 12 − 20% but this proportion increases up to 80% in tropical environments, when not treated maize grain with high moisture content (>15%) is stored in traditional structures (Dhliwayo and Pixley, 2003; Boxall, 2002; CIMMYT, 2001). The two insects' damage leads directly to the loss of food as well as limiting the next planting area for the farmers who use saved grain (Cugala *et al.*, 2007b). Storage pests contribute to a public health by the risk associated with eating infested grain, since it has higher levels of *Aspergillus flavus* contamination over non-infested grain (Smalley, 1998).

2.6. Biology, ecology and distribution of maize weevil

The maize weevil (*Sitophilus zeamais* Motschulsky) (Plate 2-1) is an insect belonging to the order *Coleoptera* and family Curculionidae. It is distributed in tropical environments and it is the dominant *Sitophilus* species in tropical subsistence agricultural systems where its infestation starts in the field before harvesting. Maize weevil can be found throughout the warm and humid regions worldwide, especially where maize is grown. In Africa, it can be found in all sub-Saharan countries (CABI, 2018). However, maize weevil is also established in temperate environments where it is commonly associated with feeding on maize, rice and other raw or processed cereals (Canadian Grain Comission, 1999).

The adults are small weevils around 2.4 to 6.0 mm in length, dark brown to black in colour with four reddish-brown spots on the wing covers (elytra), elbowed antennae and head protruded into a long and thin snout (proboscis) which is used to bore holes into grain kernels. They have a pair of mandibles or jaws at the end of the snout, usually used to chew and crush the kernel (Roberts and Douce, 1999; Tefera *et al.*, 2010).

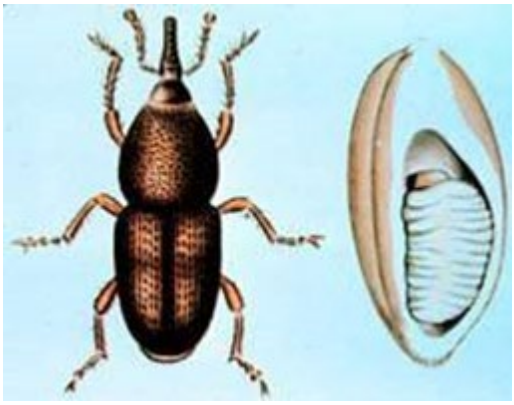

Plate 2-1. Adult and pupa of Maize weevil (*Sitophilus zeamais* Motschulsky)
Source: (Robert and Douce, 1999

The life cycle of *Sitophilus zeamais* is composed of egg, larvae, pupa and adult stages. Some stages happen inside the grain, so are rarely seen, this includes the egg, larva and pupa stages. The fully-grown larva has a length of about 4 mm, white, legless and grub-like (Cotton, 1956). It develops and pupates in the kernel. Under optimal temperature conditions of 27 - 31°C and 70% RH, the maize weevil's life cycle is completed in 5 to 8 weeks. Development stops if the temperature falls below 17°C or above 32°C (Canadian Grain Commission, 1999; Tefera *et al.* 2010). However, adults and larvae can survive at 0°C for several hours (Roberts and Douce, 1999). Once the female adult emerges from the kernel, it needs 3 days for pre-oviposition period and then it mates and lays eggs. The newly developed weevils have a sex ratio of 1:1. The female maize weevils have a longer life than the males (Tefera *et al.*, 2010).

The fecundity of female weevils is high, and if the weevils are not controlled, the population increases very fast (Tefera *et al.*, 2010). Adults can live from 4 to 12 months depending on the diet and environmental conditions (Adams, 1976; Dobie and Kilminster, 1978; Gomez *et al.*, 1983b; Canadian Grain Comission, 1999). During feeding, the adult maize weevil creates a roughly circular hole of approximately 1.5 mm in diameter in the grain by eating

through the outer layer of it (Kranz *et al.*, 1997). They can fly and the female lays most of the eggs in the kernels within the first four weeks after its emergence. It chews a small hole into the kernel to lay eggs, where it lays up to four eggs in a single hole and then covers it with a waxy secretion, which rapidly stabilizes to make a defensive plug. It lays approximately 300 - 400 eggs in its lifetime. The eggs hatch in a short period of time and upon hatching, the larvae burrows and feeds in the grain forming winding tunnels that increase in size as they grow (Roberts and Douce, 1999; Tefera *et al.*, 2010).

2.7. Maize weevil damage

The detection of early maize weevil infestation is difficult because very little external evidence can be seen when the larvae starts chewing and developing inside the kernel until about 30 days when the adult gnaws throughout the kernel coat and surfaces (Roberts and Douce, 1999). Total destruction of grain and reduced market value of the maize is observed frequently because of infestation by larvae and adult maize weevils (Tefera *et al.*, 2010).The larvae complete the growth inside the kernel and consume the endosperm, often leaving only hollow kernel shells (Roberts and Douce, 1999; Tefera *et al.*, 2010). The adults leave large, ragged exit holes in the kernel and feed on damaged kernels and flour (Roberts and Douce, 1999). Maize weevils produce heat and moisture through their metabolic activities, which lead to mold development and invasion by other insect species (Canadian Grain Commission, 1999). Sometimes, the seed germ (embryo) is not affected; therefore, germination process can occur, but the smaller endosperm leads to the production of weak seedlings, prone to attack by insects, fungus, bacteria and molds. According to DegeschInc (2009) grain damaged by weevils is easily identified by the presence of large holes, used as exit holes by emerging adults.

2.8. Biology and ecology of larger grain borer

The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Plate 2-2), belongs to the order *Coleoptera* and family *Bostrichidae*, affiliates of the group that are recognized as powder post beetles (CTA, 1998, Booth *et al.*, 1990). Larger grain borer originated from Central America as an endemic pest (Farrell and Schulten, 2002). It has long been known as the most damaging pest of maize stored on cob form. *Bostrichidae* insects are widely known as "pests of timber". Most species attack and live on dried wood, but there are some, which also attack green wood/timber (Tooke and Scott, 1994).

Plate 2-1. Larva and adult of LGB (*Prostephanus truncatus* Horn)
Source: CTA, 1998

The adults are dark brown, small with 1.0 to 1.5 mm width and 2.0 to 3.5 mm length, with a cylindrical body, deflexed head, and strong mandibles and squared terminal part, typical features of xylophagous insects, which feed in the heartwood of woody plants (Tefera *et al.*, 2010). The pronotum defends the head throughout tunnelling, providing robust care for the mandibular muscles (Li, 1988). LGB has an extraordinary capability to tunnel throughout tough things, such as 35 mm heavy plastic (Li, 1988).

Larger grain borer breeds on dry cassava, maize ears, grain and any additional stored commodity. The life cycle is composed of egg, larvae, pupa and adult stages. LGB has three larval instar stages, where the last instar stage makes a pupal case within the grain (Hodges, 1986). The eggs, larva, and pupal stages are hardly seen, because they occur inside the kernels. The pre-oviposition period is 5 - 10 days. The females deposit eggs in lateral tunnels at a right angle to the main tunnel and the egg clutch is typically secured by tightly packed frass when laid on loose maize grain (Bell and Watters, 1982). The larva emerges on average after 5 days and the average larval and pupal periods are 25 and 5 days, respectively (Subramanyam *et al.*, 1985). The larva is immobile, pale in colour and easily distinguished by their C-shaped body (scarabeiform) and head retracted into the prothorax (Subramanyam *et al.*, 1985; CTA, 1998; Tefera *et al.*, 2010).

Optimum conditions for growth and development are 27 - 32ºC and 70 - 80% RH. Temperatures less than 25ºC or more than 35ºC reduce the rate of growth of the population significantly. Under optimum conditions and in rich substrata as corn-cobs, the development

of the egg phase to adults can take 24 days with the females ovipositing an average of 430 eggs during their life period (Bell and Watters, 1982). The adults LGB can fly. The male: female reproductive ratio is 1:1. The male LGB have a shorter life-span than the female ones (Birkinshaw and Hodges, 2000). LGB can grow in grain with low moisture content, such as 9%, lower than other storage insects (Haines, 1991).

2.8.1. Larger grain borer in Africa

Larger grain borer has been reported from late 1970s. Its introduction is alleged to have been unintentional from Central America through maize donated to the refugee camps in Tanzania at Urambo, Tabora in western part of the country (Dunstan and Magazini, 1981). Years later in 1984, it was reported in Togo (Krall, 1984; Richter and Biliwa, 1991). From these two initial locations, the pest has spread rapidly to cover various countries in Africa, causing tremendous maize and dried cassava losses (Shires, 1977; Hodges, 1994). Since then, larger grain borer has been observed in 19 African countries namely: Tanzania (1981), Kenya (1983), Burundi, Togo and Benin (1984), Guinea and Guinea Conakry (1987), Ghana (1989), Burkina Faso (1991), Nigeria and Malawi (1992), Rwanda (1993), Zambia and Niger (1994), Uganda (1997), Namibia and South Africa (1998), Mozambique (1999) and Zimbabwe (2004) (CTA, 1998; Farrell and Schulten, 2002; Rwegasiram *et al.*, 2003; Cugala *et al.*, 2007b; Ridley and Bartlett, 2010; Tefera *et al.*, 2010).

After reports of LGB in Africa, the grain weight loss caused by storage pests increased dramatically, for instance, the losses in Togo increased from 11% to more than 35% in six months (Pantenius, 1988). In Benin losses increased to more than 30% in five months (Fandohan *et al.*, 1992), in Tanzania, in three months, losses increased up to 34% (Hodges *et al.*, 1984), in Kenya losses increased to more than 15% (Ndiso *et al.*, 2007) and in South Africa, the losses increased up to 30% on average (ARC-LRN, 2010).

2.8.2. Larger grain borer damage

Larger grain borer is a critical storage pest of maize and it is amongst the primary pests of storage maize. Weight losses of 9% to 90% have been observed in many African countries due to its infestation (Pantenius, 1988; Markham *et al.*, 1991; Schneider *et al.*, 2004; Bett and Nguyo, 2007; Gueye *et al.*, 2008). It can attack all kernels on the cob before and after harvest. Larvae and adults attack stored grains, often causing total damage of grain, reducing many kernels to powder and drastically reducing the market value of the maize (Compton *et al.*, 1998). It has been reported that it can bore into the maize husks, cobs

and/or grain, creating many round holes and burrowing widely, making huge amounts of grain flour (CTA, 1998). The adults LGB have preference to grain on cobs over shelled grain, probably because the loose grain is more difficult to penetrate (CTA, 2003; Tefera *et al.*, 2010).

2.9. Management of storage pests in Mozambique

The main control method of larger grain borer and maize weevil attack is the use of cultural methods. However, these methods are not efficient (Mariquele, 2006). Chemical control using synthetic insecticides, mostly Actellic Super®, Actellic Gold® and Shumba® (all as a cocktail of Pirimiphos-methyl and Permethrin) have been tested and extensively promoted by extension services, but adoption by the small-scale farmers is very low (INE, 2003; Mariquele, 2006). This is mostly due to the high costs of pesticides (TIA, 2007). Only 4.5% of the small-scale farmers use pesticides in Mozambique.

The use of biological control agents against LGB has been tried on an experimental basis. In 2007, about 550 individual *Teretrius nigrescens* Lewis (order *Coleoptera* and family Histeridae), a historic predator of larger grain borer, were introduced in three districts in Mozambique (Manica, Barue and Manicathe). Two months later, pheromone traps were placed in the locations where the predator was released to evaluate its establishment and dispersion. It was observed that the predator had multiplied. However, the number was low and the predator needed to be given a period of 3 to 4 years to multiply to the levels that can control LGB (Cugala *et al.*, 2007a).

Borgemeister *et al.* (2003) reported the effectiveness of *T. nigrescens* in controlling LGB, when well established in Togo and Niger while Nang'ayo (1996) reported in Kenya. The adults and larvae of this insect kill the LGB eggs and larvae, even though the larvae are more effective than adults (Pöschko, 1993).

2.10. Breeding for storage pests

Development of storage pest resistant genotypes plays an essential part in an integrated pest management (IPM) strategy to reduce storage damage and effect on grain quality. Three-resistance mechanisms of plants to insect pests have been reported by Painter (1951) namely antibiosis, non-preference and tolerance. These mechanisms were reported to be significant factors of grain resistance to the maize weevil (Gomez *et al.*, 1982; Gomez *et al.*, 1983a; Horber, 1989; Arnason *et al.*, 1997).

Dent (1991) defined antibiosis resistance as the capability or capacity of a host to harm the pest, reducing its potential reproduction, retarding the development frequency or eradicating the pest. Non-preference is defined as the genetic transmissible feature of the grain, which discourages the insects from feeding, colonizing and ovipositing and tolerance refers to when the plant shows an ability to develop, reproduce and repair an injury caused by the pest (Derera *et al.*, 2001b). Antibiosis resistance of grain to storage pests was reported by Schoonhoven *et al.* (1975), Derera *et al.* (2001a) and Nhamucho *et al.* (2014) while non-preference has been reported by Schoonhoven *et al.*, 1976, Gomez *et al.*, 1982, Gomez *et al.*, 1983a, Kang *et al.*, 1995, Derera *et al.*, 2001b. There are huge changes in the desirability of maize grain to attack by storage pests but grain texture is the basis of non-preference resistance (Tipping *et al.* 1986, 1987). In this case, the smooth pericarp deterred weevils from feeding and ovipositing (Tipping *et al.*, 1988).

In case of grain in storage, tolerance is inapplicable, since the grain is in latent stage and cannot repair its injury; therefore, the damage incurred is terminal (Horber, 1989). Thus, evaluation and selection of maize resistance to storage pests must emphasise on evaluating antibiosis and non-preference (Derera *et al.*, 2001a). Good levels of resistance in inbred and hybrid maize have been reported by Derera *et al.*, 2001a, 2001b, Bergvinson *et al.*, 2002, Mwololo *et al.*, 2013 and Nhamucho *et al.*, 2014, 2017. Genetic diversity to storage pests is a critical aspect for the progress in appreciating the genetic basis, biophysical and biochemical components of host plant resistance, which is crucial in guaranteeing that the selected traits meet customer needs.

Biochemical and physical characteristics of the maize varieties were recognized as mechanisms of kernels to resist storage pests (Arnason *et al.*, 1997; Derera *et al.*, 2001a; Mwololo *et al.*, 2010), and they have been used as secondary traits for breeding selection. The most important physical attributes are grain hardness and pericarp surface texture while the most important nutritional components are quantities of amylose, lipid, protein and sugar content in the grain (Arnason *et al*., 1997; Dhliwayo and Pixley, 2001; Garcia-Lara *et al*., 2004). On the biochemical factors, phenolic compounds related to grain hardness are considered the most important (Serratos *et al*., 1987). Grain hardness, sugar and protein content are positively correlated with resistance to the maize weevil (Arnason *et al.*, 1994; Garcia-Lara *et al.*, 2004; Abebe *et al.*, 2009).

Good heritability of the insect resistance has been reported, suggesting the possibility to introduce resistance into elite germplasm (Derera *et al.*, 2001a; Dhliwayo and Pixley, 2001, 2003, Dhliwayo *et al.*, 2005; Abebe *et al.*, 2009; Derera *et al.*, 2010; Mugo *et al.*, 2010; Mwololo *et al.*, 2010; Matewele, 2014). Most recently, Mwololo *et al*. (2018) identified quantitative traits loci (QTL) associated with postharvest insect resistance traits, especially for larger grain borer. They, reported also that the chromosomal regions containing genes involved in the synthesis of cell wall components could be associated with resistance to different insect species in maize and marker assisted recurrent selection would be useful in transferring the QTL alleles into susceptible and promising inbred lines. The identification of the QTLs associated with post-harvest insect resistance in tropical maize will enable breeders to exploit the genetic variation and increase the efficiency in delivering maize varieties resistant to storage pests increasing food security for small-scale farmers.

2.11. Inheritance of important traits in maize breeding

Quantitative traits such as grain yield are highly affected by the environment, which includes temperature, water availability, soil fertility and sunlight (Bänziger *et al.*, 2000). Many researchers have reported that both general and specific combining abilities for grain yield in maize are highly influenced by the environment (Egesel *et al.*, 2003; Ojo *et al.*, 2007), suggesting that performance of the inbred lines and their behaviour in a combination may differ according to the environmental conditions where they are grown. Bhatnagar *et al.* (2004) researching combining ability in quality protein maize (QPM), did not find significant effects of GCA for grain yield but observed significant effects on the interaction with the environment.

Traits that are not yield or yield components have mostly been reported to be controlled primarily by additive gene action in various crop species, although in many cases non-additive gene effects have also been reported to play a role. Wegary *et al.* (2014) reported prevalence of GCA over SCA effects for most maize agronomic traits evaluated under drought, low-nitrogen and optimum environmental conditions. The importance of additive gene effects was also reported for maize lethal necrosis and storage pests' resistance parameters (Derera *et al.*, 1999; Dhliwayo and Pixley, 2003; Dari *et al.*, 2010; Beyene *et al.*, 2017).

Non-additive gene effects, except under drought, mainly control grain yield and its components. Machida *et al.* (2010) studying nine quality protein maize inbred lines crossed

in a diallel design and tested under optimum conditions, observed that SCA effects were dominant over GCA effects for grain yield. Wegary *et al.* (2014) reported that SCA effects were more important under optimal conditions and low-nitrogen for grain yield while Betrán *et al.* (2003b), Derera *et al.* (2008) and Wegary *et al.* (2014) reported the predominance of additive gene effects for grain yield under drought conditions.

It was reported by Gamble (1962) that when the material used to obtain the genetic variance estimates become more restricted (reduced genetic variation), the additive variance for grain yield in maize may be reduced, giving more predominance of non-additive gene effects and epistasis, especially the additive × additive and additive × dominance interactions, but only a few crosses can exhibit dominance × dominance or the three types of epistasis simultaneously. In the current study, two methods of estimating gene action were used; generation mean analysis and diallel mating design.

2.12. Generation means analysis

The generation means analysis (GMA) gives detailed information on the genetic inheritance for quantitative traits as mean [m], main gene effect (additive [a], and dominance [d]) and digenic or non-allelic interactions (epistasis) such as additive × additive [aa], additive × dominance [ad] and dominance × dominance [dd] effects in a cross between two divergent inbred lines for a particular trait (Kearsey and Pooni, 1998; Dabholkar, 1999; Bernardo, 2002; Mather and Jinks, 2013). The digenic non-allelic interactions (epistasis) are broadly classified as complementary when the [d] and [dd] show the same sign and duplicate when they show a different sign, while the positive [a] indicates gene association and negative [a] gene dispersion (Hayman and Mather, 1955). This analysis measures the mean of different generations derived from the cross of two inbred parents and interprets the means in terms of different genetic effects (Hayman, 1958; Gamble, 1962; Bernardo, 2002). Usually, only main effects are assumed to exist and only parent 1 (P1), parent 2 (P2) and F1 generation are used in this case (Chahal and Gosal, 2002; Mather and Jinks, 2013). However, any deviation in the observed and expected or significance of the linear regression ANOVA within families, would indicate that there are other parameters apart from the main effects, which will require six generations; P1, P2, F1, F2 and backcross generations, BCP1, BCP2 to be used for estimation (Kearsey and Pooni, 1998).

Variation among individual plants in each generation has been used to assess additive and dominance variances, which in turn have been used to obtain heritability estimates (Mather

and Jinks, 1982). Generation means analysis has an advantage that the used population for genetic analyses provides generations that can be included in a breeding programme (Coates and White, 1998) and all the genetic effects can be estimated at the same time with smaller sampling error due to being estimated from the generation means rather than the variances (Bernardo, 2002). However, the main disadvantage of GMA is that it requires divergent inbreds, thus it focuses on one trait at a time and one set of inbred lines and the inference of the results is restricted to the inbred lines used in the cross (Sibiya, 2009).

In maize, GMA has been used mostly to study the disease inheritance. It has also been applied for grain yield and its components, maturity and some other quantitative traits, such as plant and ear height, ear length and diameter (Mushongi *et al.*, 2013; Sher *et al.,* 2012; Haq *et al.,* 2013; Derera and Musimwa, 2015; Fahad *et al.,* 2018). Several studies have been reported using GMA for diseases, including brown spot caused by *Physoderma maydis* (Moll *et al.*, 1963), Northern corn leaf blight (Hughes and Hooker, 1971), common rust (Kim and Brewbaker, 1977), anthracnose leaf blight (Carson and Hooker, 1981), corn leaf aphid (Homoptera: Aphididae) (Bing and Guthrie, 1991), aspergillus ear rot and aflatoxin (Campbell and White, 1995), Grey Leaf Spot (Coates and White, 1998; Crowley *et al.*, 2002), Phaeosphaeria leaf spot (Carson, 2001; Sibiya, 2009), diplodia ear rot (Ndaruhutse, 2016). Limited studies on resistance to storage pests using GMA are available. Serratos *et al.* (1993) reported high correspondence of phenolic compounds with maize weevil susceptibility.

2.13. Diallel mating design

Diallel mating design is the most used among all other mating designs for assessing genetic information (Hallauer *et al.*, 2010). The design allows making all possible crosses among a group of parents, mostly inbred lines (Sprague and Tatum, 1942). Four methods of the diallel are available; method I (full diallel), method II (half diallel, which includes parents and one set of the F1 crosses), method III (exclude the parents) and method IV (includes only F1s crosses) (Griffing, 1956a; Griffing, 1956b; Hallauer *et al.*, 2010; Schlegel, 2010). In addition, two models for analysis; fixed (Model 1) and random (Model 2) models (Griffing, 1956a, Griffing, 1956b) are used. A random model includes parents, which are randomly selected from a random large mating population while in a fixed model the parents are considered fixed, specifically selected in a small population. The random model allows estimation of general and specific combining ability variances, and inferences can be

extrapolated for the whole population while in the fixed model; allows measuring the combining abilities effects, for each parent (GCA) and for each pair of parents (SCA). The variance estimates obtained from the analysis of diallel are translated into genetic variance components, additive or dominance and from these variance components, random and fixed effects are analysed using either analysis of variance or combining ability estimates (Hallauer, 2007; Hallauer *et al.*, 2010).

In this study, since the parents were selected carefully for drought tolerance, maize lethal necrosis and insect resistance traits, a fixed model was used for estimating both GCA and SCA effects (Shattuck *et al.*, 1993). Various studies on maize genetics have been conducted using diallel analysis for different traits, including disease resistance, drought tolerance, aflatoxin accumulation in maize grain and insect resistance. Borges and Orangel (1987) researching on downy mildew resistance concluded that both additive and non-additive were important although additive was predominant, while Ulrich *et al.* (1990) researching grey leaf spot concluded that only additive gene effects were important. On drought, several studies on tropical maize have reported the prevalence of additive effects in controlling yield under stress. This includes studies by Derera *et al.* (2008) and Betrán *et al.* (2003a,b), on the gene action controlling grain yield and secondary traits, and genetic analysis of maize under stress and non-stress conditions.

Williams and Windham (2015) used diallel to investigate aflatoxin accumulation in maize, where they reported positive and significant GCA effects on susceptible maize inbred lines, while Betrán *et al.* (2002) reported that yellow maize was more prone to aflatoxin infection as compared to white, with a strong environmental influence. Additive and non-additive gene effects were essential depending on the method used for screening aflatoxin accumulation.

For insect resistance studies, the diallel mating design was used by Alvarez and Miranda (2002). They reported the importance of both additive and non-additive effects for resistance to *S. frugiperda*. Butrón *et al.* (1999) while investigating maize ear resistance to pink stem borer observed that additive, non-additive and cytoplasmic effects were important for the resistance, although additive effects were predominant. Other studies, involving storage pests' resistance have been carried out using diallel-mating design. Dhliwayo *et al.* (2005) investigated combining ability effects for resistance to maize weevil and showed that both general and specific combining ability effects were important for maize weevil

resistance but general combining ability was more significant. No studies have reported GCA or SCA effects of two combined traits using diallel-mating design.

2.14. Summary of literature review

The reviewed literature showed that field pests and foliar diseases are the main biotic constraints while drought and soil fertility are the major abiotic stress; and some of the problems have already been addressed by different breeding projects in the region. Although breeding for improved grain yield under low soil fertility has shown tremendous results, research is still going on since 1995. Currently, there are regionally different projects addressing it, in different perspectives, breeding and agronomy.

In contrast, drought, maize lethal necrosis and storage pests, have been well addressed with good results but as single traits. A combination of tolerance to the aforementioned stresses in maize and more focus on the mechanisms of resistance to storage pests is a relatively new research area and little has been done in terms of genetic studies and practical breeding. No detailed genetics of maize resistance to storage pests and combining these traits has been reported. Control of MLN and storage pests has been well described in literature, where some simple and applicable methods can be used to minimize the negative effects of these stress, including their propagation or spread. Additive gene effects were reported by many researchers, as the most important gene action to be considered when developing varieties resistant to storage pests, which suggests that both parents should be tolerant while for maize lethal necrosis, both additive and non-additive gene action were important, although additive was predominant. MLN is a new disease in Africa and limited genetic studies have been reported. Therefore, specific research is required in order to fill these gaps in information for scientists addressing the challenge caused by climate change worldwide, especially in the tropical lowland environments. This study will thus contribute to supporting available or reporting new findings.

References

Abebe, F., Tefera, T., Mugo, S., Beyene, Y. and Vidal, S. 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (*Coleoptera*: Curculionidae). African Journal of Biotechnology 8: 5937-5943.

Adams, I., Harju, V., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M., Smith, J., Fox, A. and Uzayisenga, B. 2014. First report of maize lethal necrosis disease in Rwanda. New Disease Reports 29: 22- 22.

Adams, J. 1976. Weight loss caused by development of Sitophilus zeamias Motsch. in maize. Journal of Stored Products Research 12: 269-72.

Adebayo, M., Menkir, A., Blay, E., Gracen, V., Danquah, E. and Hearne, S. 2014. Genetic analysis of drought tolerance in adapted× exotic crosses of maize inbred lines under managed stress conditions. Euphytica 196: 261-270.

Alvarez, M. and Miranda, J. 2002. Diallel crossing among maize populations for resistance to fall armyworm. Scientia Agricola 59: 731-741.

Almeida, G. D., Nair, S., Borém, A., Cairns, J., Trachsel, S., Ribaut, J.-M., Bänziger, M., Prasanna, B. M., Crossa, J. and Babu, R. 2014. Molecular mapping across three populations reveals a QTL hotspot region on chromosome 3 for secondary traits associated with drought tolerance in tropical maize. Molecular breeding 34: 701-715.

Araus, J. L., Slafer, G. A., Royo, C. and Serret, M. D. 2008. Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Science 27: 377-412.

ARC-LRN. 2010. Post-harvest constraints facing small-scale farmers and to improve post-harvest management strategies for improving household food security [Online]. Available: http://www.arc.agric.za/home.asp?pid=945#further_info [Accessed 10 October 2010].

Arnason, J., Baum, B., Gale, J., Lambert, J., Bergvinson, D., Philogene, B., Serratos, J., Mihm, J. and Jewell, D. 1994. Variation in resistance of Mexican landraces of maize

to maize weevil *Sitophilus zeamais*, in relation to taxanomic and biochemical parameters. Euphytica 74: 227-236.

Arnason, J., Conilh De Beyssac, B., Philogene, B., Bergvinson, D., Serratos, J. and Mihm, J. 1997. Mechanisms of resistance in maize grain to the maize weevil and the larger grain borer. In: MIHM, J. (ed.) pp 91-95. Insect Resistant Maize.- Recent Advances and Utilization. A Proceedign of an International Symposium. Mexico City, 27 Nov.–3 Dec. 1997: CIMMYT.

Banziger, M. and Diallo, A. 2001. Stress tolerant maize for farmers in sub-Saharan Africa. CIMMYT Maize Research Highlights 1999–2000. Mexico D.F: CIMMYT.

Bänziger, M., Edmeades, G., Beck, D. and Bellon, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice, Mexico D.F.: CIMMYT.

Bänziger, M., Edmeades, G. and Lafitte, H. 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Research 75: 223-233.

Bänziger, M., Setimela, P. S., Hodson, D. and Vivek, B. 2006. Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. Agricultural Water Management 80: 212-224.

Baum, M., Von Korff, M., Guo, P., Lakew, B., Hamwieh, A., Lababidi, S., Udupa, S. M., Sayed, H., Choumane, W. and Grando, S. 2007. Molecular approaches and breeding strategies for drought tolerance in barley. In Genomics-assisted crop improvement. Springer 51-79.

Bell, R. and Watters, F. 1982. Environmental factors influencing the development and rate of increase of *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*) on stored maize. Journal of Stored Products Research 18: 131-142.

Bergvinson, D., Vasal, S., Singh, N., Panwar, V. and Sekhar, J. 2002. Advances in Conventional Breeding for Insect Resistance in Tropical Maize. 8th Asian Regional Maize Workshop. Bangkok, Thailand.

Bernardo, R. 2002. Breeding for Quantitative traits in Plant. Stemma Press. Woodbury, Minnesota. 369pp.

Betrán, F., Beck, D., Bänziger, M. and Edmeades, G. 2003a. Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Science 43: 807-817.

Betrán, F., Beck, D., Benziger, M. and Edmeades, G. 2003b. Secondary traits in parental inbreds and hybrids under stress and non-stress environments in tropical maize. Field Crops Research 83: 51-65.

Betrán, F., Isakeit, T. and Odvody, G. 2002. Aflatoxin accumulation of white and yellow maize inbreds in diallel crosses. Crop Science 42: 1894-1901.

Betrán, F., Ribaut, J., Beck, D. and De Leon, D. 2003c. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Science 43: 797-806.

Bett, C. and Nguyo, R. 2007. Post-harvest storage practices and Techniques used by Farmers in Semi-arid Eastern and Central Kenya. African Crop Science Conference Proceedings 8: 1023 - 1027.

Beyene, Y., Gowda, M., Suresh, M., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Tarekegne, A. and Prasanna, B. 2017. Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. Euphytica 213: 224 - 236

Beyene, Y., Semagn, K., Crossa, J., Mugo, S., Atlin, G. N., Tarekegne, A., Meisel, B., Sehabiague, P., Vivek, B. S. and Oikeh, S. 2016. Improving maize grain yield under drought stress and non-stress environments in sub-Saharan Africa using marker-assisted recurrent selection. Crop Science 56: 344-353.

Beyene, Y., Semagn, K., Mugo, S., Tarekegne, A., Babu, R., Meisel, B., Sehabiague, P., Makumbi, D., Magorokosho, C. and Oikeh, S. 2015. Genetic gains in grain yield through genomic selection in eight bi-parental maize populations under drought stress. Crop Science 55: 154-163.

Bhatnagar, S., Betrán, F. and Rooney, L. 2004. Combining Abilities of Quality Protein Maize Inbreds. Crop Science 44: 1997-2005.

Bing, J. and Guthrie, W. 1991. Generation mean analysis for resistance in maize to the corn leaf aphid (Homoptera: Aphididae). Journal of economic entomology 84: 1080-1082.

Birkinshaw, L. and Hodges, R. 2000. Improving IPM approaches for LGB control in Africa: PhAction News, 3 [Online]. Available: http://www.iita.org [Accessed 10 October 2010].

Bohnert, H. J., Gong, Q., Li, P. and Ma, S. 2006. Unraveling abiotic stress tolerance mechanisms–getting genomics going. Current opinion in plant biology 9: 180-188.

Bolaños, J. 1995. Physiological bases for yield differences in selected maize cultivars from Central America. Field Crops Research 42: 69-80.

Bolaños, J. and Edmeades, G. 1996. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Research 48: 65-80.

Booth, R., Cox, M. and Madge, R. 1990. IIE Guide to insects of Importance to Man: 3. *Coleoptera*. Wallingford: CAB International.

Borgemeister, C., Schneider, H., Affognon, H., Schulthess, F., Bell, A., Zweigert, M., Poehling, H. and Sétamou, M. 2003. Impact Assessment of Teretrius nigrescens Lewis (COL.: HISTERIDAE) In West Africa, a predator of the larger grain borer, *Prostephanus truncatus* (Horn) (COL.: *BOSTRICHIDAE*). 1st International Symposium on Biological Control of Arthropods. USDA-Forest Service FHTET-03-05. June 2003,1: 343-350.

Borges, F. and Orangel, L. 1987. Diallel Analysis of Maize Resistance to Sorghum Downy Mildew. Crop science 27: 178-180.

Boxall, R. 2002. Damage and loss caused by the larger grain borer *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*). Integrated Pest Management Reviews 7: 105-121.

Brandes, E. 1920. Artificial and insect transmission of sugarcane mosaic. Journal of Agricultural Research 19: 131-138.

Butrón, A., Malvar, R., Velasco, P., Vales, M. and Ordás, A. 1999. Combining abilities for maize stem antibiosis, yield loss, and yield under infestation and non infestation with pink stem borer. Crop science 39: 691-696.

Cabanas, D., Watanabe, S., Higashi, C. and Bressan, A. 2013. Dissecting the mode of Maize chlorotic mottle virus transmission (Tombusviridae: Machlomovirus) by Frankliniella williamsi (Thysanoptera: Thripidae). Journal of Economic Entomology 106: 16-24.

CABI. 2018. Invasive Species Compendium: Detailed coverage of invasive species threatening livelihoods and the environment worldwide: Maize weevil [Online]. Available: https://www.cabi.org/isc/datasheet/10926 [Accessed 04 October 2019].

CABI. 2019. Invasive Species Compendium: Detailed coverage of invasive species threatening livelihoods and the environment worldwide: Maize lethal necrosis disease [Online]. Available: https://www.cabi.org/isc/datasheet/119663 [Accessed 04 October 2019].

Cakir, R. 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crops Research 89: 1-16.

Campbell, K. and White, D. 1995. Inheritance of resistance to Aspergillus ear rot and aflatoxin in corn genotypes. Phytopathology 85: 886-896.

Campos, H., Cooper, M., Edmeades, G., Loffler, C., Schussler, J. and Ibanez, M. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the US corn belt. Maydica 51: 369-381.

Campos, H., Cooper, M., Habben, J., Edmeades, G. and Schussler, J. 2004. Improving drought tolerance in maize: a view from industry. Field crops research 90: 19-34.

Canadian Grain Comission. 1999. Maize weevil *Sitophilus zeamais* (Motsch.) [Online]. Canada. Available: http://www.grainscanada.gc.ca/storage-entrepose/pip-irp/mw-cr-eng.htm [Accessed 12th, August 2011].

Carson, M. 2001. Inheritance of resistance to Phaeosphaeria leaf spot of maize. Plant disease 85: 798-800.

Carson, M. and Hooker, A. 1981. Inheritance of resistance to anthracnose leaf blight in five inbred lines of corn. Phytopathology 71: 488-491.

Castillo, J. and Hebert, T. 1974. A new virus disease of maize in Peru. Fitopatologia 9: 79-84.

Chahal, G. S. and Gosal, S. S. 2002. Principles and Procedures of Plant Breeding: Biotechnology and Conventional Approaches. Alpha Science International, Pangbourne, UK. 604.

Chaúque, P. 2009. Combining Ability for grain yield and related traits of early, flint and drought tolerant maize inbred lines in Mozambique. MPhil Thesis. MOI University, Kenya.

Chaúque, P. 2016. Genetic and path coefficient analyses and heterotic orientation of maize germplasm under combined heat and drought stress in sub-tropical lowland environments. PhD Thesis, University of KwaZulu-Natal, SA.

Chen, J., Xu, W., Burke, J. and Xin, Z. 2010. Role of phosphatidic acid in high temperature tolerance in maize. Crop science 50: 2506-2515.

Chimenti, C., Marcantonio, M. and Hall, A. 2006. Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. Field Crops Research 95: 305-315.

CIMMYT. 2001. Maize research highlights 1999-2000. CIMMYT. Mexico City.

CIMMYT. 2008. Annual Report 2007-2008. International Maize and Wheat Improvement Center, CIMMYT., Mexico, D.F.

Coates, S. and White, D. 1998. Inheritance of resistance to gray leaf spot in crosses involving selected resistant inbred lines of corn. Phytopathology 88: 972-982.

Compton, J. a. F., Floydb, S., Ofosua, A. and Agboa, B. 1998. The modified count and weigh method: an improved procedure for assessing weight loss in stored maize cobs Journal of Stored Products Research 34: 277-285

Cotton, R. 1956. Pests of Stored Products, Hutchinson, London, UK, 234pp.

Crowley, J., Hallauer, A. and Martinson, C. 2002. Inheritance of gray leaf spot resistance in corn. Journal of the Iowa Academy of Science 109: 25-29.

CTA. 1998. Larger grain borer- Techinical leaflet n.1, Netherlands: CTA.

CTA. 2003. Insect pests in field and Storage: Larger grain borer, *Prostephanus truncatus* (*Coleoptera*: *Bostrichidae*) [Online]. Available: http://www.naturalcropprotection.margraf-verlag.de/borer.htm [Accessed 10 August 2011].

Cugala, D., Omwega, C., Ogol, C. and Santos, L. 2003. Establishment of Cotesia flavipes population in Mozambique. African Crop Science Journal 6: 241-245.

Cugala, D., Omwega, C., Ogol, C. and Schulthess, F. 2009. Incidence of cereal stem borer egg parasitoids and their relative importance in small scale farmers' maize fields in Mozambique. African Crop Science Journal 9: 641-645.

Cugala, D., Sidumo, A., Santos, L. and Givá, N. 2007a. Uso do método de controlo biológico contra a broca maior do grão do milho armazenado, *Prostephanus truncatus* (horn) (*Coleoptera*: *Bostrichidae*) nos celeiros das famílias rurais em Moçambique. Moçambique- Maputo: Universidade Eduardo Mondlane.

Cugala, D., Sidumo, A., Santos, L., Mariquele, B., Cumba, V. and Bulha, M. 2007b. Assessment of status, distribuition and weight lost due to *Prostephanus trancutus* (Horn) (*Coleoptera*: *Bostrichidae*) in Mozambique. African Crop Science Journal 8: 975-979.

Dabholkar, A. R. 1999. Elements of Biometrical Genetics. In: Mittal, A. K. (ed.). New Delhi: Concept Publishing Company.

Dari, S., Pixley, K. and Setimela, P. 2010. Resistance of early generation maize inbred lines and their hybrids to maize weevil [*Sitophilus zeamais* (Motschulsky)]. Crop Science Society of America 50: 1310-1317.

Degeschinc. 2009. Stored Grain Insects: Greator Rice Weevil (*Sitophilus zeamais*) [Online]. Available: http://agspsrv34.agric.wa.gov.au/ento/pestweb/Query1_1.idc?ID=-1055010548 [Accessed 12th. August 2011].

Degroote, H., Oloo, F., Tongruksawattana, S. and Das, B. 2016. Community-survey based assessment of the geographic distribution and impact of maize lethal necrosis (MLN) disease in Kenya. Crop protection 82: 30-35.

Denic, M., Chaúque, P., Fato, P., Haag, W., Mariote, D., Langa, M. and Carlos, J. 2001. Maize Screening for multiple stress tolerance and agronimic traits. In: Frisen, D. & Palmer, A. (eds.) Seventh Eastern and Sourthern Africa Regional Maize Conference. 5-11 February 2001, Nairobi- Kenya, .

Denic, M., Chaúque, P., Fato, P., Senete, C., Mariote, D. and Haag, W. 2007. Breeding approaches in simultaneous selection for multiple stress tolerance of maize in tropical environments. Genetika 39: 113-124.

Denic, M., Chaúque, P., Fato, P., Senete, C., Mariote, D. and Haag, W. 2008. Approaches in breeding for high quality protein maize. Genetika 40: 237-247.

Dent, D. 1991. Insect pest management, CAB International, United Kingdom. 604 pp.

Derera, J. and Musimwa, T. 2015. Why SR52 is such a great maize hybrid? I. Heterosis and generation mean analysis. Euphytica 205: 121-135.

Derera, J., Pixley, K. and Giga, D. 1999. Inheritance of maize weevil resistance in maize hybrids among maize lines from Southern Africa, Mexico and CIMMYT Zimbabwe. Maize Production Technology for the Future: Challenges and Opportunities. Proceedings of the Eastern and Southern Africa Regional Maize Conference 6; 21-25 Sep 1998, CIMMYT EARO, Addis Ababa (Ethiopia).

Derera, J., Pixley, K. and Giga, D. 2001a. Resistance of maize to the maize weevil. I. Antibiosis. African Crop Science Journal 9: 431-440

Derera, J., Pixley, K. and Giga, D. 2001b. Resistance of maize to the maize weevil: II. non-preference. African Crop Science Journal 9: 441-450.

Derera, J., Pixley, K. and Giga, D. 2010. Appraisal of protocol for the rapid screening of maize genotypes for maize weevil resistance African Entomology 18: 8-16.

Derera, J., Tongoona, P., Vivek, B. S. and Laing, M. D. 2008. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. Euphytica 162: 411-422.

Desai, S. and Singh, R. 2001. Combining ability studies for some morphophysiological and biochemical traits related to drought tolerance in maize (*Zea mays* L.). The Indian Journal of Genetics and Plant Breeding 61: 34-36.

Dhliwayo, T. and Pixley, K. 2001. Breeding for resistance to maize weevil (*Sitophilus zeamais* Motsch.): Is it feasible? Seventh Eastern and Southern Africa Regional Maize Conference, 11- 15th February, pp. 134-138.

Dhliwayo, T. and Pixley, K. 2003. Divergent Selection for Resistance to Maize Weevil in Six Maize Populations. Crop Science Society of America 43: 2043-2049.

Dhliwayo, T., Pixley, K. and Kazembe, V. 2005. Combining Ability for Resistance to Maize Weevil among 14 Southern African Maize Inbred Lines. Crop Science Society of America 45: 662-667.

Dhliwayo, T., Pixley, K., Menkir, A. and Warburton, M. 2009. Combining ability, genetic distances, and heterosis among elite CIMMYT and IITA tropical maize inbred lines. Crop science 49: 1201-1210.

Dobie, P. and Kilminster, A. 1978. The susceptibility of triticale to post-harvest infestation by *Sitophilus zeamais* Motsch., Sitophilus oryzea (L) and Sitophilus granarius (L). Journal of Stored Products Research 14: 87-93.

Doupnik Jr, B. 1979. Status of corn lethal necrosis [virus diseases in the United States]: update. Proceedings of the 34th Annual Corn and Sorghum Research Conference, 1979 Chicago (USA). American Seed Trade Association, 16-34.

Dunstan, W. and Magazini, I. 1981. Out breaks and new records, Tanzania. The Larger Grain Borer in Stored Products. FAO Plant Protection Bulletin 29: 80-81.

Dwyer, S., Ghannoum, O., Nicotra, A. and Von Caemmerer, S. 2007. High temperature acclimation of C4 photosynthesis is linked to changes in photosynthetic biochemistry. Plant, Cell & Environment 30: 53-66.

Eberhart, S., Salhuana, W., Sevilla, R. and Taba, S. 1995. Principles for tropical maize breeding. Maydica 40: 339-355

Egesel, C., Wong, J., Lambert, R. and Rocheford, T. 2003. Combining ability of maize inbreds for carotenoids and tocopherols. Crop Science 43: 818-823.

Erdal, S., Pamukcu, M., Ozturk, A., Aydinsakir, K. and Soylu, S. 2015. Combining abilities of grain yield and yield related traits in relation to drought tolerance in temperate maize breeding. Turkish Journal of Field Crops 20: 203-212.

Ertiro, B. T., Beyene, Y., Das, B., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Labuschagne, M. and Prasanna, B. M. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. Plant breeding 136: 197-205.

Fahad, S., Noor, M., Shahwar, D., Alam, M., Ullah, H., Adnan, M., Jamal, Y., Wahid, F., Ur Rahman, H. and Yasir, M. 2018. Generation mean analysis for grain yield and its components in popcorn. Open Agriculture 3: 451-458.

Fandohan, P., Langner, B. and Mutlu, P. 1992. Distribuition, recherché et controle du grand Capucin du Mais *Prostephanus truncatus* (Horn) au Benin. FAO/GTZ Coord. Meet. Lomé-Togo.

FAO. 2019. FAO in Mozambique: Mozambique at a glance [Online]. Available: http://www.fao.org/mozambique/fao-in-mozambique/mozambique-at-a-glance/en/ [Accessed 01 October 2019].

Farrell, G. and Schulten, G. 2002. Larger Grain Borer in Africa; a history of efforts to limits its impact. Integrated Pest Management Reviews 7: 67-84.

Fato, P. 2010. Investigation of heterotic patterns and genetic analysis of downy mildew resistance in Mozambican lowland maize (*Zea mays* L.) germplasm. PhD Thesis, University of KwaZulu-Natal, SA.

Fato, P., Chaúque, P., Ecole, C. and Cugala, D. 2008. The Status of Development of Maize Resistant to Field and Storage Pests in Mozambique. In: Mugo, S., Gethi, J., Ouma, J., Murenga, G., Mulaa, M., Likhayo, P., Gichuki, V., Kega, V., DeGroote, H. & Chavangi, A. (eds.) Book of abstract of the Insect Resistant Maize for Africa (IRMA) .(2008) " Consolidating Experiences from IRMA I and II: Achievements, Prospects and Lessons", IRMA project ect End-of-Phase II Conference, 28-30 October. Nairobi- Kenya KARI and CIMMYT.

Feder, M. and Hofmann, G. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual review of physiology 61: 243-282.

Flett, B. and Mashingaidze, K. 2016. Maize Lethal Necrosis: Possible threat to local maize production [Online]. Grain SA, ARC-Grain Crops Institute, Potchefstroom. Available: https://www.grainsa.co.za/maize-lethal-necrosis:-possible-threat-to-local-maize-production [Accessed 20 June 2019].

Fu, F., Feng, Z., Gao, S., Zhou, S. and Li, W. 2008. Evaluation and quantitative inheritance of several drought-relative traits in maize. Agricultural Sciences in China 7: 280-290.

Gamble, E. E. 1962. Gene effects in corn (*Zea mays* L.) I. Separation and relative importance of gene effects for yield. Canadian Journal of Plant Science 42: 339-348.

Garcia-Lara, S., Bergvinson, D., Burt, A., Ramputh, A., Diaz-Pontones, D. and Arnason, J. 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science Society of America 44: 1546-1552.

Gill, D. and Raj, T. 2009. Engineering temperature tolerance in agricultural crops. Agricultural Reviews, 30.

Gomez, L., Rodriguez, J., Poneleit, C. and Blake, D. 1982. Preference and utilisation of maize endosperm variants by the rice weevil. Journal of Economic Entomology 75: 363-367.

Gomez, L., Rodriguez, J., Poneleit, C., Blake, D. and Smith, C. 1983a. Chemosensory responses of the rice weevil (Coleptera: Curculionidae) to a susceptible and a resistant corn genotype. Journal of Economic Entomology 76: 1044-1048.

Gomez, P., Rodriguez, J. and Poneleit, C. 1983b. Influence of nutritional characteristics of selected corn genotypes on food utilization of the rice weevil. Journal of Economic Entomology 76: 728-731.

Griffing, B. 1956a. Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science 9: 463-493.

Griffing, B. 1956b. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10: 31-50.

Grime, J. and Campbell, B. 1991. Growth rate, habitat productivity, and plant strategy as predictors of stress response. In Response of plants to multiple stresses. Elsevier, 143-159

Guei, R. and Wassom, C. 1992. Inheritance of some drought adaptive traits in maize [in Mexico]. 1: Interrelationships between yield, flowering and ears per plant. Maydica 37: 157-164.

Gueye, M., Goergen, G., Badiane, D., Hell, K. and Lamboni, L. 2008. First report on occurrence of the larger grain borer *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*) in Senegal. African Entomology 16,: 309-311.

Habben, J. E., Bao, X., Bate, N. J., Debruin, J. L., Dolan, D., Hasegawa, D., Helentjaris, T. G., Lafitte, R. H., Lovan, N. and Mo, H. 2014. Transgenic alteration of ethylene biosynthesis increases grain yield in maize under field drought-stress conditions. Plant biotechnology journal 12: 685-693.

Haines, C. 1991. Insects and arachnids of tropical stored products: their biology and identification - A training manual. Natural Resources Institute (NRI).

Hallauer, A., Russell, W. and Lamkey, K. 1988. Corn breeding. In: Sprague, G. & Dudley, J. (eds.) Corn and corn improvement. Crop Science Society of America, Madison, pp 463-564.

Hallauer, A. R. 2007. History, contribution, and future of quantitative genetics in plant breeding: lessons from maize. Crop Science 47: 4-19.

Hallauer, A. R., Carena, M. J. and Filho, J. B. 2010. Quantitative genetics in maize breeding New York ; London, Springer.

Hao, Z., Li, X., Xie, C., Weng, J., Li, M., Zhang, D., Liang, X., Liu, L., Liu, S. and Zhang, S. 2011. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. Journal of integrative plant biology 53: 641-652.

Haq, M., Ajmal, S., Kamal, N., Khanum, S., Siddique, M. and Kiani, M. 2013. Generation mean analysis for grain yield in maize. Journal of Animal & Plant Sciences 23: 1146-1151

Hayman, B. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12: 371-390.

Hayman, B. I. and Mather, K. 1955. The description of genetic interaction in continuous variation. Biometrics 11: 69-82.

Hodges, R. 1986. The Biology and Control of Prostephanus truncutus (Horn) (*Coleoptera*: *Bostrichidae*)-A destrutive storage pest with an increasing range. Journal of Stored Products Research 22: 1-14.

Hodges, R. 1994. The potential threat and means of control of *Prostephanus truncatus* (*Coleoptera*: *Bostrichidae*) in Africa. Proceedings of the African Crop Science Conference. Kampala, Uganda, 14–18 June 1993.

Hodges, R., Cork, A. and Hall, D. 1984. Aggregation pheromones for monitoring the greater grain borer, *Prostephanus truncatus*. British Crop Protection Conference- Pest and Diseases.

Horber, E. 1989. Methods to detect and evaluate resistance in maize to grain insects in the field and in storage. In Toward insect resistance maize for the Third World.

Proceedings of the International Symposium on methodologies for developing host plant resistance to maize insects. D.F. CIMMYT, Mexico, 327 pp.

Hossain, M. A., Wani, S. H., Bhattacharjee, S., Burritt, D. J. and Tran, L. S. P. 2016. Drought stress tolerance in plants, Vol 1: Physiology and biochemistry, © Springer International Publishing Switzerland 2016, Springer 543pp.

Hughes, G. and Hooker, A. 1971. Gene Action Conditioning Resistance to Northern Leaf Blight in Maize. Crop science, 11, 180-184.

Hussain, H. A., Hussain, S., Khaliq, A., Ashraf, U., Anjum, S. A., Men, S. and Wang, L. 2018. Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. Frontiers in plant science 9: 393- 413.

INE, 2003. Censo Agro-pecuário CAP 1999-2000. Resultados temáticos: Direcção Nacional de Estatísticas Sectoriais e de Empresas, Maputo, Moçambique.

IPPC 2014. New pest of maize: maize lethal necrosis in Uganda. IPPC Official Pest Report, No. UGA-01/2, No. UGA-01/2. Rome, Italy: FAO. https://www.ippc.int/

Isabirye, B. E. and Rwomushana, I. 2016. Current and future potential distribution of maize chlorotic mottle virus and risk of maize lethal necrosis disease in Africa. Journal of crop protection 5: 215-228.

Jensen, S., Wysong, D., Ball, E. and Higley, P. 1991. Seed transmission of maize chlorotic mottle virus. Plant Disease 75: 497-498.

Jiang, X., Meinke, L., Wright, R., Wilkinson, D. and Campbell, J. 1992. Maize chlorotic mottle virus in Hawaiian-grown maize: vector relations, host range and associated viruses. Crop Protection 11: 248-254.

Kang, M., Zhang, Y. and Magari, R. 1995. Combining ability for maize weevil preference of maize grain. Crop Science Society of America 35: 1556-1559.

Kearsey, M. J. and Pooni, H. S. 1998. The Genetical Analysis of Quantitative Traits Abingdon, Oxon, OX14 4RN, New York NY10016, Springer: Taylor & Francis Group.

Kikuchi, A., Huynh, H. D., Endo, T. and Watanabe, K. 2015. Review of recent transgenic studies on abiotic stress tolerance and future molecular breeding in potato. Breeding science 65: 85-102.

Kim, S. and Brewbaker, J. 1977. Inheritance of General Resistance in Maize to Puccinia sorghi Schw. Crop science 17: 456-461.

Kim, S., Efron, Y., Khadr, F., Fajemisin, J., Lee, M., Wann, E., Schulz-Schaeffer, J., Haller, S., Stuthman, D. and Rothman, P. 1987. Registration of 16 maize-streak virus resistant tropical maize parental inbred lines. Crop science 27: 824-825.

Krall, S. 1984. A new threat to farm-level maize storage in West Africa: *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*). Tropical Stored Products Information 50: 26–31.

Kranz, J., Shchmutterer, H. and Koch, W. 1997. Diseases, pest and weeds in tropical crops, Wesley, New York, USA, 666pp.

Kondwakwenda, A., Sibiya, J., Zengeni, R., Musvosvi, C. and Tesfay, S. 2019. Screening of Provitamin-A Maize Inbred Lines for Drought Tolerance Using β-carotene Content: Morphophysiological and Biochemical Traits. Agronomy 9: 692-709.

Langyintuo, A. S., Mwangi, W., Diallo, A. O., Macrobert, J., Dixon, J. and Bänziger, M. 2010. Challenges of the maize seed industry in eastern and southern Africa: a compelling case for private-public intervention to promote growth. Food Policy 35: 323-331.

Li, L. 1988. Behavioral ecology and life history evolution in the Larger Grain Borer, *Prostephanus truncatus* (Horn), Ph.D. thesis. PhD, University of Reading, UK.

Li, W., Liu, Z., Shi, Y., Song, Y., Wang, T., Xu, C. and Li, Y. 2010. Detection of consensus genomic region of QTLs relevant to drought-tolerance in maize by QTL meta-analysis and bioinformatics approach. Acta Agronomica Sinica 36: 1457-1467.

Liu, Y., Qin, L., Han, L., Xiang, Y. and Zhao, D. 2015. Overexpression of maize SDD1 (ZmSDD1) improves drought resistance in *Zea mays* L. by reducing stomatal density. Plant Cell, Tissue and Organ Culture (PCTOC) 122: 147-159.

Lopes, M., Araus, J., Van Heerden, P. and Foyer, C. 2011. Enhancing drought tolerance in C4 crops. Journal of Experimental Botany 62: 3135-3153.

Louie, R. 1980. Sugarcane mosaic virus in Kenya. Plant Disease 64: 944-947.

Lukanda, M., Owati, A., Ogunsanya, P., Valimunzigha, K., Katsongo, K., Ndemere, H. and Kumar, P. 2014. First report of Maize chlorotic mottle virus infecting maize in the Democratic Republic of the Congo. Plant disease 98: 1448-1448.

Maazou, A., Tu, J., Qiu, J. and Liu, Z. 2016. Breeding for Drought Tolerance in Maize (*Zea mays* L.). American Journal of Plant Sciences 7: 1858-1870.

Machida, L., Derera, J., Tongoona, P. and Macrobert, J. 2010. Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. Crop science 50: 1708-1717.

Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Nguyen, H. T. and Marmiroli, N. 2002. Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant molecular biology 48: 667-681.

Mahuku, G., Lockhart, B., Wanjala, B., Jones, M., Kimunye, J., Stewart, L., Cassone, B., Sevgan, S., Nyasani, J., Kusia, E., Kumar, P., Niblett, C., Kiggundu, A., Asea, G., Pappu, H., Wangai, A., Prasanna, B. and Redinbaugh, M. 2015a. Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-Saharan Africa. Phytopathology 105: 956-965.

Mahuku, G., Wangai, A., Sadessa, K., Teklewold, A., Wegary, D., Ayalneh, D., Adams, I., Smith, J., Bottomley, E. and Bryce, S. 2015b. First report of maize chlorotic mottle virus and maize lethal necrosis on maize in Ethiopia. Plant Disease 99: 1870-1870.

Makumbi, D. and Wangai, A. 2013. Maize lethal necrosis (MLN) disease in Kenya and Tanzania: Facts and actions. CIMMYT/KARI. http://www.cimmyt.org/en/where-we-work/africa/item/maize-lethal-necrosismln-disease-in-kenya-and-tanzania-factsand-actions. [Accessed 28 Januarty 2014].

Mariquele, B. 2006. Ocorrência da broca maior do grão *Prostephanus truncatus*: Horn (*Coleoptera*: *Bostrichidae*) em Moçambique: O caso do distrito de Manica. Lincenciatura Projecto final, Universidade Eduardo Mondlane.

Markham, R., Wright, V. and Rios Ibarra, R. 1991. A selective review of research on *Prostephanus truncatus* (Horn) (Col.: *Bostrichidae*) with an annotated and updated bibliography. CEIBA 32, 90pp.

Matewele, M. 2014. Diversity Analysis and Breeding for Maize Weevil (*Sitophilus zeamais* Motschulsky) and Larger Grain Borer (*Prostephanus truncatus* Horn) Resistance in Productive Maize Germplasm in Malawi. PhD Thesis, University of KwaZulu-Natal, SA.

Mather, K. and Jinks, J. L. 1982. Biometrical Genetics: The study of Continuos variation 3rd edition, London. UK., Chapman and Hall Ltd.

Mather, K. and Jinks, J. L. 2013. Biometrical genetics: The study of continuous variation, Springer.

Mdrat 2012. The Status of Maize Lethal Necrosis Disease and General Maize Performance in Kenya. Multi-Disciplinary Rapid Assessment Team, Ministry of Agriculture, Kenya.

Menkir, A. and Akintunde, A. 2001. Evaluation of the performance of maize hybrids-improved open-pollinated and farmers' local varieties under well-watered and drought stress conditions. Maydica 46: 227-238.

Meseka, S., Menkir, A. and Ajala, S. 2011. Genetic analysis of performance of maize inbred lines under drought stress. Journal of crop improvement 25: 521-539.

Messmer, R., Fracheboud, Y., Bänziger, M., Vargas, M., Stamp, P. and Ribaut, J. 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theoretical and Applied Genetics 119: 913-930.

Messmer, R., Fracheboud, Y., Bänziger, M., Stamp, P. and Ribaut, J. 2011. Drought stress and tropical maize: QTLs for leaf greenness, plant senescence, and root capacitance. Field Crops Research 124: 93-103.

Mir, R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125: 625-645.

Moll, R., Thompson, D. and Harvey, P. 1963. A quantitative genetic study of the inheritance of resistance to brown spot (Physoderma maydis) of Corn. Crop science 3: 389-391.

Monneveux, P., Sanchez, C., Beck, D. and Edmeades, G. 2006. Drought tolerance improvement in tropical maize source populations. Crop Science 46: 180-191.

Mugo, S., Likhayo, P., Karaya, H., Gethi, J., Njoka, S., Ajanga, S., Shuma, J. and Tefera, T. 2010. Screening maize germplasm for resistance to maize weevil (*Sitophilus zeamais* Motschulsky) and Larger grain borer (*Prostephanus truncatus* (Horn)) pests in Kenya. In: KARI (ed.) 12th KARI biennial Scientific Conference. KARI headquarters, Keptaget Road, Nairobi, Kenya.

Mushongi, A., Derera, J., Tongoona, P. and Lyimo, N. 2013. Generation mean analysis of leaf chlorophyll concentration from mid-silking to physiological maturity in some tropical maize (*Zea mays* L.) genotypes under low and high nitrogen dosages. Euphytica 189: 111-122.

Mwololo, J., Mugo, S., Okori, P., Tefera, T. and Munyiri, S. 2010. Genetic diversity for resistance to larger grain borer in maize hybrids and open pollinated varieties in Kenya. Second RUFORUM Biennial Meeting , Entebbe, Uganda, 20 - 24 September.

Mwololo, J., Mugo, S., Tefera, T. and Munyiri, S. 2013. Evaluation of traits of resistance to postharvest insect pests in tropical maize. Internaltional Journal of Agriculture and Crop Science 6: 926–933.

Mwololo, J., Mugo, S., Otim, M., Munyiri, S. and Okori, P. 2018. Quantitative Trait Loci Mapping in Maize for Resistance to Larger Grain Borer. Maydica 63: 1-9.

Nang'ayo, F. 1996. Ecological studies on Larger Grain Borer in savanna woodlands of Kenya. Ph.D. dissertation, Imperial College, London. England, UK.

Nations encycloedia. 2019. Mozambique - Agriculture [Online]. Available: https://www.nationsencyclopedia.com/Africa/Mozambique-AGRICULTURE.html [Accessed 01 October 2019].

Ndaruhutse, F. 2016. Generation mean analysis for diplodia ear rot resistance in tropical maize inbred lines. MSc Thesis. MSc, Nairobi University, Kenya.

Ndiso, J., Mugo, S., Kibe, A., Pathak, R. and Likhayo, P. 2007. Characterization for Phenotypic Drought Tolerance and Resistance to Storage Pests in Local Coastal Maize Landraces in Kenya. African Crop Science Journal 8: 245-250.

Nelson, S., Brewbaker, J. and Hu, J. 2011. Maize chlorotic mottle virus. Plant Disease 79: 1-6.

Nesmith, D. and Ritchie, J. 1992. Effects of soil water-deficits during tassel emergence on development and yield component of maize (*Zea mays* L.). Field Crops Research 28: 251-256.

Nhamucho, E., Mugo, S., Kinyua, M., Gohole, L., Tefera, T. and Mulima, E. 2014. Antibiosis mechanism of resistance to larger grain borer *Prostephanus truncatus* (Horn), (*Coleoptera*: *Bostrichidae*) in Maize. Journal of Entomology 11: 248-260.

Nhamucho, E., Mugo, S., Gohole, L., Tefera, T., Kinyua, M. and Mulima, E. 2017. Resistance of selected Mozambican local and improved maize genotypes to maize weevil, *Sitophilus zeamais* (Motschulsky). Journal of Stored Products Research 73: 115-124.

Niblett, C. and Claflin, L. 1978. Corn lethal necrosis-a new virus disease of corn in Kansas. Plant disease reporter 62: 15-19.

Ojo, G., Adedzwa, D. and Bello, L. 2007. Combining ability estimates and heterosis for grain yield and yield components in maize (*Zea mays* L.). Journal of sustainable development in agriculture and environment 3: 49-57.

Painter, R.1951. Insect Resistance in Crop Plants. McMillan, New York, USA, 520pp.

Pantenius, C. 1988. Storage losses in traditional maize granaries in Togo. Insect Science and its Applications 9: 725-735.

Pöschko, M. 1993. Biologie und Wirtsspezifität von Teretriosoma nigrescens Lewis. PhD dissertation, Technical University Berlin, Germany.

Prasanna, B. M., Huesing, J. E., Eddy, R. and Peschke, V. M. 2018. Fall Armyworm in Africa: A Guide for Integrated Pest Management, First Edition. Mexico, CDMX: CIMMYT.

Quan, R., Shang, M., Zhang, H., Zhao, Y. and Zhang, J. 2004. Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. Plant Biotechnology Journal 2: 477-486.

Redinbaugh, M. G. and Pratt, R. C. 2008. Virus resistance. In: Hake, S. & Bennetzen, J. (eds.) The maize handbook (2nd ed, 255-270). New York: Springer-Verlag.

Reynolds, M. and Trethowan., R. 2007. Physiological interventions in breeding for adaptation to abiotic stress. Wageningen UR Frontis Series 21: 127-144.

Ribaut, J., Betrán, J., Monneveux, P. and Setter, T. 2009. Drought tolerance in maize. Handbook of maize: its biology. Springer.

Ribaut, J., Edmeades, G. and Hoisington, D. 2000. The genetic basis of drought tolerance in maize and options for improvement via marker-assisted selection. Plant genetic engineering: towards the third millennium: Proceedings of the International Symposium on Plant Genetic Engineering, Havana, Cuba, 6-10 December, 1999. Elsevier Science Publishers, 35-41.

Richardson, C. 2005. The loss of property rights and the collapse of Zimbabwe. Cato Journal 25: 541-565.

Richter, J. and Biliwa, A. 1991. A countrywide survey for the presence of *Prostephanus truncatus* (Horn) (*Coleoptera*, *Bostrichidae*) in Togo using pheromone traps. Anzeiger fuer Schaedlingskunde Pflanzenschutz Umweltschutz 64: 89-92.

Ridley, A. and Bartlett, J. 2010. Diagnostic Methods for larger grain borer (*Prostephanus truncatus*), Plant Biosecurity Toolbox pp 33.

Roberts, P. and Douce, G. 1999. Weevils and Borers. A County Agent's Guide to Insects Important to Agriculture in Georgia, USA.

Rwegasiram, M., Jowah, P. and Mvumi, B. 2003. The potential invasion areas by the larger grain borer in Zimbabwe. African Crop Science Journal 6: 254-259.

Sari-Gorla, M., Krajewski, P., Di Fonzo, N., Villa, M. and Frova, C. 1999. Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. Theoretical and Applied Genetics 99: 289-295.

Schlegel, R. 2010. Dictionary of plant breeding 2 ed. CRC Press,Taylor & Francis Group, Boca Raton

Schneider, H., Borgemeister, C., Sétamou, M., Affognon, H., Bell, A., Zweigert, M., Poehling, H. and Schulthess, F. 2004. Impact assessment of Teretrius nigrescens Lewis (*Coleoptera*: Histeridae), an introduced predator of the larger grain borer *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*) in Togo and Benin. . Biological Control 30: 241-255.

Schoonhoven, A., Horber, E. and Mills, R. 1976. Conditions modifying expression of resistance of maize kernels to the maize weevil. Environmental Entomology 5: 163-168.

Schoonhoven, A., Horber, E., Wassom, C. and Mills, R. 1975. Selection for resistance to the maize weevil in kernels of maize. Euphytica 24: 639-644.

Segeren, P., Van Den Oever R. and J., C. (eds.) 1994. Pragas, Doenças e Ervas Daninhas nas culturas Alimentares em Moçambique, Moçambique- Maputo: Minísterio de Agricultura- Instituto Nacional de Investigação Agronómica.

Semagn, K., Beyene, Y., Warburton, M. L., Tarekegne, A., Mugo, S., Meisel, B., Sehabiague, P. and Prasanna, B. M. 2013. Meta-analyses of QTL for grain yield and anthesis silking interval in 18 maize populations evaluated under water-stressed and well-watered environments. BMC genomics 14: 313-329.

Serratos, A., Arnason, J., Nozzolillo, C., Lambert, J., Philogiène, B., Fulcher, G., Davidson, K., Peacock, L., Atkinson, J. and Morand, P. 1987. Factors contributing to resistance of exoticmaize populations to maize weevil, *Sitophilus zeamais*. Journal of Chemical Ecology 13: 751-762.

Serratos, J., Blanco-Labra, A., Mihm, J., Pietrzak, L. and Arnason, J. 1993. Generation means analysis of phenolic compounds in maize grain and susceptibility to maize weevil *Sitophilus zeamais* infestation. Canadian Journal of Botany 71: 1176-1181.

Setimela, P. S., Vivek, B., Bänziger, M., Crossa, J. and Maideni, F. 2007. Evaluation of early to medium maturing open pollinated maize varieties in SADC region using GGE biplot based on the SREG model. Field Crops Research 103: 161-169.

SETSAN. 2010. Relatório da Monitoria da Situação de Segurança Alimentar e Nutricional em Moçambique. Secretariado Tecnico para Seguranca alimentar e Nutricional (SETSAN). Maputo.

Shattuck, V., Christie, B. and Corso, C. 1993. Principles for Griffing's combining ability analysis. Genetica 90: 73-77.

Sher, H., Iqbal, M. and Khan, K. 2012. Genetic analysis of maturity and flowering characteristics in maize (*Zea mays* L.). Asian Pacific journal of tropical biomedicine 2: 621-626.

Shires, S. 1977. Ability of *Prostephanus truncatus* (Horn) (*Coleoptera*:*Bostrichidae*) to damage and breed on several stored food commodities. Journal of Stored Products Research 13: 205- 208.

Shiri, M., Aliyev, R. and Choukan, R. 2010. Water stress effects on combining ability and gene action of yield and genetic properties of drought tolerance indices in maize. Research Journal Environmental Science 4: 75-84.

Shuja, M., Ali, W., Iqbal, A., Ali, I., Munir, I., Ahmad, D., Ahmad, G., Khan, M. and Swati, Z. 2011. Maize breeding for marginal lands: Physiological and molecular approach to decipher response and selection of maize recombinant inbred lines (RILs) under water deficit at early growth stage. African Journal of Biotechnology 10: 3521-3527.

Sibiya, J. 2009. Breeding Investigations for resistance to Phaeosphaeria Leaf spot (PLS) and other important foliar diseases and a study af yield stability in African Maize Germplasm PhD Thesis, University of KwaZulu-Natal, SA.

Smalley, E. 1998. Identification of mycotoxin producing fungi and conditions leading to aflatoxin contamination of stored foodgrains. In: Semple *et al*.1999 (ed.) Mycotoxin prevention and control in foodgrains. United Nations Development Programme and Food and Agriculture Organization. Bankok- Thailand.

Sprague, G. and Tatum, L. A. 1942. General vs. specific combining ability in single crosses of corn. Journal of American Society of Agronomy 34: 923-932.

Subramanyam, B., Cutkomp, L. and Darveaux, B. 1985. A new character for identifying larval instars of *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*). Journal of Stored Products Research 21: 101-104.

Takeda, S. and Matsuoka, M. 2008. Genetic approaches to crop improvement: responding to environmental and population changes. Nature Reviews Genetics 9: 444.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2010. Mass Rearing of Stem Borers, Maize Weevil and Larger Grain Borer Insect Pests of Maize, CIMMYT: Nairobi- Kenya, 36pp.

TIA 2007. Trabalho de Inquerito Agricola 2007. Ministerio de Agricultura. Direcção Nacional de Economia Agraria. Maputo.

Tilahun, T., Ayana, G., Abebe, F. and Wegary, D. Maize Pathology Research in Ethiopia: 2001. A review. In: Negusie, M., Tanner, D. & Twuwasi-Afriyie, F., eds. Enhancing the Contribution of Maize to Food Security in Ethiopia, 2001 Second National Maize Workshop of Ethiopia 12-16 November 2001, Addis Ababa, Ethiopia. EARO/CIMMYT, 97-105.

Tipping, P., Legg, D., Rodriguez, J. and Poneleit, C. 1988. Influence of maize pericarp surface relief on resistance to the maize weevil (*Coleoptera*: Curculionidae). Journal of Kansas Entomological Society 61: 237-241.

Tipping, P., Mikolajczak, K., Rodriguez, J., Poneleit, C. and Legg, D. 1987. Effects of whole corn kernels and extracts on behaviour of maize weevil (*Coleoptera*: Curculionidae). Journal of Economic Entomology 80: 1010-1013.

Tipping, P., Mikolajczak, K., Rodriguez, J., Zilkowski, B. and Legg, D. 1986. Attraction of Sitophilus oryzae L. and *S. zeamais* Motsch. (*Coleoptera*: Curculionidae) to extracts from two corn genotypes. Journal of the Kansas Entomological Society 59: 190-194.

Toker, C., Canci, H. and Yildirim, T. 2007. Evaluation of perennial wild Cicer species for drought resistance. Genetic Resources and Crop Evolution 54: 1781-1786.

Tollenaar, M. and Lee, E. 2006. Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. Maydica 51: 399.

Tooke, F. and Scott, M. 1994. Wood-boring beetles in South Africa. Department of Agriculture Technical Bulletin No. 247 (Entomology Series No. 14).

Trading economics. 2019. Mozambique - Agriculture, value added (% of GDP) [Online]. Available: https://tradingeconomics.com/mozambique/agriculture-value-added-percent-of-gdp-wb-data.html [Accessed 01 October 2019].

Tuberosa, R. and Salvi, S. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends in plant science 11: 405-412.

Ulrich, J., Hawk, J. and Carroll, R. 1990. Diallel analysis of maize inbreds for resistance to gray leaf spot. Crop Science 30: 1198-1200.

Uyemoto, J. 1983. Biology and control of maize chlorotic mottle virus. Plant Disease 67: 7-10.

Uyemoto, J., Bockelman, D. and Claflin, L. 1980. Severe outbreak of corn lethal necrosis disease in Kansas. Plant Disease (formerly Plant Disease Reporter) 64: 99-100.

Uyemoto, J., Claflin, L., Wilson, D. and Raney, R. 1981. Maize chlorotic mottle and maize dwarf mosaic viruses; effect of single and double inoculations on symptomatology and yield. Plant Disease 65: 39-41.

Vaezi, B., Bavei, V. and Shiran, B. 2010. Screening of barley genotypes for drought tolerance by agro-physiological traits in field condition. African Journal of Agricultural Research 5: 881-892.

Van Gioi, H., Mallikarjuna, M. G., Shikha, M., Pooja, B., Jha, S. K., Dash, P. K., Basappa, A. M., Gadag, R. N., Rao, A. R. and Nepolean, T. 2017. Variable level of dominance of candidate genes controlling drought functional traits in maize hybrids. Frontiers in plant science 8: 940- 953.

Wang, B., Liu, C., Zhang, D., He, C., Zhang, J. and Li, Z. 2019. Effects of maize organ-specific drought stress response on yields from transcriptome analysis. BMC plant biology 19: 335-354.

Wangai, A., Redinbaugh, M., Kinyua, Z., Miano, D., Leley, P., Kasina, M., Mahuku, J., Scheets, J. and Jeffers, D. 2012a. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Diseaase 96: 1582- 1586.

Wangai, A., Sikinyi, E., Ochieng, J., Miyogo, S., Karanja, T., Odour, H., Kimani, E., Irungu, J., Kinyua, Z., Ngaruiya, P., Ligeyo, D. and Kipkemboi, S. 2012b. Joint assessment report: Report on status of maize lethal necrosis disease and general maize performance. Ministry of Agriculture, Kenya. Online publication. http://www.fao.org/fileadmin/user_upload/drought/docs/Maize%20Lethal%20Necrotic%20Disease%20in%20Kenya_Joint%20Assessment%20Report%20(July%202012).pdf. [Accessed 15 November 2012]

Wegary, D., Vivek, B. and Labuschagne, M. 2014. Combining ability of certain agronomic traits in quality protein maize under stress and nonstress environments in Eastern and Southern Africa. Crop Science 54: 1004-1014.

Williams, T., Snell, R. and Cress, C. 1969. Inheritance of drought tolerance in sweet corn. Crop Science 9: 19-22.

Williams, W. and Windham, G. 2015. Aflatoxin accumulation in a maize diallel cross. Agriculture 5: 344-352.

Xie, L., Zhang, J., Wang, Q., Meng, C., Hong, J. and Zhou, X. 2011. Characterization of maize chlorotic mottle virus associated with maize lethal necrosis disease in China. Journal of Phytopathology 159: 191-193.

Xin-Hai, L., Xian-De, L., Ming-Shun, L. and Shi-Huang, Z. 2003. Identification of Quantitative Trait Loci for Anthesis-Silking Interval and Yield Components Under Drought Stress in Maize. Acta Botanica Sinica 45: 852-857.

Xiong, L., Wang, R., Mao, G. and Koczan, J. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. Plant physiology 142: 1065-1074.

Xoconostle-Cazares, B., Ramirez-Ortgega, F., Flores-Elenes, L. and Ruiz-Medrano, R. 2011. Drought tolerance in crop plants. American Journal of Plant Physiology 5: 1-16.

Xu, Y. 2010. Molecular plant breeding, United Kingdom, England, CABI. 734pp

Zacarias, A. 2018. USDA Global Agricultural Information Network- Mozambique Fall Armyworm, 5pp.

Zhu, J., Wang, X., Sun, C., Zhu, X., Meng, L., Zhang, G., Tian, Y. and Wang, Z. 2011. Mapping of QTL associated with drought tolerance in a semi-automobile rain shelter in maize (*Zea mays* L.). Agricultural sciences in China 10: 987-996.

3. CHAPTER THREE

Heritability and gene action controlling post-harvest maize weevil and larger grain borer resistance in tropical maize germplasm

Abstract

The maize weevil*, Sitophilus zeamais* Motschulsky (*Coleoptera: Curculionidae*) and the larger grain borer *Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*) are the most damaging post-harvest insect pests of maize in Africa causing losses of up to 90% when maize is stored with high moisture content and/or without use of chemical protectants. Host plant resistance is thus a vital component of an integrated pest management (IPM) strategy against *S. zeamais* and *P. truncatus.* Small scale farmers can greatly benefit from resistant maize cultivars. This study aimed at determining the genetic action influencing inheritance of resistance to *S. zeamais* and *P. truncatus* in three maize inbred lines using generation mean analysis. Six generations (P1, P2, F1, F2, BCP1 and BCP2) were developed from each cross. The parents were two inbred lines resistant to maize weevil and larger grain borer (CKDHL120731 and CKDHL120517) and one line susceptible to the same insect pests (CKDHL120918), and crosses made were CKDHL120731×CKDHL120918 (cross 1) and CKDHL120517×CKDHL120918 (cross 2). The various generations from the two crosses were evaluated in separate experiments in a post-harvest laboratory in Kiboko- Kenya over three months, in a complete randomized design, replicated twice from November 2018 to January 2019. Fifteen cobs were used for P1, P2 and F1, 30 cobs for BCP1 and BCP2 and 60 cobs for F2. Data were recorded for percentage kernel weight loss, kernel damage and the final number of living insects. The resistance traits for both crosses did not fit a simple additive-dominance model for *S. zeamais*, suggesting the existence of epistasis effects. However, for *P. truncatus* resistance, cross 1 fitted well on a simple additive-dominance model but cross 2 did not, suggesting both simple model and digenic interaction model were present in the inheritance of *P. truncatus* resistance, depending on the genetic background of the parent used. Additive, dominance and epistasis gene effects played a significant role in the inheritance of resistance to both insects in the selected maize genotypes. This was further confirmed by moderate narrow-sense heritability estimates. This suggest that it is feasible to improve maize genotypes for insect resistance, although not simple due to involvement different types of gene action.

3.1. Introduction

Farmers in Mozambique experience high maize losses in storage due to postharvest insect pests, mainly the maize weevil (*Sitophilus zeamais* Motschulsky) and the larger grain borer (*Prostephanus truncatus* Horn) (Cugala *et al.*, 2007a,b). These two pests cause an average of 12 to 30% annual maize loss, which can increase to 80% (Cugala *et al.*, 2007a,b; Fato *et al.*, 2008), thus aggravating food and seed insecurity. Damaged grains are prone to contamination by mycotoxins, including aflatoxins which when consumed result in serious health risk (Kankolongo *et al.*, 2009). The use of pesticides has been promoted to control these insect pests but adoption rates are low due to perceived high costs (INE, 2003; Mariquele, 2006). Most of the farmers resort to indigenous control methods, including the use of botanical extracts. However, these have low efficacies as they are seldom standardized in content, concentration and quantities of application (Mariquele, 2006). Host plant resistance (HPR) is thus an attractive solution and resistant maize varieties to these postharvest insect pests have been developed in other countries.

Knowledge of the inheritance of a target trait for improvement is the first step towards a successful plant breeding program (Kearsey and Pooni, 1998). There are several studies that have reported the gene action controlling the inheritance of resistance to storage pests but mainly for *S. zeamais* (Derera *et al.*, 2001; Sodedji *et al.*, 2018), using north carolina design II and line × tester mating design, respectively, with only one reported study on *P. truncatus* (Matewele, 2014) using north carolina design II. However, the results in these reports are conflicting. Dhliwayo *et al.* (2005) and Sodedji *et al.* (2018) indicated that the variance due to specific combining ability effects (dominance effects) was more important than the general combining ability effects (additive effects) for some traits controlling *S. zeamais* resistance. On the other hand, significant additive, non-additive and maternal effects were reported by Schoonhoven *et al.* (1975), Widstrom *et al.* (1975, 1983) and Derera *et al.* (2001) for *S. zeamais* resistance in maize grain. In all these studies, additive gene effects were more important especially for the female parent in a hybrid combination. Widstrom *et al.* (1975) also reported that dominance effects were more significant for seed resistance to maize weevil among genotypes segregating for endosperm when they are females in a cross. Matewele (2014) indicated the importance of additive gene effects for resistance to *P. truncatus* on grain damage.

The objective of this study was to determine the gene effects controlling the inheritance of resistance to *S. zeamais and P. truncatus* in two selected crosses involving resistant and

susceptible maize inbred lines using generation mean analysis. Results from the study would help maize breeders in devising an effective resistance breeding strategy.

3.2. Materials and Methods

3.2.1. Germplasm

Three inbred lines, selected from previous studies (Table 3.1) were planted in June 2017, during the 2017A season at International Maize and Wheat Improvement Center (CIMMYT) drought screening site at Kenya Agricultural and Livestock Research Organization (KALRO) at Kiboko Research Station. Kiboko is located at 2°12' 50.24" S, 37°43' 30.11" E, 945 masl. These lines were crossed in the nursery to form two F_1 hybrids (Table 3.1). The female plots were planted in five rows of 5 m length at a spacing of 75 x 25 cm. Two seeds were sown per hill and thinned down to one plant per hill after emergence. The male plots were sown on one row per planting, preceding each set of female rows at three intervals (-5, 0 and +5 days) after planting the females to synchronize flowering for continued pollen supply at the time of pollination (CIMMYT, 1985). Once the pollination was done, the male plants were cut down from the field. A side nursery for increasing the inbred seed was planted and each inbred line was planted in three rows for selfing.

Table 3.1. List of the inbred lines and crosses involved in the study

Designation	Inbred line name	Attributes	Origin
Parent 1 (P1)	CKDHL120731	Resistant to both maize weevil and LGB	CIMMYT- Kenya
Parent 2 (P2)	CKDHL120517	Resistant to both maize weevil and LGB	CIMMYT- Kenya
Parent 3 (P3)	CKDHL120918	Susceptible to both maize weevil and LGB	CIMMYT- Kenya
Cross Code	**Attribute**	**Pedigree**	**Origin**
P1 x P3	R x S	CKDHL120731 x CKDHL120918	CIMMYT- Kenya
P2 x P3	R x S	CKDHL120517 x CKDHL120918	CIMMYT- Kenya

Fertilizers were applied according to the recommended rates for the Kiboko area, which includes 60 kg N and 60 kg P_2O_5 ha^{-1}, where nitrogen was split in two applications. Weeding and harvesting were done manually. The fields were kept clean from planting to harvest. Supplementary irrigation was provided when necessary. Both nurseries were harvested in October, 2017 and the seed from female rows was cleaned, dried, shelled and kept in the cold room. During the long rainy season of 2018A in May, the two F1 hybrids and all inbred lines were planted again in the nursery at the same station to produce the grain materials

for evaluation. For each cross; P1, P2, F1, F2, BCP1 and BCP2 generations were produced where; P1 = parent 1 (resistant) and P2 = parent 2 (susceptible) in each cross; F1 = single cross between P1 and P2; F2 = generation produced by selfing the F1 hybrid; BCP1 and BCP2 = generation produced by backcrossing F1 to each of the parents.

The same procedures for spacing and field management used on the 2018A nursery were used in this nursery. The F2s were produced in 15 rows of 5 metres in length, while BCP1, BCP2, F1 were produced in 10 rows for the female parent and 6 rows for the male parent, while, P1 and P2 were produced in eight rows each. The production of all generations in one season was to ensure that all seed were produced at the same time. During harvest, one best cob harvested from each plant was cleaned, sundried and kept in cob form.

3.2.2. Rearing of maize weevil and larger grain borer

The maize weevil (MW) and larger grain borer (LGB) were reared on maize grain according to the methods described by Tefera *et al.* (2010). Maize is a good culture medium for both insects. Four hundred (400) grams of susceptible hybrid (H513) grain with 11–12% moisture content was placed in one-liter glass jars covered with perforated lids. Two hundred unsexed adult MW or LGB were introduced into the jars separately. The jars were maintained at KALRO-Kiboko Post-Harvest Laboratory at ambient temperatures (27 ± 2ºC) and 65 - 70% relative humidity, 12:12 (light: dark) photoperiod. For maize weevil, after 10 days of oviposition, all introduced adult insects were removed and then the jar was kept for the eggs to hatch and progeny emergence. The emerged progeny was observed daily and emerged insects were transferred to new glass jars with fresh grain until enough insects were obtained for the experiments. For larger grain borer, after 35 days, newly emerged LGB were removed daily and replaced in a fresh grain in glass jars and kept in the CTH room until enough insects were obtained.

3.2.3. Evaluation of maize genotypes for resistance to *S. zeamais* and *P. truncatus*

Infestation of post-harvest insects starts from the field. After harvest, the maize cobs were sun dried for seven days and an application of Gastoxin™ (phosphine fumigant), was used to fumigate the maize in plastic drums for seven days. The fumigation was applied to kill insects or eggs, which might have come from the field. Phosphine is an effective fumigant in sealed storage (air tight) against insects in most types of grain and does not give any residual protection after the seven days. After the seven days, the cobs harvested from the

different generations were shelled and dried to 12 to 13% moisture content separately. The experiments were set at the post-harvest pest laboratory of KARLO/ CIMMYT's research station in Kiboko. The number of cobs used differed depending on the generation. For P1, P2 and F1 15 cobs were used, 30 cobs for BCP1 and BCP2 and 60 cobs for F2, all replicated twice, for each cross and insect.

The number of cobs in the segregating generations (F2, BCP1 and BCP2) were higher than the non-segregating generations (P1, P2 and F1). This is due to the variability expected within each generation. In each experimental set, samples of 50±1 g of clean, undamaged grains from each cob were placed in clean 250 cm^3 glass jars (Derera *et al.*, 2010). To allow ventilation inside the glass jar and prevent escape of the insects, the tops of the lids were cut out, leaving only the screw-top rings with fine wire gauze (Plate 3-1). The jars were kept in a controlled temperature and humidity (CTH) room for seven days for acclimatization at 28±2°C and 65±5% RH with 12:12 (light: dark) photoperiod to reach uniform temperature and grain moisture content among all samples.

Plate 3-1. Jars with lids cut out with fine wire gauze

After the acclimatization period, 32 unsexed and active 20 - 25 day-old *S. zeamais* and *P. truncatus* adults were chosen randomly from a laboratory culture and introduced into all jars. The jars were placed again in CTH room in shelves (Plate 3-2), laid out in a Completely Randomized Design (CRD) with four replications and kept undisturbed in the CTH room for 90 days. The Completely Randomized Design (CRD) was used because in the CTH room the environmental conditions were constant and uniform, so the observed differences on

the measured parameters were attributed to the genotype effects only. The temperature in the CTH room was maintained at 27 ± 2ºC and the relative humidity at 65 – 70% and had 12:12 (light: dark) photoperiod.

Plate 3-2. Shelves with jars in a CTH room at KALRO Kiboko post-harvest pest laboratory

3.2.4. Data collection

Data was collected after 90 days of incubation on number of living insects *S. zeamais* or *P. truncatus*, number and weight of damaged and undamaged grain from individual jars. Data on seed damage and grain weight loss was computed from this information. The glass jars were opened and the contents were separated into grains, insects and flour using 4.7 mm and 1.0 mm sieves for each jar. The grains were hand-sorted into damaged and undamaged categories.

Damaged grains were considered those with holes and/or tunnels done by insects. A precision electronic scale of 0.01g was used to weigh the damaged and undamaged grains. Seed damage was expressed as a proportion of damaged seed over the total number of seeds sampled while weight loss was calculated using the Count and Weigh method as described by Boxall (1986) and expressed by the formula below:

$$Weight\ loss\ (\%) = \frac{Wu \times Nd - Wd \times Nu}{Wu \times (Nd + Nu)} \times 100 \qquad \textbf{Equation 3.1}$$

Where, Wu = weight of undamaged grains Nu = number of undamaged grains

Wd = weight of insect damaged grains Nd = number of insect damaged grains

An index of selection (SI) based on the susceptibility parameters was computed to categorize the genotypes into resistant or susceptible types. This index was calculated by summing the ratios between values and overall mean and dividing by the number of parameters. The susceptibility parameters used were: number of living LGB, weight loss (%) and seed damage (%). The classification of the genotypes were grouped into resistance or susceptible using selection index, based on a scale developed by Bergvinson *et al.* (2002):

Index	**Classification**
≤ 0.60	Highly Resistant
0.61 - 0.8	Moderately Resistant
0.81- 1.0	Moderately Susceptible
> 1.0	Highly Susceptible

3.2.5. Data analysis

The data of weight loss, seed damage and number of living insects were analysed using unbalanced analysis of variance in Genstat 18.2 edition. The variance components were analysed using SAS version 9.4 (Hayman and Mather, 1955). The means were separated using the least significant difference (LSD) at 5%. Before the analysis of variance components, an F-test was applied to test the variance of the segregating generation (F2, BCP1 and BCP2) against the variance of non-segregating generation (P1, P2 and F1). Each one of the segregating generation was tested against environment variance which was the mean of variances of the non-segregating generation ($\sigma^2E = (\sigma^2P1 + \sigma^2P2 + \sigma^2F1)/3$). The grain weight loss (%) and seed damage (%) were transformed with angular-transformation, $\arcsin\sqrt{proportion}$, and the data for living insects were transformed with logarithm transformation base 10, ($\lg_{10}(x+1)$), where x is the observed value (Gomez and Gomez, 1984). These were done to normalize the data for analysis but the final results were presented as back transformed means (original data).

3.2.5.1. Analysis of genetic effects

Two analysis steps were carried out.

First, the differences among the mean of the six generations; P1, P2, F1, F2, BCP1, and BCP2, for each trait of the two crosses were analysed by joint scaling test (Mather, 1949; Hayman and Mather, 1955; Mather and Jinks, 1982). The scaling test parameters A, B, C and D and their variance were calculated to test adequacy of the additive-dominance model, following the below formulas:

A = $2\overline{BC}$P1-$\overline{P}$1-$\overline{F}$1

B= $2\overline{BC}$P2- $\overline{P}$2-$\overline{F}$1

C= $4\overline{F}_2$- $2\overline{F}$1-$\overline{P}$1-$\overline{P}$2

D= $2\overline{F}$2- $\overline{BC}P1$-$\overline{BCP2}$

A, B, C and D are the values of each scaling test,

The variance of the tests were:

σ^2A = 4$\sigma^2\overline{BC}$P1 + $\sigma^2\overline{P}$1 + $\sigma^2\overline{F1}$

σ^2B = 4$\sigma^2\overline{BC}$P2 + $\sigma^2\overline{P}$2 + $\sigma^2\overline{F}$1

σ^2C = 16$\sigma^2\overline{F}$2 + 4$\sigma^2\overline{F}$1 + $\sigma^2\overline{P}$1 + $\sigma^2\overline{P}$2

σ^2D = 4σ^2VF2 + $\sigma^2\overline{BC}$1 + $\sigma^2\overline{BC}$2

The significance of each scaling test was determined using t-test, which was as follows:

$\pm t = \frac{Deviation}{standard\ error} = \frac{Deviation\ (Values\ of\ A\ or\ B\ or\ C)}{\sqrt{variation\ of\ deviation}}$; $\pm tA = \frac{A}{\sqrt{\sigma^2 A}}$; $\pm tB = \frac{B}{\sqrt{\sigma^2 B}}$; $\pm tC = \frac{C}{\sqrt{\sigma^2 C}}$; $\pm tD = \frac{C}{\sqrt{\sigma^2 D}}$

Where, tA, tB, tC and tD are t-test for scaling test parameters A, B, C and D.

The significance of scaling test parameter A and B suggest presence of all types of non-allelic gene interactions and the significance of C reveals presence of dominance × dominance [dd] type of epistasis while the significance of D suggest presence of additive × additive [aa] gene interaction (Singh and Narayanan, 1993).

In general, significance of scaling test implies that the additive-dominance model is inadequate.

Secondly, the results of the scaling test, were used to fit either the three-parameter (*m*, *a*, and *d*) or six genetic parameters (*m*, *a*, *d*, *aa*, *ad*, and *dd*) model developed by (Hayman, 1958) using the notation of Gamble (1962)

The model is: $Y = m + \alpha a + \beta d + \alpha^2 aa + 2\alpha\beta ad + \beta^2 dd$ (Kang, 1994) **Equation 3.2**

The six parameters of the genetic model were computed according to Jinks and Jones (1958) where:

Y = generation mean,

m = mean of the F2 generation and intercept value,

α and β = matrix coefficients of generations;

a = pooled additive effects;

d = pooled dominance effects;

aa = pooled additive × additive (homozygote × homozygote) effects;

ad = pooled additive × dominance (homozygote × heterozygote) effects;

dd = pooled dominance × dominance (heterozygote × heterozygote) effects

The genetic effects were estimated as:

m = $\overline{F}$2,

d =a= $\overline{BC}$P1- $\overline{BC}$P2,

h =d= $\overline{F}$1- 4$\overline{F}$2- ½ $\overline{P}$1- ½$\overline{P}$2 + 2$\overline{BC}$P1 + 2$\overline{BC}$P2

I = aa = 2$\overline{BC}$P1 + 2$\overline{BC}$P2 – 4$\overline{F}$2

j = ad = $\overline{BC}$P1 – ½$\overline{P}$1 – $\overline{BC}$P2 + ½$\overline{P}$2

l = dd = $\overline{P}$1 + $\overline{P}$2 + 2$\overline{F}$1 + 4$\overline{F}$2 - 4 $\overline{BC}$P1 – 4 $\overline{BC}$P2

The coefficients that estimate the degree of relationship of several generations used to determine gene effects for the generation means (Table 3.2)

Table 3.2. Generalized expectations of the six generations mean

Generations	[m]	[a]	[d]	[aa]	[ad]	[dd]
P1	1	1	- ½	1	-1	¼
P2	1	-1	- ½	1	1	¼
F1	1	0	½	0	0	¼
F2	1	0	0	0	0	0
BCP1	1	½	0	¼	0	0
BCP2	1	- ½	0	¼	0	0

[m]: mean of F2 generation[a] =Additive effect; [d] =Dominance effect; [aa] =Pooled Additive x Additive effects; [ad] = Interaction of Additive x Dominance effect and [dd] = Pooled dominance x dominance effects.
Source: Hayman (1958); Gamble (1962) and Hallauer *et al.*, 2010.

3.2.5.2. Genetic variance components

The analysis of variance of an unbalanced model was used to calculate the mean of the generations and genetic variances (Mather and Jinks, 1971). The variance of the segregating generations (F2, BCP1 and BCP2) were tested against the non-segregating generation (P1, P2 and F1) using the pooled estimated environment variance (σ^2E) on a simple F- test. The variance components were analysed using formulae described by Kearsey and Pooni (1998). The phenotypic variance was regarded equal to the variance of the F2 generation. The variance components were determined as follows:

i) $\sigma^2 \mathrm{E} = \left[\sigma^2 \mathrm{P1} + \sigma^2 \mathrm{P2} + 2\sigma^2 F1\right]/4,$

where $\sigma 2E$ =environment variance, σ^2 P1 =variance of parent one, σ^2 P2 =variance of parent two and σ^2 F1=variance of F1 generation.

ii) $\sigma^2 G = \sigma^2 \mathrm{P} - \sigma^2 \mathrm{E},$ Where σ^2 G =genetic variance, σ^2 P =phenotypic variance (σ^2 F2) and σ^2 E =environmental variance.

iii) $\sigma^2 A = 2\sigma^2 F2 - \left(\sigma^2 BCP1 + \sigma^2 BCP2\right),$ Where $\sigma^2 A$ = additive variance, σ^2 F2 =variance of F2 generation, σ^2 BCP1 =variance of backcross with parent one, σ^2 BCP2 =variance of backcross with parent two.

iv) $\sigma^2 D = \sigma^2 BCP1 + \sigma^2 BCP2 - \sigma^2 F2 - \sigma^2 \mathrm{E},$ where $\sigma^2 D$ = dominance variance

v) $\sigma^2 AD = \frac{1}{2}\left(\sigma^2 BCP2 - \sigma^2 BCP1\right)$,

where σ^2AD =additive and dominance variance.

3.2.5.3. Heritability

The heritability values were calculated using procedures developed by Warner (1952) and Allard (1960). The broad-sense heritability (H^2) was expressed as the ratio of genetic variance (σ^2G), which in this case similar as variance of the F2 generation (σ^2F2) to phenotypic variance (σ^2 P).

$$H^2 = 100\ x\ \frac{\sigma^2 G}{\sigma^2 P} = 100x\frac{\sigma^2 A + \sigma^2 D}{\sigma^2 A + \sigma^2 D + \sigma^2 E} \qquad \textbf{Equation 3.3}$$

The narrow-sense heritability (h^2) was estimated as the ratio of additive variance (σ^2A) to phenotypic variance (σ^2P)

$$h^2 = 100\ x\ \frac{\sigma^2 A}{\sigma^2 P} = 100\ x\ \frac{\sigma^2 A}{\sigma^2 A + \sigma^2 D + \sigma^2 E} \qquad \textbf{Equation 3.4}$$

The heritability estimates were classified as low when ≤ 30%, moderate from 31- 60% and high when it was greater than 60% as high (Robinson *et al.*, 1949).

The dominance ratio (DR) used to classify the significance of dominance and additive gene effects as for inbred line selection was calculate according Kearsey and Pooni (1998), using the following formula:

$$DR = \sqrt{\frac{4\sigma^2 D}{2\sigma^2 A}} \qquad \textbf{Equation 3.5}$$

Where: DR=dominance ratio, σ^2D =dominance variance and σ^2A =additive variance.

The potence ratio were also calculated to assess the degree of dominance and the presence or absence of heterosis in the crosses. The potence ratio was calculated following the formula below:

$$PR = \frac{[d]}{[a]} \qquad \textbf{Equation 3.6}$$

Where [d] is the estimate of dominance and [a] the estimate of additive effects

3.3. Results

3.3.1. Analysis of variances for insect resistant parameters

Significant differences (p> 0.05) were observed among generations for all investigated traits for both insects, *S. zeamais and P. truncatus*, in the two crosses, indicating the existence of genetic variation (Table 3.3 and Table 3.4). Consequently, generation mean analysis could be used to estimate the genetic parameters for the traits.

The means for selection index (SI) shows the average mean of the plants for a specific generation and it revealed the contrast on resistance to the two crossed inbred lines for both insects (Table 3.5).

Table 3.3. Means squares of parameters for maize resistance to the *S. zeamais*

Cross 1- CKDHL120731/CKDHL0918					
Source of variation	**df**	**SI**	**WL**	**SD**	**MW-A**
Rep	1	15.730***	0.105***	0.412***	3.548***
Generation	5	14.445***	0.190***	0.150***	0.759***
Plants	159	1.983***	0.015***	0.015***	0.185***
Error	164	0.643	0.002	0.002	0.060
Total	329	1.139	0.012	0.012	0.140
Cross 2- CKDHL120517/CKDHL0918					
Source of variation	**df**	**SI**	**WL**	**SD**	**MW-A**
Rep	1	13.507***	0.164***	0.119***	2.451***
Generation	5	18.017***	0.163***	0.132***	1.472***
Plants	159	0.902***	0.007***	0.010**	0.140ns
Error	164	0.452	0.003	0.005	0.120
Total	329	0.84	0.007	0.008	0.151
*** significant at 0.1%, ** significant at 1% , * significant at 5% and ns non-significant					
SI- Selection index			SD- Seed damage (%)		
WL- Weight loss (%)			MW-A- # of living insects		

Table 3.4. Mean squares of parameters for maize resistance to *P. truncatus*

Cross 1- CKDHL120731/CKDHL0918					
Source of variation	**df**	**SI**	**WL**	**SD**	**LGB-A**
Rep	1	18.97**	0.12*	1.67***	11.31***
Generation	5	19.15***	0.08***	0.46***	2.53***
Plants	159	2.53*	0.02 ns	0.09 *	0.56 ns
Error	164	1.812	0.01	0.07	0.52
Total	329	2.256	0.02	0.08	0.59
Cross 2- CKDHL120517/CKDHL0918					
Source of variation	**df**	**SI**	**WL**	**SD**	**LGB-A**
Rep	1	2.84ns	0.00 ns	0.39**	2.44*
Generation	5	10.24***	0.17***	0.60***	0.75*
Plants	159	6.83**	0.03***	0.10***	0.79***
Error	164	1.54	0.01	0.04	0.45
Total	329	2.62	0.02	0.06	0.52
*** significant at 0.1%, ** significant at 1% , * significant at 5% and ns non-significant					
SI- Selection index			SD- Seed damage (%)		
WL- Weight loss (%)			LGB-A- # of living insects		

Table 3.5. Selection index (SI) and reaction of the generations to *S. zeamais* and *P. truncatus*

	S. zeamais				*P. truncatus*		
	Cross 1- CKDHL120731/CKDHL0918				Cross 1- CKDHL120731/CKDHL0918		
Generation	SI± SE	STDV	Reaction		SI ± SE	STDEV	Reaction
P1	0.31 ± 0.15	0.16	Higly resistant		0.46 ± 0.25	0.81	Highly resistant
P2	2.2 ± 0.15	1.48	Highly Susceptible		2.45 ± 0.25	2.80	Highly Susceptible
F1	0.49 ± 0.15	0.28	Moderately Resistant		0.47 ± 0.25	0.61	Highly resistant
F2	1.19 ± 0.09	1.21	Highly Susceptible		1.16 ± 0.15	1.51	Highly Susceptible
BCP1	0.76 ± 0.11	0.53	Moderately Resistant		0.73 ± 0.18	1.03	Moderately Resistant
BCP2	1.03 ± 0.11	0.87	Moderately Susceptible		0.68 ± 0.18	0.96	Moderately Resistant
	Cross 2- CKDHL120517/CKDHL0918				Cross 2- CKDHL120517/CKDHL0918		
	SI± SE	STDV	Reaction		SI ± SE	STDV	Reaction
P1	0.38 ± 0.12	0.31	Higly resistant		0.59 ± 0.23	1.35	Highly resistant
P2	2.57 ± 0.12	0.87	Highly Susceptible		2.07 ± 0.23	1.11	Highly Susceptible
F1	0.69 ± 0.12	0.57	Moderately Resistant		0.72 ± 0.23	1.08	Moderately Resistant
F2	1.04 ± 0.07	0.91	Moderately Susceptible		0.74 ± 0.14	2.05	Moderately Susceptible
BCP1	0.88 ± 0.09	0.76	Moderately Susceptible		0.71 ± 0.16	1.05	Moderately Resistant
BCP2	0.98 ± 0.09	0.58	Moderately Susceptible		1.26 ± 0.16	1.46	Highly Susceptible

i) Generation means analysis

The means, standard errors and variances of the generation means of the two crosses (CKDHL120731×CKDHL120918 and CKDHL120517×CKDHL120918) for *S. zeamais and P. truncatus* resistance were computed (Table 3.6 and Table 3.7). These tables also include test of significance for variance of the segregating generations against non-segregating ones. In all parameters recorded P1 and P2 showed distinct contrasts in their resistance levels while the other generations showed continuous variations. The lowest weight loss (WL) and seed damage (SD) due to *S. zeamais* infestation in cross 1 was observed on P1, followed by F1 with 0.28% and 1.1% for WL and 1.16 and 1.71%, respectively while the highest for the same cross was observed on F2 and P2 with 3.78 and 8.4% WL and 4.84 and 9.17%, respectively. The same observation was made in *P. truncatus* infestation. Cross 2 performed slightly different where a lower WL and SD due to the LGB were observed for P1 and BCP1, while the worst performance was observed on F2 and P2. It was observed on both crosses that the WL and SD due to *P. truncatus* were higher compared to those of *S. zeamais* infestation. The numbers of living insects on both crosses did not reveal any specific trend.

The variance of most segregating generations (F2, BCP1 and BCP2) were significant ($p<0.05$) in both crosses for the WL and SD under artificial infestation of *S. zeamais* and *P. truncatus* except BCP1 for WL in cross 1 and SD in cross 2 and BCP2 for SD in cross 2 under artificial infestation of *P. truncatus* which were not significant. The variance of the number of living insects for all segregating generations did not show any trend and it was significant and non-significant in the same generation cross for the different insects. It was significant ($p<0.05$) for F2 and BCP2 in cross 1 and only F2 in cross 2 under *S. zeamais* infestation. Under *P. truncatus* infestation the scenario was different, the three segregating generations were significantly different ($p<0.05$) from the non-segregating ones in cross 1 and only BCP2 in cross 2. The significant variance components of the segregating generations revealed the genetic variation that exists in the generations derived from the cross between resistant and susceptible inbred lines in both crosses.

Table 3.6. Means and variances of the traits collected for *S. zeamais* resistance

		Cross 1- CKDHL120731/CKDHL0918					
		WL		SD		MW-A	
Generation	n	Mean ± SE	Variance	Mean ± SE	Variance	Mean ± SE	Variance
P1	30	0.28 ± 0.04a	0.06	1.16 ± 0.18a	1.02	7.43 ± 0.71a	15.29
P2	30	8.4 ± 1.47e	5.09	9.17 ± 1.06e	9.66	22.57 ± 2.48e	184.81
F1	30	1.1 ± 0.19d	1.04	1.71 ± 0.29d	2.44	9.53 ± 0.96d	27.64
F2	120	3.78 ± 0.56c	12.3***	4.84 ± 0.48c	18.04***	13.8 ± 1c	119.07*
BCP1	60	1.72 ± 0.23b	5.09***	3.08 ± 0.35b	7.31*	11.88 ± 1.04b	68.51ns
BCP2	60	2.8 ± 0.42b	10.48***	4.28 ± 0.55bc	17.9***	14.3 ± 1.4d	117.6*
	LSD	0.01562		0.01272		0.07387	
	CV	35.13		22.85		22.87	
	SE	0.05003		0.04074		0.2366	
		Cross 2- CKDHL120517/CKDHL0918					
		WL		SD		MW-A	
Generation	n	Mean ± SE	Variance	Mean ± SE	Variance	Mean ± SE	Variance
P1	30	0.56 ± 0.1c	0.33	1.23 ± 0.31a	2.94	6.77 ± 0.94a	26.46
P2	30	7.71 ± 0.46a	4.23	8.64 ± 0.85c	10.76	23.9 ± 1.56d	72.78
F1	30	1.49 ± 0.27ab	2.14	2.29 ± 0.46ab	6.43	9.37 ± 1.2ab	42.86
F2	120	2.26 ± 0.28b	5.29***	3.29 ± 0.33b	13.27***	11.07 ± 0.7bc	88.86**
BCP1	60	1.69 ± 0.25b	3.69**	3.06 ± 0.48b	13.71***	11.65 ± 1.05bc	65.72ns
BCP2	60	1.93 ± 0.2b	4.29***	3.21 ± 0.3b	6.48*	13.53 ± 1.13c	77.23ns
	LSD	0.01928		0.02536		0.1149	
	CV	44.36		46.95		34.65	
	SE	0.05811		0.07643		0.3464	

ii) The joint scaling test

The joint scaling test for the six generations of the two crosses (CKDHL120731×CKDHL120918 and CKDHL120517×CKDHL120918) infested with *S. zeamais* and *P. truncatus* is shown in Tables 3.8. Scaling parameter B was not significant for all traits in both crosses for the two insects. The scaling parameters A, C and D were significant ($p<0.05$) in most traits for both insects while all the scaling parameters A, B, C and D were non-significant for all traits in cross 2 for *P. truncatus*.

Table 3.7. The means and variances of the traits collected for *P. truncatus* resistance

		Cross 1- CKDHL120731/CKDHL0918					
		WL		SD		LGB-A	
Generation	n	Mean ± SE	Variance	Mean ± SE	Variance	Mean ± SE	Variance
P1	30	1.23 ± 0.4 a	4.85	5.58 ± 1.94 a	112.9	10.67 ± 4 a	479
P2	30	7.31 ± 1.66 c	10.63	28.36 ± 5.41 b	179.4	64.3 ± 15.38 c	395
F1	30	1.69 ± 0.33 ab	3.32	5.96 ± 1.39 a	58.3	9.5 ± 3.24 a	315
F2	120	4.16 ± 0.65 bc	13.92***	14.86 ± 1.84 a	275.5***	25.55 ± 3.07 bc	828***
BCP1	60	2.01 ± 0.34 ab	6.77ns	10.5 ± 1.99 a	237.7**	17.13 ± 3.24 abc	532**
BCP2	60	2.43 ± 0.47 ab	13.12***	8 ± 1.66 a	165.6ns	15.38 ± 3.37 ab	680***
	LSD	0.04039		0.0853		0.2384	
	CV	92.77		96.23		89.56	
	SE	0.1215		0.2571		0.7184	
		Cross 2- CKDHL120517/CKDHL0918					
		WL		SD		LGB-A	
Generation	n	Mean ± SE	Variance	Mean ± SE	Variance	Mean ± SE	Variance
P1	30	1.13 ± 0.45 a	6.02	5.04 ± 2.07 a	128.90	11.97 ± 5.19	808.7
P2	30	7.05 ± 0.54 c	8.81	22.78 ± 1.94 d	112.40	17.33 ± 5.26	829.6
F1	30	1.71 ± 0.44 ab	5.68	7.74 ± 2.03 abc	123.60	10.37 ± 3.22	311.1
F2	120	2.86 ± 0.64 a	17.16***	7.42 ± 1.47 ab	259.3***	15.62 ± 3.26	817.7ns
BCP1	60	1.61 ± 0.42 a	10.66*	6.23 ± 1.29 abc	99.9ns	10.47 ± 2.28	311.1ns
BCP2	60	2.88 ± 0.51 b	15.49***	10.67 ± 2.2 ac	289.3***	18.47 ± 3.22	1121.2**
	LSD	0.03383		0.06914		0.222	
	CV	90.67		98.89		114.49	
	SE	0.102		0.2084		0.669	

Table 3.8. Estimates of scaling test for the two crosses infested with *S. zeamais* and *P. truncatus*

S. zeamais						*P. truncatus*					
		A	B	C	D			A	B	C	D
WL	Cross 1	2.088 **	-3.876	5.358*	3.043*	WL	Cross 1	1.104	-4.149	6.397*	3.875**
	Cross 2	1.337*	-5.34	-0.72	0.90		Cross 2	0.39	-2.994	1.56	1.228
SD	Cross 1	3.285**	-2.325	7.336**	2.332*	SD	Cross 1	9.46*	-18.32	19.54*	11.22*
	Cross 2	2.614*	-4.50	1.01	0.30		Cross 2	-0.328	-9.183	-5.871	-2.049
MW-A	Cross 1	6.8**	-3.50	15.67**	1.42	LGB-A	Cross 1	14.09	-43.04	17.73	18.59*
	Cross 2	6.692**	9.00	12.58	4.34		Cross 2	-1.4	9.24	22.81	2.3

iii) Analysis of variance of genetic effects

The analysis of variance of the genetic effects for the crosses showing at least one significant (p> 0.05) scaling test parameter are presented in Table 3.9 for *S. zeamais* and Table 3.10 for *P. truncatus*. The genetic estimates of digenic interaction model were highly significant (p<0.001) for all the parameters collected. Different genetic parameters performed differently in different crosses for resistance to the post-harvest insects. However, the additive effects and pooled dominance × dominance effects did not have significant effects for both crosses in all traits under *S. zeamais* infestation while the interaction effects of additive × dominance had significance effects (P< 0.05). Dominance effects were significant (P< 0.05) for weight loss and seed damage in cross 1 and only for weight loss in cross 2 for *S. zeamais* resistance.

Table 3.9. Estimates of gene effects of 2 crosses infested with *S. zeamais*

	WL		SD		MW-A	
Parameter	**Cross 1**	**Cross 2**	**Cross 1**	**Cross 2**	**Cross 1**	**Cross 2**
Model	0.18***	0.16***	0.20***	0.13***	1.24***	1.63***
m ± SE	4.24 ± 0.5***	1.65 ± 0.24***	6.1 ± 0.43***	2.62 ± 0.35***	16.52 ± 1.01***	9.47 ± 0.8***
[a] ± SE	-1.08 ± 0.86ns	-0.24 ± 0.4ns	-1.2 ± 0.74ns	-0.15 ± 0.6ns	-2.42 ± 11.73ns	-1.8 ± 1.38ns
[d] ± SE	-9.37 ± 2.64**	-4.43 ± 1.25***	-8.12 ± 2.3***	-3.25 ± 1.64ns	-8.3 ± 5.34ns	-1.33 ± 4.24ns
[aa] ± SE	-6.09 ± 2.42*	-1.79 ± 1.15ns	-4.67 ± 2.11*	-0.61 ± 1.69ns	2.83 ± 4.9ns	4.63 ± 3.89ns
[ad] ± SE	2.98 ± 1.05**	3.34 ± 0.5***	2.81 ± 0.91**	3.56 ± 0.73***	5.15 ± 2.12*	6.77 ± 1.69***
[dd] ± SE	7.88 ± 4.36 ns	5.79 ± 2.06ns	3.71 ± 3.8ns	2.49 ± 3.05ns	-0.47 ± 8.83ns	-5.43 ± 7.02ns
*** significant at 0.1%, ** significant at 1% , * significant at 5% and ns non-significant						
m - mean						
[a]- additive gene effects				[ad]- additive x dominance gene effects		
[d]- dominance gene effects				[dd]- dominance x dominance gene effects		
[aa]- additive x additive gene effects				SE- standard error		

For *P. truncatus* resistance, cross 1 showed significant (p< 0.05) dominance, interaction of additive × dominance and pooled additive × additive in all traits while additive effects were non-significant in all traits. The pooled dominance × dominance effects were significant at 5% only for the weight loss.

On simple additive-dominance model, applied only for cross 2, both additive and dominance parameters were significant (p< 0.05). Additive effects were significant for all parameters while dominance effects were non-significant only for the number of living larger grain borer insects.

Table 3.10. Estimates of gene effects of two crosses infested with *P. truncatus*

	WL		SD		LBG-A	
Parameter	Cross 1	Cross 2	Cross 1	Cross 2	Cross 1	Cross 2
Model	0.09***	-	0.67***	-	4.00***	-
m ± SE	4.5 ± 0.58***	3.87 ± 0.32**	18.8 ± 1.85***	13 ± 1.25**	32.24 ± 3.87***	16.16 ± 2.89**
[a] ± SE	-0.41 ± 1.00ns	-2.59 ± 0.31**	2.5 ± 3.17ns	-7.9 ± 1.21**	1.75 ± 6.63ns	-5.12 ± 2.66*
[d] ± SE	-10.27 ± 3.08***	-2.38 ± 0.55**	-33.46 ± 9.77***	-7.27 ± 2.38*	-65.15 ± 20.43**	-4.67 ± 4.87ns
[aa] ± SE	-7.69 ± 2.82**	--	-22.45 ± 8.97*	--	-37.17 ± 18.75*	--
[ad] ± SE	2.63 ± 1.22*	--	13.88 ± 3.88***	--	28.57 ± 8.12***	--
[dd] ± SE	10.74 ± 5.09*	--	31.13 ± 16.17ns	--	66.10 ± 33.79ns	--
Epistasis type	Duplicate decreasers	n/a	-	n/a	-	n/a
*** significant at 0.1%, ** significant at 1% , * significant at 5% and ns non-significant						
m - mean						
[d] = [a]- additive gene effects				[J] = [ad]- additive x dominance gene effects		
[h] = [d]- dominance gene effects				[L] = [dd]- dominance x dominance gene effects		
[i] = [aa]- additive x additive gene effects				SE- standard error		

iv) Variance components and heritability

The variance components, heritability, dominance and potence ratios of the two crosses evaluated under artificial infestation of *S. zeamais* and *P. truncatus* were estimated (Tables 3.11 and Table 3.12). The number of living insects for both crosses and both insects showed higher variances compared to other traits and the variances under *P. truncatus* infestation were highest compared to the variances under *S. zeamais* infestation for the same traits.

High broad sense heritability was observed in cross 1, for weight loss (85.3% and 60.27%) and seed damage (78.44% and 62.90%) for the *S. zeamais* and *P. truncatus*, respectively. All other heritability estimates (broad- and narrow-sense) were moderate for all traits, in the two crosses for the two insects except weight loss in the cross 2 under larger borer infestation which showed high broad sense heritability (61.84%). The dominance ratio ranged from 0.3 to 0.78 in different traits under *S. zeamais* infestation and from 0.19 to 0.77 under *P. truncatus* artificial infestation. Under *S. zeamais* infestation, the lowest potence ratio was observed on the number of living insects (0.74) and highest on the seed damage (22.33) both on the cross 2 while under *P. truncatus* infestation, the lowest and highest were observed on the number of living insects in cross 1 and 2, respectively.

Table 3.11. Variance components, heritability, dominance and potence ratios under *S. zeamais* infestation

		Cross 1			Cross 2		
Iten	**Genetic parameters**	**WL**	**SD**	**MW-A**	**WL**	**SD**	**MW-A**
Variance components	σ^2_E	1.81	3.89	63.845	2.21	6.64	46.24
	σ^2_A	9.03	10.87	52.03	2.60	6.35	34.77
	σ^2_D	1.46	3.28	3.195	0.48	0.28	7.85
	σ^2_{AD}	2.70	5.30	24.545	0.30	3.62	5.76
	σ^2_G	10.49	14.15	55.225	3.08	6.63	42.62
	σ^2_P	12.30	18.04	119.07	5.29	13.27	88.86
Heritability	H^2	85.30	78.44	46.38	58.22	49.96	47.96
	h^2	73.41	60.25	43.70	49.15	47.85	39.13
Dominance Ratio	DR	0.57	0.78	0.35	0.61	0.30	0.67
Potence ratio	[d]/[a]	1.37	6.67	3.43	18.46	22.33	0.74

σ^2_E - Environment variance; σ^2_{AD} - Additive x Dominance variance

σ^2_A - Additive variance; σ^2_G - Genetic variance

σ^2_D - Dominance varience; σ^2_P - Phenotypic variance

Table 3.12. Variance components, heritability, dominance and potence rations under *P. truncatus* infestation

		Cross 1			Cross 2		
Iten	**Genetic parameters**	**WL**	**SD**	**LGB-A**	**WL**	**SD**	**LGB-A**
Variance components	σ^2_E	5.53	102.23	376.00	6.55	122.13	565.13
	σ^2_A	7.95	147.70	444.00	8.17	129.40	203.10
	σ^2_D	0.44	25.58	8.00	2.44	7.78	49.48
	σ^2_{AD}	3.18	36.05	74.00	2.42	94.70	405.05
	σ^2_G	8.39	173.28	452.00	10.61	137.18	252.58
	σ^2_P	13.92	275.51	828.00	17.16	259.30	817.70
Heritability	H^2	60.27	62.90	54.59	61.84	52.90	30.89
	h^2	57.11	53.61	53.62	47.61	49.90	24.84
Dominance Ratio	DR	0.33	0.59	0.19	0.77	0.35	0.70
Potence ration	[d]/[a]	25.05	13.38	37.23	0.92	0.92	0.91

σ^2_E - Environment variance; σ^2_{AD} - Additive x Dominance variance

σ^2_A - Additive variance; σ^2_G - Genetic variance

σ^2_D - Dominance varience; σ^2_P - Phenotypic variance

3.4. Discussion

This study estimated the gene effects controlling resistance and heritability of the main maize storage pests, maize weevil (*Sitophilus zeamais* Motsch) and larger grain borer

(*Prostephanus truncatus* (Horn) (*Coleoptera*: *Bostrichidae*) in two crosses. Mechanisms of resistance for storage pests vary among maize germplasm and resistance cannot focus on a single trait (Mwololo *et al.*, 2013). The present study based the analysis on three traits, including the percentage of weight loss and damaged grain due to *S. zeamais* and *P. truncatus* infestation and the total number of living insects. These traits were among the most used in several studies on resistance to *S. zeamais* and *P. truncatus* to classify resistance among maize genotypes (Dobie, 1977; Dhliwayo *et al.*, 2005; Siwale *et al.*, 2009; Tefera *et al.*, 2011; Mwololo *et al.*, 2013; Nhamucho *et al.,* 2014).

The significant difference observed in the analysis of variance among the generations for all investigated traits of both crosses in the two insects, *S. zeamais* and *P. truncatus*, indicates the presence of genetic variation. Therefore, generation means analysis method could be used to estimate the genetic parameters. The large difference in reaction for *S. zeamais* and *P. truncatus* resistance for the different traits including the selection index between parents P1 and P2, indicates that P1 and P2 used in the crosses were divergent for the studied characters. P1 was resistant and P2 was susceptible, which is a requirement for generation mean analysis (Mather, 1949, Mather and Jinks, 1982). The average reaction of the different generations varied from trait to trait, and cross to cross under the infestation of the two insects. However, it was observed that the reaction of F1 generation varied from one cross to other from moderately to highly resistant for the two insects in different traits, F2 varied from moderately to highly susceptible in both crosses for the two insects while the backcross generation did not show any trend between the crosses for the two insects.

The significance of any one of the scaling test parameters indicates the presence of non-allelic interaction, meaning that the simple additive-dominance model was inappropriate to elucidate most of genetic variation observed on the expression of the traits. This suggest that the inheritance of these parameters are complex and polygenic (Warnock *et al.*, 1998), hence a six parameter model (digenic interaction model) was necessary to clarify the genetic variation, since there was contribution of epistatic effects.

On the other hand, the non-significance of all scales observed in cross 2 under infestation of *P. truncatus* suggests that the simple additive-dominance model was adequate to estimate the genetic components of variance. From the results, it was evident that additive and dominant gene effects were highly significant for all parameters measured, suggesting

that both were essential for the inheritance. This also suggests, that to improve these traits, selection could be effective when practiced in F2 generation.

Similar results, on the significance of additive and non-additive gene effects on controlling most of the *S. zeamais* resistance traits were reported by Kang *et al.* (1995), Dhliwayo *et al.* (2005) and Gafishi *et al.* (2012) but there is little information in literature on gene action responsible for resistance *P. truncatus.* Various studies done on resistance to *P. truncatus* were mostly for phenotypic evaluation (Tefera *et al.*, 2011; Mwololo *et al.*, 2013; Nhamucho *et al.*, 2014). However, Matewele (2014) in his study showed the importance of additive gene effects on resistance to larger grain borer on grain damage. The estimated mean effects [m], was significant for all studied traits in all crosses for the two insects, indicating that these traits were quantitatively inherited. The mean [m] reflects the contribution due to the locus effects, interaction of the fixed loci plus the over-all mean.

The additive [a] gene effect and the pooled dominance × dominance [dd] did not show any influence on the inheritance of the collected traits for the two crosses under infestation of the two insect pests, except cross 1 for weight loss under larger grain borer. These results are in contrast with those of Kang *et al.* (1995); Derera *et al.* (2001); Kim and Kossou (2003). However, Dhliwayo *et al.* (2005) found that the variance due to SCA effects (dominance effects) was more important than the GCA effects (additive effects) for F1 maize weevil insects emerged. Sodedji *et al.* (2018) reported that non-additive genetic effects were relatively more important for F1 maize weevil insects emerged and index of susceptibility, indicating the importance of the contribution of the specific combining ability (dominance effects) in the responses of the maize hybrids against *S. zeamais.*

No complementary interaction was observed in the genetic control of the studied traits on the two crosses for the two post-harvest insects, since none of the signs of [d] were similar to the [dd]. The dominance [d], the pooled dominance × dominance [dd] and the interaction additive × dominance [ad], which is referred to as non-additive genetic variance were the most important for cross 1 for weight loss and seed damage while dominance [d] and the interaction additive × dominance [ad] were the most important gene effects for cross 2 for the same traits for both insect pests. In both crosses, the number of the living insects were mainly controlled by the interaction additive × dominance [ad]. This suggests that, these were mostly controlled by dominance as main effect with non-allelic interactions as epistatic effects.

Significant additive, non-additive and maternal effects determining *S. zeamais* resistance in maize grain have been reported by Schoonhoven *et al.* (1975); Widstrom *et al.* (1975); Widstrom *et al.* (1983); Tipping *et al.* (1989) and Derera *et al.* (2001). According to Widstrom *et al.* (1975) dominance effects were important for seed resistance to weevils in maize sources segregating for endosperm, while Derera *et al.* (2001) reported additive effects as more important for the female parent in a cross. For rapid crop improvement, in a trait which shows significant epistatic effects, adoption of recurrent selection to handle desirable segregates through inter-mating in early segregations would be effective (Dong *et al.*, 2006; El-Beially and Mohamad, 2008; El-Refaey and El-Razek, 2013).

The negative values observed in most cases either with main effects [a], [d] and non-allelic interactions, [aa], [ad] and [dd], may suggest that the alleles responsible for lower trait values were dominant over the alleles controlling the high trait value. However, the direction of additive and dominance effects was the same where the signs were similar. This was observed for all traits in the two crosses except for seed damage and number of living insects in cross 1 under *P. truncatus* infestation. It was also observed that dominance effects were much higher than additive effects and this might indicate that dominance gene effects play a bigger role in governing the genetic variation of most of studied traits. When additive effects are greater over non-additive effects, it suggests that selection in early segregating generations can be effective, whereas if non-additive portion is greater over additive component, the intensive selection can be effective during later generations (Japtap, 1986).

The observed results are in line with that reported by Dhillon and Singh (1980), Lin and Zhao (1988), Singh and Narayanan (1993), Mert *et al.* (2003) and Esmail (2007). Overall, for the two crosses, it was detected that the signs of dominance (d) and dominance × dominance (dd) gene effects were opposite for most of the parameters except for number of living insects under *S. zeamais* infestation, indicating duplicate type of non-allelic interaction. However due to insignificance of [dd] of most traits, the duplicate epistasis was only observed on weight loss in cross 1 under *P. truncatus* infestation. Since [d] was negative and [dd] positive, the observed duplicate epistasis is classified as duplicate epistasis between dominant decreasers. Furthermore, the opposite directions of dominance and dominance × dominance effects resulted in lower overall dominance.

The positive or negative effect on additive × additive gene effects indicate association and dispersion of alleles in parents (Kearsey and Pooni, 1998). The negative and significant additive × additive gene effects observed in cross 1 for weight loss and seed damage under artificial infestation of both insects revealed allele dispersion in parents for resistance indicating the potential that the resistance can be fixed and exploited in later generations.

Narrow-sense heritability estimates were generally lower, suggesting the presence of non-additive gene action (Dhliwayo *et al.*, 2005; El-Refaey and El-Razek, 2013). This was also observed using the dominance ratio, which showed partial dominance in both crosses for the two insect pests (Checa *et al.*, 2006; Kearsey and Pooni, 1998).

The potence ratio showed the presence of heterosis for cross 1 for both insects and cross 2 only for the weight loss and seed damage under *S. zeamais* infestation. Weight loss and seed damage presented high heritability estimates, indicating that those traits can be used in selection of the genotypes to improved *S. zeamais* and *P. truncatus* resistance, as observed by other researchers including Derera *et al.* (2001), Nhamucho *et al.* (2014, 2017) and Matewele (2014).

3.5. Conclusion

The genetic effects on the different traits used to assess resistance to *S. zeamais* and *P. truncatus* in maize grains mainly depended on the cross. Additive and non-additive, including an epistasis gene effects play a role in the inheritance of resistance to both insects in the selected maize genotypes. This was further confirmed by the heritability estimates, where moderate narrow-sense heritability estimates were observed, suggesting involvement of additive and non-additive gene effects in the expression of resistance to both insects.

References

Allard, R. W. 1960. Principles of plant breeding. John Willey and Sons Inc, New York.

Bergvinson, D., Vasal, S., Singh, N., Panwar, V. and Sekhar, J. 2002. Advances in Conventional Breeding for Insect Resistance in Tropical Maize. 8th Asian Regional Maize Workshop. Bangkok, Thailand.

Boxall, R. 1986. A critical review of the methodology for assessing farm level grain losses after harvest. Report of the TDR G191.

Checa, O., Ceballos, H. and Blair, M. 2006. Generation Means Analysis of Climbing Ability in Common Bean (*Phaseolus vulgaris* L.). Journal of Heredity 97: 456-465.

CIMMYT. 1985. Managing trials and reporting data for CIMMYT's International maize testing program. , CIMMYT-Int., Mexico DF. p. 20.

Cugala, D., Sidumo, A., Santos, L. and Givá, N. 2007a. Uso do método de controlo biológico contra a broca maior do grão do milho armazenado, *Prostephanus truncatus* (horn) (*Coleoptera*: *Bostrichidae*) nos celeiros das famílias rurais em Moçambique. Moçambique- Maputo: Universidade Eduardo Mondlane.

Cugala, D., Sidumo, A., Santos, L., Mariquele, B., Cumba, V. and Bulha, M. 2007b. Assessment of status, distribuition and weight lost due to *Prostephanus trancutus* (Horn) (*Coleoptera*: *Bostrichidae*) in Mozambique. African Crop Science Journal 8: 975-979.

Derera, J., Pixley, K. and Giga, D. 2001. Resistance of maize to the maize weevil. I. Antibiosis. African Crop Science Journal 9: 431- 440

Derera, J., Pixley, K. and Giga, D. 2010. Appraisal of protocol for the rapid screening of maize genotypes for maize weevil resistance. African Entomology 18: 8-16.

Dhillon, S. S. and Singh, T. H. 1980. Genetic control of some quantitative characters in upland cotton (*Gossypium hirsutum* L.). The Journal of Agricultural Science 94: 539-543.

Dhliwayo, T., Pixley, K. and Kazembe, V. 2005. Combining Ability for Resistance to Maize Weevil among 14 Southern African Maize Inbred Lines. Crop Science Society of America, 45: 662-667.

Dobie, P. 1977. The contribution of the tropical stored products center to the study of insect resistance in stored maize. Tropical Stored Products Information 34: 7-22.

Dong, F., Wu, Z., Jin, Z. and Huang, Y. 2006. Heterosis for yield and some physiological traits in hybrid cotton Cikangza. Euphytica 151: 17-77.

El-Beially, I. and Mohamad, G. 2008. Estimates of genetic parameters using six populations in Egyptian cotton (*Gossypium barbadense* L.). Al-Azher Journal of Agriculture Research 4: 51 - 64.

El-Refaey, R. A. and El-Razek, U. A. 2013. Generation Mean Analysis for Yield, its Components and Quality Characteristics in Four Crosses of Egyptian Cotton (*Gossypium barbadense* L.). Asian Journal of Crop Science 5: 153 - 166.

Esmail, R. 2007. Genetic analysis of yield and its contribuiting traits in two intra-specific cotton crosses. Journal of Applied Sciences Research 3: 2075 - 2080.

Fato, P., Chaúque, P., Ecole, C. and Cugala, D. 2008. The Status of Development of Maize Resistant to Field and Storage Pests in Mozambique. In: Mugo, S., Gethi, J., Ouma, J., Murenga, G., Mulaa, M., Likhayo, P., Gichuki, V., Kega, V., Degroote, H. & Chavangi, A. (eds.) Book of abstract of the Insect Resistant Maize for Africa (IRMA) .(2008) " Consolidating Experiences from IRMA I and II: Achievements, Prospects and Lessons", IRMA project ect End-of-Phase II Conference, 28-30 October. Nairobi- Kenya KARI and CIMMYT.

Gafishi, K. M., Karungi, J., Asea, G. and Gibson, P. 2012. Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to the maize weevil. African Crop Science Journal 20: 99-104.

Gamble, E. E. 1962. Gene effects in corn (*Zea mays* L.) II. Relative importance of gene effects for plant height and certain component attributes of yield. Canadian Journal of Plant Science 42: 349-358.

Gomez, K. and Gomez, A. 1984. Statistical producedures for agricultural research, J. Wiley and sons, New York, USA.

Hallauer, A. R., Carena, M. J. and Filho, J. B. 2010. Quantitative genetics in maize breeding New York ; London, Springer.

Hayman, B. 1958. The separation of epistatic from additive and dominance variation in generation means. Heredity 12: 371-390.

Hayman, B. I. and Mather, K. 1955. The description of genetic interaction in continuous variation. Biometrics 11: 69-82.

INE. 2003. Censo Agro-pecuário 1999-2000. Resultados temáticos: Direcção Nacional de Estatísticas Sectoriais e de Empresas, Maputo, Moçambique.

Japtap, D. R. 1986. Combining ability in uploand cotton. Indian Journal of Science 56: 833 - 840.

Jinks, J. and Jones, R. 1958. Estimation of the components of heterosis. Genetics 43: 223-234.

Kang, M., Zhang, Y. and Magari, R. 1995. Combining ability for maize weevil preference of maize grain. Crop Science Society of America 35: 1556-1559.

Kang, M. S. 1994. Applied quantitative genetics: Diallel analysis, Louisiana State University, Baton Rouge, pp 33-77, Department of Agronomy.

Kankolongo, M., Hell, K. and Nawa, I. 2009. Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. Journal of Food Science and Agriculture 89: 1366 – 1375.

Kearsey, M. J. and Pooni, H. S. 1998. The Genetical Analysis of Quantitative Traits Abingdon, Oxon, OX14 4RN, New York NY10016, Springer: Taylor & Francis Group.

Kim, S. and Kossou, D. 2003. Responses and genetics of maize germplasm resistant to the maize weevil *Sitophilus zeamais* Motschulsky in West Africa. Journal of Stored products Research 39: 489- 505.

Lin, Y. and Zhao, L. 1988. Estimation of genetic effects on the main fibre quality characteristics in upland cotton. Acta Genetica Sinica 15: 401- 408

Mariquele, B. 2006. Ocorrência da broca maior do grão *Prostephanus truncatus*: Horn (*Coleoptera*: *Bostrichidae*) em Moçambique: O caso do distrito de Manica. Lincenciatura Projecto final, Universidade Eduardo Mondlane.

Matewele, M. 2014. Diversity Analysis and Breeding for Maize Weevil (*Sitophilus zeamais* Motschulsky) and Larger Grain Borer (*Prostephanus truncatus* Horn) Resistance in Productive Maize Germplasm in Malawi. Ph.D Thesis., University of KwaZulu-Natal, Pietermaritzburg, SA.

Mather, K. 1949. Biometrical Genetics: The study of Continuous Variation London, Dover Publication, Inc. New York. Ltd.

Mather, K. and Jinks, J. L. 1971. The study of continuos variation, London. UK., Chapman and Hall Ltd.

Mather, K. and Jinks, J. L. 1982. Biometrical Genetics: The study of Continuos variation 3rd edition, London. UK., Chapman and Hall Ltd.

Mert, M., Gencer, O., Akscan, Y. and Boyac, K. 2003. Inheritance of yield and yield components in cotton (*Gossypium hirsutum* L.). Turkish Journal of Field Crops 8: 62 - 67.

Mwololo, J., Mugo, S., Tefera, T. and Munyiri, S. 2013. Evaluation of traits of resistance to postharvest insect pests in tropical maize. Internaltional Journal of Agriculture and Crop Science 6: 926–933.

Nhamucho, E., Mugo, S., Gohole, L., Tefera, T., Kinyua, M. and Mulima, E. 2017. Resistance of selected Mozambican local and improved maize genotypes to maize weevil, *Sitophilus zeamais* (Motschulsky). Journal of Stored Products Research 73: 115-124.

Nhamucho, E., Mugo, S., Kinyua, M., Gohole, L., Tefera, T. and Mulima, E. 2014. Antibiosis mechanism of resistance to larger grain borer *Prostephanus truncatus* (Horn), (*Coleoptera*: *Bostrichidae*) in Maize. Journal of Entomology 11: 248 - 260.

Robinson, H., R. , Comstock, E. and Harvey, P. 1949. Estimates of heritability and the degree of dominance in corn. Agronomy Journal 41: 353-359.

Schoonhoven, A., Horber, E., Wassom, C. and Mills, R. 1975. Selection for resistance to the maize weevil in kernels of maize. Euphytica 24: 639-644.

Singh, P. and Narayanan, S. 1993. Biometrical Techniques in Plant breeding, New Delhi, India, 182pp, Kalyani Publishers.

Siwale, J., Macrobert, J. and Lungu, D. 2009. Comparative resistance of improved maize genotypes and landraces to maize weevil. African Crop Science Journal 17: 1-16.

Sodedji, F., Kwemoi, D., Kasozi, C., Asea, G. and Kyamanywa, S. 2018. Genetic analysis for resistance to *Sitophilus zeamais* (Motschulsky) among provitamin-A maize germplasm. Maydica electronic publication 1 - 8.

Tefera, T., Mugo, S., Likhayo, P. and Beyene, Y. 2011. Resistance of three-way cross experimental maize hybrids to post-harvest insect pests, the large grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*). International Journal of Tropical Insect Science 31: 3-12.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2010. Mass Rearing of Stem Borers, Maize Weevil and Larger Grain Borer Insect Pests of Maize, CIMMYT: Nairobi- Kenya, 36pp.

Tipping, P. W., Cornelius, P. L. and Legg, D. E. 1989. Inheritance of resistance in whole kernel maize to oviposition by the maize weevil (*Coleoptera*: Curculionidae). Journal of Economic Entomology 82: 1466-1469.

Warner, J. N. 1952. A method for estimating heritability. Agronomy Journal 44: 427-430.

Warnock, D. F., Davis, D. W. and Gengera, G. R. 1998. Inheritance of ear resistance to European corn borer in apache sweet corn. Crop Science 38: 1451 - 1457.

Widstrom, N. W., Hanson, W. D. and Redlinger, L. M. 1975. Inheritance of maize weevil resistance in maize. Crop Science 15: 467- 470.

Widstrom, N. W., Mcmillian, W. W., Redlinger, L. M. and Wiser, W. J. 1983. Dent corn inbred sources of resistance to the maize weevil (*Coleoptera*: Curculionidae). Journal of Economic Entomology 76: 31-33.

4. CHAPTER FOUR

Genetic analyses and potential of combining drought tolerance and maize lethal necrosis resistance in tropical maize germplasm

Abstract

Drought and maize lethal necrosis disease (MLND) are among the most important stresses impacting maize production in sub-Saharan Africa (SSA), although maize lethal necrosis has not been reported in Mozambique as yet. Host plant resistance and tolerance have been achieved in maize hybrids and inbred lines for each of these stresses individually, but no combined drought tolerance and maize lethal necrosis resistance has been reported. This study aimed at combining resistance to the two stresses in hybrids developed from a half-diallel involving eight parents and to assess gene action controlling maize grain yield and other agronomic traits. Hybrids were evaluated in Kenya during seasons 2017A and 2018A across six locations under optimal conditions and over two locations under MLN infestation, and during season 2017B and 2018B under managed drought over two locations, resulting in ten environments. There were highly significant genotype and genotype × environment interaction effects ($p \leq 0.01$) for grain yield under stress and optimum conditions. Hybrids differed significantly ($p \leq 0.01$) for MLND resistance and drought tolerance traits including MLN scores, senescence, days to anthesis and anthesis-silking interval. The yield reduction due to MLND was 93% of the optimum (6.04 t/ha), while reduction due to drought was 67%. Genetic analysis detected highly significant mean squares ($p < 0.01$) due to general combining ability (GCA) and specific combining ability (SCA) for most of the recorded traits, including grain yield under all environments, suggesting the importance of both additive and non-additive gene effects. However, additive gene action was generally predominant across all research conditions. Hybrids tolerant to drought and resistant to MLND were identified. The results suggest that it is possible to improve tropical maize for combined drought and MLN tolerance and it can be faster when evaluation is conducted under combined drought and MLN conditions.

4.1. Introduction

Maize (*Zea mays* L.) is the major food crop for the majority of households in sub-Saharan Africa (FAOSTAT, 2019). However, its production is below the demand because of yield losses caused by different field stresses, including drought and maize lethal necrosis (Meseka *et al.*, 2011; Mahuku *et al.*, 2015a). Drought and maize lethal necrosis (MLN) can occur simultaneously during the main cropping season and sometimes cause complete maize crop failure in many tropical and subtropical environments. Drought occurs when seasonal rainfall stops during the growing period of the crops or when its distribution is erratic in the same period (Mir *et al.*, 2012). Drought stress can occur at all stages of plant growth. In maize its negative effects are severe when it occurs at flowering and grain-filling periods (Cakir, 2004; Zaidi *et al.*, 2004; Toker *et al.*, 2007; Hao *et al.*, 2011; Mir *et al.*, 2012). During flowering, drought disturbs the synchronization between pollen shed and silking, which is the major reason for yield reduction (Grime and Campbell, 1991), while when it occurs at grain filling stage, it reduces endoreduplication, endosperm cell division and other actions of the cell related to storage-product synthesis, thus reducing grain weight (Bänziger *et al.*, 2002).

Grain yield loss of 17 to 90% has been reported when drought occurs in these critical stages (Edmeades *et al.*, 1992; NeSmith and Ritchie, 1992; Menkir and Akintunde, 2001; Campos *et al.*, 2006). For example, a maize production loss of 60% was reported in southern Africa, during the severe drought of 1991-92 (Rosen and Scott, 1992) and an estimation of 80% of the total maize crop grown in developing countries is lost due to drought (Bolaños and Edmeades, 1993). Small-scale farmers mostly with limited access to irrigation facilities grow drought susceptible hybrids and in southern Africa, dryland production represents around 95% (Banziger and Diallo, 2001). The occurrence of drought is unpredictable over space and time (Campos *et al.*, 2004) and it its negative effects are difficult to minimize without irrigation. The low production of maize, being a staple food, has negative effects on regional and international economies, reflecting low annual gross domestic product throughout the drought years (Richardson, 2005).

Maize lethal necrosis is a devastating new disease in Africa, where it was first observed in Kenya in September 2011, with incidences recorded from 2012 (Wangai *et al.*, 2012a; Wangai *et al.*, 2012b). The MLN disease was earlier reported in Kansas, USA in 1977, where it was known as corn lethal necrosis (CLN) disease (Niblett and Claflin, 1978). Thereafter, it was observed in Nebraska, USA (Doupnik Jr, 1979), Hawaii, USA (Jiang *et*

al., 1992) and China (Xie *et al.*, 2011), Peru, Argentina, Mexico, Brazil and Thailand (Flett and Mashingaidze, 2016). Since then, the disease has been observed in Uganda and Tanzania (Wangai *et al.*, 2012b), Democratic Republic of Congo (DRC) (Lukanda *et al.*, 2014) and Rwanda (Adams *et al.*, 2014). Most recently, MLN related symptoms have been observed in Ethiopia and South Sudan (Mahuku *et al.*, 2015b, Flett and Mashingaidze, 2016).

Maize lethal necrosis and corn lethal necrosis are both caused by dual infection with synergistic interaction on maize leaf plants by two viruses, namely *maize chlorotic mottle virus* (MCMV) (*Machlomovirus: Tombusviridae*) in co-infection with any cereal viruses from the Potyviridae group, including *maize dwarf mosaic virus* (MDMV), *sugarcane mosaic virus* (SCMV) (*Potyvirus: Potyviridae*) or *wheat streak mosaic virus* (WSMV). In China, MCMV was observed in combination with SCMV (Xie *et al.*, 2011). In all African countries where the presence of MLN has been reported to date, MCMV has been associated with SCMV (Louie, 1980, Wangai *et al.*, 2012a). MCMV is transmitted mostly by thrips (*Frankliniellawilliamsi* Hood) and beetles (Cabanas *et al.*, 2013) but some transmission and spread through seeds from infected plants has been reported at a rate of 0.0003% which might transform to a high number of infected plants (Jensen *et al.*, 1991). The SCMV, on the other hand, is transmitted by aphids (Brandes, 1920). MCMV is a threat on its own causing huge yield loss even in the non-appearance of other viruses.

The MLN disease is a threat to food security in Africa. Observations from the field made in Kenya indicated that nearly all commercial maize varieties were affected by MLN in 2012, causing yield losses from 30 to 100% depending on the crop stage when the disease came in. Approximately 77,000 ha of maize was affected, translating into a projected yield loss of 126 MMT equivalent of USD 52 million (Wangai *et al.*, 2012b). Being a new disease, scanty information on the genetics of host resistance is available. Beyene *et al.* (2017) reported that combining ability estimates for MLN resistance suggested a predominance of additive over non-additive gene action. This implies that recurrent selection can be employed as a breeding strategy. Since both drought and MLN are devastating stresses in maize-growing areas and can occur simultaneously, high yielding varieties which are drought-tolerant and MLN resistant are thus required.

In efforts to address this challenge, this study was carried out to: i) determine the gene action controlling various traits of maize in the hybrids developed from a half diallel mating

scheme involving 8 inbred lines with various levels of resistance/tolerance to the two stresses and ii) identify maize hybrids with multiple resistance/tolerance to both drought and MLN without compromising the yield. The findings can be used to devise the strategy for breeding multiple stress tolerance/resistance, and thus increase food security and improve the livelihoods of the small-holder farmers in Africa.

4.2. Materials and Methods

4.2.1. Germplasm

Eight tropical maize inbred lines from the International Maize and Wheat Improvement Center (CIMMYT) developed from different projects (Table 4.1) were crossed in a half diallel combination.

Table 4.1. List of the inbred lines used as parents in the diallel-cross and their attributes.

Designation	Inbred line name	Attributes	Origin
Parent 1 (P1)	CKSBL10027	Stem borer resistant	CIMMYT- Kenya
Parent 2 (P2)	CKSBL10011	Stem borer resistant	CIMMYT- Kenya
Parent 3 (P3)	CKDHL120172	Drought tolerant	CIMMYT- Kenya
Parent 4 (P4)	CKDHL121230	Drought tolerant	CIMMYT- Kenya
Parent 5 (P5)	CML395	Resistant to MSV	CIMMYT- Kenya
Parent 6 (P6)	CML442	Drought and Low N tolerant	CIMMYT- Kenya
Parent 7 (P7)	CKDHL120918	Maize lethal Necrosis (MLN) resistant	CIMMYT- Kenya
Parent 8 (P8)	CML494	Maize lethal Necrosis (MLN) resistant	CIMMYT- Kenya

4.2.2. Testing environments and field management

The nursery with the eight inbred lines was planted in June 2016, during the 2016B season at CIMMYT´s new site at Kenya Agricultural and Livestock Research Organization (KALRO) in Kiboko (2°12' 50.24" S, 37°43' 30.11" E, 945 masl). A total of 28 F1 crosses were generated. The procedure used to manage the crosses and pollination is similar to that described in chapter 3, section 3.2.1.

Table 4.2. Geographical locations, agro-climatic and soil description of the sites used for hybrid evaluation

Site	Latitude	Longitute	Elevation m asl	Rain fall (mm)	Temperature (°C) min	max	Soil texture
KALRO- Kiboko	2°15'S	37°75'E	975	530	14.3	35.1	Sandy clay
KALRO- Kakamega	0°16'N	34°45'E	1585	1916	12.8	28.6	Sandy loam
KALRO- Embu	0° 49'S	37° 42'E	1510	1200	14.1	25.0	Clay loam
KALRO/CIMMYT- Naivasha	0° 41'S	36° 23'E	1904	131	8.4	27.6	Clay loam

Four sites, Kiboko, Kakamega, Embu and Naivasha (Table 4.2) were used to evaluate the resulting 28 F1 hybrids. Kakamega, Kiboko and Embu were used to evaluate the F1 hybrids under optimum conditions, while Kiboko was used to evaluate the hybrids under managed drought and Naivasha used to evaluate the same materials under artificial MLN infestation. At all sites, the evaluation was done in two main seasons, 2017A and 2018A, resulting in a total of six optimum, two managed drought and two artificial MLN infestation environments. The choice of these sites was based on specific constraints: Kakamega is a well-known hot spot for foliar diseases mainly grey leaf spot and turcicum leaf blight, while Embu is a known natural hot spot for maize streak virus and occasionally good natural infestation of stem borers occurs. Although, the main focus was on drought and MLN, evaluation of the hybrids in other environments was an added advantage that is useful for cultivar development.

In the optimum environments, all routine agronomic practices for maize production; weeding, fertilizer and pesticides application were followed. The fertilization for the field was done using nitrogen (N) at the rate of 60 kg/ha, in two splits and potassium phosphate (P_2O_5) at rate of 60 kg/ha, a general recommendation for the Kiboko area. In the managed drought environments, the planting was done during the dry season. All routine agronomic practices of maize production; weeding, fertilizer and pesticides application were followed and drip irrigation was used. The irrigation was stopped two weeks before flowering to impose the drought stress at flowering period.

In the maize lethal necrosis environments, artificial infestation of MLN was done by infecting the maize plants with the two viruses, *maize chlorotic mottle virus* (MCMV) and *sugarcane mosaic virus* (SCMV). Preparation of the inoculum for artificial inoculation of MLN was done as reported by Gowda *et al.* (2015) and the maintenance of the viruses (MCMV and SCMV). Seedlings were inoculated two times with a 1: 4 (MCMV: SCMV) mixture of MLN viruses. At the 4–6 leaf stage, the first inoculation was done and seven days thereafter, the second

was done. A motorized backpack sprayer was used to dispense the inoculum at a rate of 120 L per hectare (Plate 4-1).

Two weeks after inoculation each plot was scored for reaction to MLN using a 1–9 rating scale. In the scale, 1 = clean, no MLN disease symptom on leaves; 3= fine chlorotic streaks on leaves; 5- chlorotic mottling and mosaic throughout the whole plant; 7 = excessive chlorotic mottling and dead heart and 9 = dead plant and complete plant necrosis. MLN scores were taken four times at 2-week intervals from the first assessment. The 1st and 2nd scores were considered as MLN-early, while the 3rd and 4th scores were considered MLN-late. Resistant hybrids had scores 1- 3, tolerant ones had scores 4-5 and susceptible hybrids had scores higher than 5.

Plate 4-1. Application of MLN inoculum at Naivasha, Kenya (Suresh, 2018)

4.2.3. Experimental design and planting

The trial consisted of 32 hybrids made up of: 28 experimental F1 hybrids, two commercial hybrid checks, two internal hybrid checks (from the CIMMYT breeding program). The checks from the two groups included one drought tolerant hybrid check and one MLN resistant hybrid check. The trial design was a 4 x 8 α-lattice, with two rows per plot, replicated three times, except under MLN infestation where one-row plots were used. The rows were 4.5 m long, with 19 hills per row, inter-row spacing of 0.75 m and intra-row spacing of 0.25 m, corresponding to a density of 53,333 plants ha^{-1}. Two seeds were planted

per hill, and later thinned to one plant per hill two weeks after emergence, except for the border hills which maintained two plants per hill.

4.2.4. Data collection

Data were collected on a per plot basis. Recommended procedures by CIMMYT (CIMMYT, 1985) and Magorokosho *et al.* (2008) were followed for data collection. The first and last plant in each plot were considered as border plants and therefore, they were not used for the assessment of the traits.

Data were collected on: days to anthesis (AD), days to silking (SD), plant height (PH) and ear height (EH), grain yield per plot, number of plants at harvest (NP), number of ears at harvest, field weight, grain weight (GW), grain moisture, ear aspect (EA) and plant aspect (PA). Anthesis-silking interval (ASI), ear position (EPO) and ears per plant (EPP) were calculated or estimated from the collected data. At the MLN evaluation sites, MLN disease reaction was scored four times (MLN1 - MLN4), while for drought trials, leaf senescence (SEN) was the added trait.

The grain yield (GY) in tonnes per hectare (t ha^{-1}), from g/m^2, was calculated as follows:

$$GY\ (t\,ha^{-1}) = \frac{GW\ (g)}{1000} \times \left[\frac{100 - G.\ moisture\ (\%)}{100 - 12.5}\right] \times \frac{10}{Net\ plot\ area}$$

4.2.5. Analysis of agronomic performance

Single and combined environments analyses were carried out for the 4×8 α-lattice design (Bänziger *et al.*, 2000) in Fieldbook-IMIS5 (Banziger *et al.*, 2012) statistical software developed by CIMMYT, following the REML procedure, mixed model. Hybrid effects were considered as fixed while the effects of the rest of the sources of variations were random.

The following statistical models was used for the single site analysis:

$$Y_{ijk} = \mu + H_i + r_j + B_{K(j)} + \varepsilon_{ijk}$$

Where, Y_{ijkl} = main effect; μ = overall mean; H_i = the effect of the i^{th} hybrid (I=1,2,…32);

r_j = Effect of the replication (j=1,2,3); $B_{k(j)}$= estimate of the incomplete block within replication and ε_{ijk} = overall random error.

The following statistical model was used for the combined analysis:

$$Y_{ijkl} = \mu + r_j + B_K + S_l + SH_{il} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = main effect; μ= overall mean; r_j = effect of the replications; $B_{k=}$ effect of the k^{th} block nested in j^{th} replication and k=1,2,3…8, while j=1,2,3; S_l =the effect of the l^{th} environment and H_i = the effect of the i^{th} hybrid and i =1,2,3…32; SH_{il} = interaction effect of the i^{th} hybrid and l^{th} environment and ε_{ijkl} = random error. The hybrids means were ranked according to yield and other specific traits in each environment, which was the principal selection criterion.

4.2.6. Genetic analysis

Genetic analyses were carried out for all the traits following the Griffings' Method IV (which excluded the parents and the reciprocal crosses), model I (fixed) (Griffing, 1956a, 1956b) to estimate the combining ability effects using PROC GLM of SAS version 9.4 (Zhang *et al.*, 2005). The genetic analysis was done using only the 28 hybrids that were generated for the half diallel mating design.

Mean squares due to general and specific combining ability parameters were estimated and used to make inferences about the type of gene action involved in the phenotypic expression of the traits (Hallauer, 2007; Hallauer *et al.*, 2010). The mathematical model for genetic analysis at single environment was as follows:

$$Y_{ijk} = \mu + R_K + G_i + G_j + S_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the individual observation recorded on cross X_{ij} in replication R_k of environment E_m subject to the peculiar experimental error $\varepsilon_{ijk,}$

μ is the trial mean in single environment or overall mean across environment

G_i *and* G_j are the General combining ability of parent $_i$ and parent $_j$ respectively

S_{ij} is the Specific combining ability between parent $_i$ and parent $_j$

The mathematical model for combined genetic analysis across environments was:

$$Y_{ijk} = \mu + E_e + g_i + g_j + s_{ij} + gE_{eg} + sE_{es} + \varepsilon_{ijk}$$

in which Y_{ijk} is the observed measurement for the ijth cross grown in the kth environment (E_e), μ is the grand mean, g_i and g_j are the GCA effects of the parents, s_{ij} is the SCA effect, gE_{eg} is the interaction effect between GCA and the environment (E), sE_{es} is the interaction effect between SCA and the environment E, and ε_{ijk} is the error term associated with the ijth cross evaluated in the kth replication and Ee environment (Hallauer and Miranda, 1988).

A combined analysis across the environments was performed taking into consideration that environments are random effects and genotypes are fixed effects (Zhang *et al.*, 2005; SAS-Institute Inc., 2013). General and specific combining ability mean squares were tested against the pooled error mean square (MSE), since the genotypes were considered fixed effects (Hallauer *et al.*, 2010). Individual parent GCA (g_i) and cross SCA (s_{ij}) effects were calculated as follows:

$$gi = \frac{nYi.-2Y..}{n\,(n-2)}$$

$$Sij = Yij - \frac{Yi.-Y.j}{n-2} + \frac{2Y..}{(n-1)(n-2)}$$

where: *gi* is the general combining ability effect of the *ith* parent, s_{ij} is the specific combining ability of the cross between ith and jth parents, n is the number of parents, $Y_{i.}$ is the total of the crosses involving parent i as female, $Y_{.j}$ is the total of the crosses involving parent j as male, and Y.. is the grand total.

Sum of squares due to GCA (SS*gca*) were divided by the total sum of squares (SS*total*) to assess the proportion of the general combining ability effects on the total genetic variability (SS*gca*/SS*total*). To determine the relative importance of general and specific combining ability effects in the observed variation among the crosses, the following three ratios using sum of squares (SS*gca*/SS(*gca*+*sca*), SS*gca*/SS*entry* and SS*sca*/SS*entry*) were used (Malacarne and Vicente, 2003).

4.3. Results

Analysis of variance for each environmental condition (Table 4.3 to Table 4.5) showed highly significant differences ($p < 0.001$) among the crosses in respect of the different traits, except for number of ears per plant (EPP) under the optimum environment and plant aspect (PA) under drought. Environmental effects were also statistically significant for all traits ($p < 0.001$). Genotype × environment interaction effects were significant for all traits except for first score of MLN under MLN environments, ears per plant (EPP) under optimum and anthesis date (AD) under managed drought. Statistical models significantly ($p < 0.001$) explained the total variation observed for all traits in all individual and across environments. The coefficients of determination (R^2) for grain yield (GY) ranged from 0.86 under optimum to 0.95 under MLN. Grand means for GY were 6.04, 2.00 and 0.38 t/ha under optimum, managed drought and MLN environments, respectively.

4.3.1. Performance under maize lethal necrosis environments

Due to the severe MLN infestation in the season 2017A, the trial did not reach the harvesting stage, more that ¾ of the trial was completely wiped out, and as a result the data for grain yield (GY), ears per plant (EPP), number of harvested plants (NP), plant height and ear position (EPO) that was analysed was only from the season 2018A. The results revealed that for early MLN scores, more hybrids tend to show resistance compared to the late MLN scores. It was observed that out of the 28 hybrids evaluated, 12 of these (42.8%) were resistant at early stage with scores ranging from 2.3 to 3.4, and the remaining 16 hybrids (57.2%) were tolerant with scores ranging from 3.5 to 4.8 (appendix 4-1). As for the late MLN scores, only one hybrid was resistant with a score of 2.8; 10 hybrids (35.7%) were tolerant with scores ranging from 4.6 to 5.3 and 17 hybrids (60.7%) were susceptible with scores ranging from 5.5 to 7.8 (Table 4.3).

The mean squares due to general combining ability (MSgca) were highly significant ($p < 0.001$) for all traits except for ear position (EPO) ($p > 0.05$). The mean squares due to specific combining ability (MSsca) were significant for GY, number of harvested plants (NP), MLN scores 2, 3 and 4 at $p < 0.001$, for MLN score 1 and EPP at $p<0.01$, EPO at $p<0.05$, but were not significant for plant height (PH). The ratios for sum of squares [(SSgca/ SS(gca + sca)], ranged from 0.2 for EPO to 0.77 for MLN score 1. The lowest proportion of GCA effects to the total observed genetic variability (SSgca/SStotal) was 0.1 for EPO and the maximum was 0.6 for GY. In this environment the coefficients of determination (R^2) varied from 0.51 to 0.92, having the lowest for EPO and the highest for GY. On the other hand, the coefficient of variation varied from 8.45% to 40.32% for the same traits.

Table 4.3. Mean squares for MLN scores, grain yield and other traits of diallel cross hybrids evaluated under MLN artificial infestation during 2017A and 2018A in Kenya

Source	Df	MLN1	MLN2	MLN3	MLN4	Df	GY	EPP	NP	PH	EPO
ENV	1	17.36***	6.10***	5.01***	5.72***	-	-	-	-	-	-
REP(ENV)	4	0.83**	0.98**	0.30ns	0.52ns	2	0.059ns	0.04ns	2.58ns	1024.54*	0.001ns
Cross	27	1.49***	3.04***	3.13***	5.77***	27	0.57***	0.26***	12.43***	831.53***	0.01*
Cross x ENV	27	0.28ns	0.56**	0.83*	0.76**	-	-	-	-	-	-
GCA	7	4.41***	8.71***	7.77***	15.20***	7	1.43***	0.57***	23.25***	2085.4***	0.004ns
SCA	20	0.47**	1.06***	1.51***	2.47***	20	0.26***	0.15**	8.64***	392.68ns	0.005*
GCA*ENV	7	0.25ns	0.82**	0.78ns	0.81*	-	-	-	-	-	-
SCA*ENV	20	0.29ns	0.46*	0.85*	0.74*	-	-	-	-	-	-
Error	108	0.22	0.28	0.44	0.38	54	0.02	0.06	2.15	229.53	0.00
R^2		0.75	0.78	0.70	0.82		0.92	0.70	0.75	0.66	0.51
CV (%)		15.43	12.42	12.85	10.13		40.32	37.14	17.21	10.87	8.43
Trial mean		3.01	4.25	5.16	6.10		0.43	0.64	8.52	139.38	0.62
Min		2.17	2.33	2.83	2.83		0.00	0.18	2.33	107.08	0.51
Max		4.17	5.50	6.67	8.50		2.32	1.52	10.33	175.67	0.71
SSgca/SS(gca + sca)		0.77	0.74	0.64	0.68		0.65	0.57	0.49	0.65	0.20
SSgca/Ssentry		0.77	0.74	0.64	0.68		0.65	0.57	0.49	0.65	0.20
SSsca/Ssentry		0.23	0.26	0.36	0.32		0.35	0.43	0.51	0.35	0.80
Ssgca/Sstotal		0.34	0.44	0.34	0.47		0.60	0.40	0.36	0.40	0.10

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

MLN1- MLN4= MLN scores, GY = grain yield; EPP = ears/plant, NP= number of plants, PH = plant height; EPO = ear position

4.3.1. Performance under managed drought stress conditions

The leaf senescence (SEN) and plant aspect (PA) data were only recorded in 2018A season due to severe drought which resulted in the plants drying up much early. The climatic data of the two seasons during the trial growth, are shown in Figures 4-1 and 4-2 for 2017A and 2018A, respectively. It was observed that generally, the temperatures in 2017A were slightly higher compared to 2018A, which affected some of the recorded traits. In 2017A, the trial anthesis date (AD) varied from 63 to 72 days with ASI ranging from 3 to 13 days while in 2018A the AD ranged from 69 to 75 days, with ASI ranging from -2 to 4 days (Table 4.4). This negatively affected the yields, where in 2017A the grain yield ranged from 0.06 to 1.00 t/ha with an average of 0.42 t/ha while in 2018A, the grain yield ranged from 2.17 to 4.58 t/ha with an average of 3.6 t/ha (Appendix 4-2). The senescence, ear position and anthesis date showed the highest values of the proportion of SSgca/SStotal.

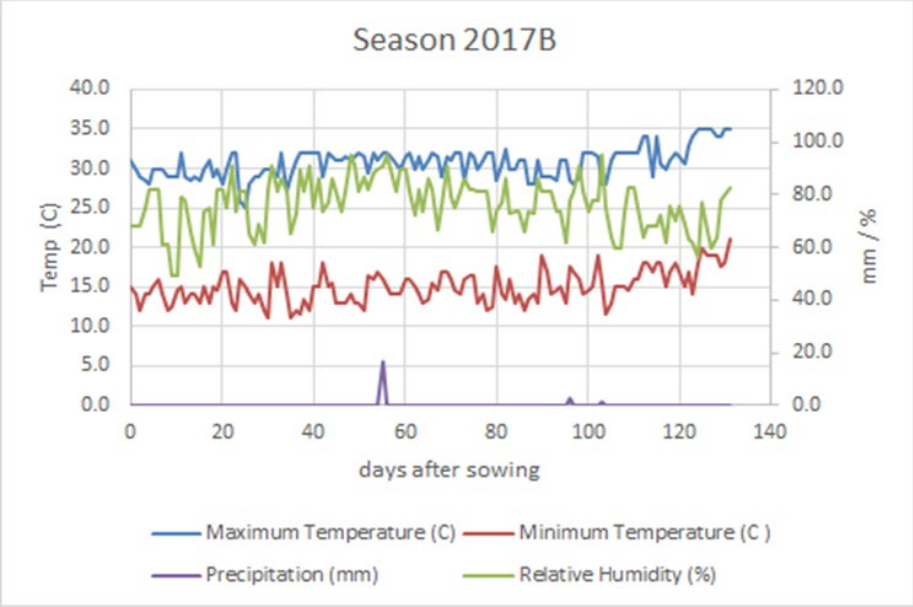

Figure 4-1. Daily precipitation, relative humidity, minimum and maximum temperatures during the dry season 2017B at Kiboko

However, it was observed that out of the 28 experimental hybrids evaluated, 19 (67.9%), in the two seasons yielded above 2.0 t/ha, 12 (42.9%) showed an ASI below 4 days and 12 (42.9%) showed an average senescence score of 4, showing that there is some good tolerance under drought. Genetic analyses at this environment showed significant MSgca at $p< 0.05$ for all measured traits, except for EPP and ASI which were not significant while MSsca was significant ($p< 0.05$) for all recorded traits. The environment had significant influence on both MSgca and MSsca for all traits, except for AD in both, EPO for MSgca

and EPP for MSsca which were not significant. Calculated combining ability effects ratios (SSgca/SStotal) ranged from a minimum of 0.02 for EPP to a maximum of 0.50 for EPO. The lowest proportions of SSgca/SS(gca+sca) were obtained for ASI (0.41) while the highest was obtained AD (0.85).

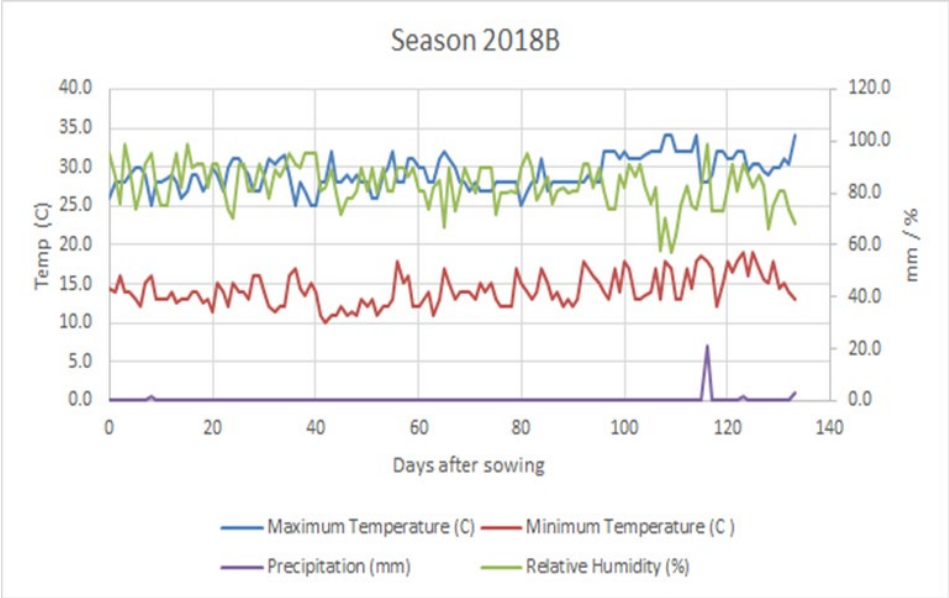

Figure 4-2. Daily precipitation, relative humidity, minimum and maximum temperatures during the dry season 2018B at Kiboko

4.3.3. Performance under optimum conditions

In the optimum environment, the genotypes showed more pronounced differences and higher yields compared to stress environments (Appendix 4-3). The yields varied from 2.91 to 7.31 t/ha. The ASI were also lower compared to the drought conditions. All traits showed medium to high coefficients of determination (R^2) which ranged from 0.59 to 0.98, PA with the lowest R^2 and AS having the highest (Table 4.5). Out of the 28 evaluated hybrids 15 (53.6%) showed mean yield across all environments above 6 t/ha. The mean squares due to general (MSgca) and specific (MSsca) combining ability were significant at $p< 0.05$ for all traits except for EA for the MSgca and PA for the MSsca. MSgca were relatively more influenced by the environment compared to the MSsca. MSsca for AD, PA and HC were not influenced by the environment. The ratios for sum of squares (SSgca / SS(gca + sca), ranged from 0.18 for EA to 0.92 for EPO and PA. The lowest proportion of GCA effects to the total observed genetic variability (SSgca / SStotal) was 0.03 for EA and the maximum was 0.70 for grain texture (TEX).

Table 4.4. Mean squares for grain yield and other traits of diallel cross hybrids evaluated under managed drought during 2017A and 2018A in Kenya

Source	Df	GY	EPP	EA	AD	ASI	PH	EPO	Df	SEN	PA
ENV	1	417.53***	18.98***	65.63***	793.01***	1354.34***	74550.72***	0.05***	-	-	-
REP(ENV)	4	6.91***	0.066*	2.94***	4.02*	1.24ns	2296.53***	0.00ns	2	13.95***	0.90ns
Cross	27	1.29***	0.053***	1.01***	21.67***	14.76***	600.38***	0.01***	27	3.34***	0.82ns
Cross x ENV	27	1.01***	0.04**	1.06***	1.87ns	14.85***	256.41**	0.00*	-	-	-
GCA	7	2.73*	0.085ns	1.73***	70.92***	23.41ns	1070.45***	0.04***	7	6.05*	-
SCA	20	0.79***	0.042**	0.76*	4.44***	11.73***	435.85***	0.00*	20	2.39***	-
GCA*ENV	7	2.10***	0.063**	1.08*	1.82ns	14.74***	409.66**	0.00ns	-	-	-
SCA*ENV	20	0.63**	0.032ns	1.05***	1.89ns	14.88***	202.77*	0.00*	-	-	-
Error	108	0.26	0.40	0.41	1.30	3.45	116.75	0.00	54	0.67	0.51
R^2		0.95	0.75	1	0.91	0.85	0.89	0.72		0.77	0.47
CV (%)		25.75	21.47	22	1.64	42.91	5.98	8.12		16.92	33.50
Trial mean		2.00	0.60	3	69.29	4.33	180.64	0.52		4.83	2.13
Min		0.62	0.43	2	66.67	1.17	159.33	0.44		3.83	1.00
Max		2.67	0.80	4	73.00	7.33	198.33	0.64		9.00	3.33
SSgca/SS(gca + sca)		0.55	0.42	0.44	0.85	0.41	0.46	0.80		0.47	-
SSgca/Ssentry		0.55	0.42	0.44	0.85	0.41	0.46	0.80		0.47	-
SSsca/Ssentry		0.45	0.58	0.56	0.15	0.59	0.54	0.20		0.53	-
SSgca/SStotal		0.04	0.02	0.07	0.31	0.06	0.06	0.40		0.27	-

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

GY = grain yield; EPP = ears/plant, EA = ear aspect; AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position; SEN= senascence; PA = plant aspect

Table 4.5. Mean squares for grain yield and other traits of diallel cross hybrids evaluated under optimum conditions during 2017A and 2018A in Kenya

Source	Df	GY	EPP	EA	AD	ASI	PH	EPO	PA	Df	HC	Df	TEX
ENV	5	223.62***	0.53**	14.54***	9377.38***	70.97***	35327.45**	0.16***	3.47***	3	2679.36***	-	-
REP(ENV)	12	2.42**	0.23ns	0.65*	9.30***	1.88ns	1162.3***	0.00***	0.39ns	8	144.71ns	2	0.25ns
Cross	27	13.26***	0.18ns	2.06***	86.11***	25.28***	2767.48***	0.02***	2.23***	27	373.48***	27	6.57***
Cross x ENV	135	2.32***	0.16ns	0.78***	5.44***	4.58***	363.87***	0.00***	0.50***	81	147.01***	-	-
GCA	7	22.80***	-	1.43ns	292.47***	86.89***	5628.91***	0.07***	7.88***	7	925.57**	7	20.00***
SCA	20	9.92***	-	2.28***	13.89***	3.71***	1765.98***	0.00***	0.26ns	20	180.24**	20	1.87***
GCA*ENV	35	5.43***	-	1.84***	13.25***	9.16***	575.93***	0.00***	0.90***	35	179.97***	-	-
SCA*ENV	100	1.23*	-	0.40*	2.70ns	2.98***	289.65*	0.00*	0.36ns	100	56.09ns	-	-
Error	324	0.94	0.16	0.30	2.75	1.38	221.77	0.00	0.32	216	82.82	-	-
R^2		0.86	0.39	0.71	0.98	0.79	0.81	0.90	0.59		0.64		0.89
CV (%)		16.04	40.12	21.15	2.39	107.81	6.47	4.62	21.13		99.56		22.15
Trial mean		6.04	1.00	2.60	69.29	1.09	230.16	0.49	2.68		9.14		2.89
Min		2.91	0.85	2.11	65.06	-1.61	204.00	0.42	1.78		0.60		1.00
Max		7.35	1.48	3.89	74.72	3.67	255.17	0.53	3.33		23.65		5.00
SSgca/SS(gca + sca)		0.49	-	0.18	0.88	0.89	0.53	0.92	0.92		0.64		0.79
SSgca/Ssentry		0.49	-	0.18	0.88	0.89	0.53	0.92	0.92		0.64		0.79
SSsca/Ssentry		0.51	-	0.82	0.12	0.11	0.47	0.08	0.08		0.36		0.21
SSgca/Sstotal		0.08		0.03	0.04	0.29	0.10	0.28	0.22		0.13		0.70

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

GY = grain yield; EPP = ears/plant, EA = ear aspect; AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position; PA= plant aspect; HC= husk cover

4.3.4. Mean performance for yield, MLN scores and drought parameters

The highest yielding entry for grain yield under optimum conditions where the trial mean was 7.35 t/ha was entry 17 (CKDHL120172×CML494) with mean of 6.04 t/ha, while under MLN infestation the highest grain yield was 2.32 t/ha for entry 7 (CKSBL10011×CKDHL120918) with trial mean of 0.43 t/ha; and under drought conditions the highest was 2.67 t/ha observed on entry 16 (CML442×CML494) with a trial mean of 1.99 t/ha. The single cross CKSBL10011×CKDHL120918 also had the lowest MLN late score (2.83) under MLN environment, a grain yield of 2.01 t/ha under managed drought and 5.50 t/ha under optimum conditions.

On the other hand, entry 2 (CKSBL10011×CML494) was the only entry which was in the top ten rank in all environments. It was the 3rd in yield under optimum, 4th under managed drought and the 10th under severe MLN infestation. Entry 18 (CKDHL120918×CML494), on the other hand, was the only entry which showed good results across drought and MLN environments. It was ranked 8th in yield under drought with 2.26 t/ha and was the 3rd under MLN with 0.88 t/ha. It was classified as tolerant to MLN based on the late MLN scores. Entry 17 (CKDHL120172×CML494) had the highest yield under optimum conditions and had a MLN score of 5 (tolerant) while entries 16 (CML442×CML494), 14 (CKSBL10027×CML494) and 12 (CKDHL120172×CKDHL121230) had yields above 6.60 t/ha under optimum and above 2.25 t/ha under drought conditions (Table 4.6).

Table 4.6. Means of GY, ASI, SEN and MLN for the top and bottom 10 hybrids under optimum, drought and MLN conditions during 2017A and 2018A in Kenya

	OPTIMUM				Drought						Maize Lethal necrosis			
Rank	Entry	GY	Entry	ASI	Entry	GY	Entry	ASI	Entry	SEN	Entry	GY	Entry	MLN3
1	17	7.35	18	-1.61	*16*	2.67	**2**	1.17	11	3.83	**7**	2.32	**7**	2.83
2	15	7.05	8	-0.78	*14*	2.42	8	2.17	21	3.83	25	0.93	25	4.00
3	**2**	6.90	7	-0.44	1	2.40	27	2.17	8	4.17	17	0.90	4	4.50
4	8	6.87	**2**	-0.39	**2**	2.36	1	2.33	9	4.17	***18***	0.88	1	4.67
5	4	6.84	17	0.11	*12*	2.29	14	2.83	14	4.17	3	0.81	13	4.67
6	6	6.72	15	0.22	9	2.27	22	2.83	16	4.17	15	0.80	21	4.67
7	*12*	6.69	14	0.28	13	2.26	**7**	3.00	26	4.17	8	0.78	22	4.67
8	*14*	6.66	25	0.33	***18***	2.26	28	3.00	27	4.17	5	0.71	6	4.83
9	*16*	6.62	22	0.50	11	2.25	9	3.17	1	4.33	22	0.63	28	4.83
10	5	6.49	16	0.61	23	2.22	***18***	3.50	**2**	4.33	**2**	0.59	**2**	5.17
18	19	5.79	6	1.50	17	2.06	3	5.00	13	4.67	10	0.00	9	5.50
19	21	5.75	12	1.50	**7**	2.01	13	5.00	24	4.67	11	0.00	14	5.50
20	20	5.70	10	1.61	22	1.90	24	5.00	5	4.83	12	0.00	15	5.50
21	25	5.68	4	1.67	20	1.84	12	5.17	25	5.17	13	0.00	19	5.50
22	27	5.67	3	1.89	4	1.84	23	5.17	19	5.33	14	0.00	27	5.50
23	**7**	5.50	20	2.11	21	1.79	4	5.50	22	5.33	16	0.00	20	5.67
24	3	5.37	21	2.28	28	1.78	15	5.50	28	5.33	19	0.00	5	5.83
25	13	5.29	23	2.44	25	1.72	11	6.33	4	5.83	20	0.00	16	5.83
26	28	5.24	19	2.61	19	1.23	25	6.83	7	6.17	23	0.00	23	6.00
27	22	5.22	26	3.00	24	0.74	6	7.00	20	6.50	24	0.00	26	6.00
28	24	2.91	24	3.67	3	0.62	19	7.33	3	9.00	26	0.00	24	6.67
Mean		6.04		1.09		2.00		4.33		4.83		0.43		5.16
Min		2.91		-1.61		0.62		1.17		3.83		0.00		2.83
Max		7.35		3.67		2.67		7.33		9.00		2.32		6.67
LSD (5%)		0.65		0.77		0.74		2.11		0.95		0.24		0.76

GY= grain yield; ASI= anthesis-silking interval; SEN = senascence; MLN3= MLN score at late stage

4.3.5. General combining ability effects

Parents 2 (CKSBL10011) and 8 (CML494) had positive and significant (p< 0.05) general combining ability (GCA) effects for grain yield under optimum conditions and MLN infested conditions and negative and significant GCA effects for maize lethal necrosis score under MLN infestation. Parent 7 (CKDHL120918) also showed positive and significant (p< 0.05) GCA effects for grain yield under MLN infested conditions and negative and significant effects for maize lethal necrosis scores under the same conditions. Positive GCA effects for grain yield were observed under managed drought, although their effects were not significant for all the parents. However, negative and significant GCA effects for leaf senescence were observed for parents 3 (CKDHL120172), 4 (CKDHL121230) and 8 (CML494). CML494 also showed negative and significant GCA effects on anthesis-silking interval (ASI). Under optimum conditions, parent 7 (CKDHL120918) and parent 8 (CML494) had negative and significant GCA effects on ASI (Table 4.7).

Table 4.7. General combing ability of parents for grain yield, anthesis-silking interval, senescence and MLN score under optimum, drought and MLN conditions during 2017A and 2018A in Kenya

	Optimum		Drought			MLN	
Genotype	**GY**	**ASI**	**GY**	**ASI**	**SEN**	**GY**	**MLN3**
CKSBL10027	***-0.32****	***0.58*****	-0.32	0.09	***0.75*****	***-0.24*****	0.06
CKSBL10011	**0.32***	-0.18	-0.08	-0.38	***0.86*****	**0.48*****	**-0.55*****
CKDHL120172	-0.23	***0.86*****	-0.19	0.48	**-0.39***	***-0.14****	0.2
CKDHL121230	0.15	-0.15	0.29	-0.24	**-0.61****	***-0.29*****	-0.07
CML395	-0.27	***0.82*****	-0.33	***1.62****	0.08	***-0.14****	0.17
CML442	0.09	***0.63*****	0.24	0.12	-0.31	***-0.3*****	***0.67*****
CKDHL120918	***-0.61*****	**-1.04*****	0.02	-0.66	0.22	**0.47*****	**-0.77*****
CML494	**0.87*****	**-1.53*****	0.36	**-1.02***	**-0.61****	**0.15****	***0.28****

GY= grain yield, ASI= anthesis-silking interval, SEN=senescence, MLN= MLN score at late stage

4.3.6. Specific combining ability effects

The single crosses CKSBL10011×CML395 and CKDHL120172×CML494 had positive and significant (p< 0.05) specific combining ability (SCA) effects of 0.74 and 0.66 for yield under optimum conditions while four single crosses CKSBL10011×CKDHL120918, CKDHL120172×CML494, CKDHL121230×CML494 and CML395×CML494 had positive and significant SCA for grain yield under maize lethal necrosis infestation. The single cross CKDHL120172×CML395 had unfavorable and significant SCA effects for grain yield under optimum and MLN scores under MLN infestation.

Five single crosses, namely CKSBL10027×CKDHL120172, CKSBL10027×CKDHL121230, CKSBL10027×CML494, CKSBL10011×CKDHL121230 and CKSBL10011×CML494 had favorable and significant SCA effects for leaf senescence under managed drought conditions, while four single crosses, CKSBL10027×CKDHL120172, CKSBL10011×CKDHL120918, CKDHL121230×CML442 and CML442×CKDHL120918 showed negative and significant SCA effects for maize lethal necrosis scores under MLN infestation (Table 4.8).

Table 4.8. Specific combining ability of crosses for grain yield, anthesis-silking interval, senescence and MLN score under optimum, drought and MLN conditions during 2017A and 2018A in Kenya

Genotypes		Optimum		Drought			MLN	
SC	**Pedigree**	**GY**	**ASI**	GY	ASI	SEN	**GY**	**MLN**
S12	CKSBL10027 × CKSBL10011	**-0.68***	0.4	-0.99	0.96	**2.56*****	0.13	0.33
S13	CKSBL10027 × CKDHL120172	0.25	-0.25	0.30	-0.06	**-1.36****	0.11	**-0.76****
S14	CKSBL10027 × CKDHL121230	0.03	-0.08	0.30	-1.01	**-0.81***	0.09	0.35
S15	CKSBL10027 × CML395	0.33	0.12	-0.11	1.30	-0.33	-0.05	0.1
S16	CKSBL10027 × CML442	-0.11	-0.2	-0.08	0.30	**1.22****	0.1	-0.23
S17	CKSBL10027 × CKDHL120918	0.11	-0.13	0.20	-0.92	-0.47	-0.04	0.21
S18	CKSBL10027 × CML494	0.07	0.14	0.38	-0.56	**-0.81***	**-0.35*****	-0.01
S23	CKSBL10011 × CKDHL120172	0.58	-0.27	0.39	**2.58***	**-0.81***	**-0.24***	0.02
S24	CKSBL10011 × CKDHL121230	-0.08	0.07	0.18	-1.37	**-0.75***	-0.12	0.13
S25	CKSBL10011 × CML395	**0.74***	-0.07	0.24	-0.06	0.06	**-0.32****	-0.29
S26	CKSBL10011 × CML442	0.03	-0.05	0.03	-0.06	-0.56	0.09	**0.55***
S27	CKSBL10011 × CKDHL120918	-0.26	0.32	0.07	-0.29	0.25	**0.93*****	**-1.01*****
S28	CKSBL10011 × CML494	-0.33	0.23	0.07	-1.76	**-0.75***	**-0.48*****	0.27
S34	CKDHL120172 × CKDHL121230	0.72*	-0.3	0.18	0.60	0.67	0.00	-0.29
S35	CKDHL120172 × CML395	**-2.64*****	**0.90****	-0.74	-1.42	0.14	-0.15	**1.13*****
S36	CKDHL120172 × CML442	0.4	0.42	0.03	-1.09	0.03	0.01	-0.04
S37	CKDHL120172 × CKDHL120918	0.03	-0.19	-0.05	-1.15	0.67	-0.19	0.24
S38	CKDHL120172 × CML494	**0.66***	-0.31	-0.11	0.55	0.67	**0.46*****	-0.31
S45	CKDHL121230 × CML395	0.28	-0.15	0.11	-1.04	0.19	0	-0.26
S46	CKDHL121230 × CML442	-0.47	**-0.58***	-0.28	2.13	-0.08	0.16	**-0.42***
S47	CKDHL121230 × CKDHL120918	-0.29	**1.21*****	-0.05	1.58	0.22	-0.61	0.35
S48	CKDHL121230 × CML494	-0.19	-0.19	-0.44	-0.90	0.56	**0.48*****	0.13
S56	CML395 × CML442	0.37	-0.1	0.31	-0.90	-0.28	0.01	-0.01
S57	CML395 × CKDHL120918	0.51	-0.54	0.03	1.55	0.03	0.17	**-0.56***
S58	CML395 × CML494	0.41	-0.16	0.15	0.58	0.19	**0.36*****	-0.12
S67	CML442 × CKDHL120918	0.15	0.09	-0.08	-1.62	-0.58	-0.08	**-0.44***
S68	CML442 × CML494	-0.38	0.42	0.07	1.24	0.25	**-0.29****	-0.29
S78	CKDHL120918 × CML494	-0.25	-0.13	-0.12	0.85	-0.11	-0.18	0.33

GY= grain yield, ASI= anthesis-silking interval, SEN=senescence, MLN= MLN score at late stage

4.4. Discussion

4.4.1. Grain yield, drought, MLN tolerance and resistance parameters

The highly significant environmental mean squares observed for all traits in all environments indicate that the experimental growing conditions were very different, each environment was unique and suitable for evaluating the test hybrids (Bello and Olaoye, 2009; Allinne *et al.*, 2009; Aly *et al.*, 2011; Beyene *et al.*, 2012). Significance of test genotypes (entries) suggests that these hybrids were variable in their genetic constitution, a prerequisite for phenotypic selection. Significant environmental effects for all traits combined with genotype × environment interactions for most of the trials including the main trait (GY) suggest that and hybrid stability across the environments for general adaptation can be explored as well as determining genotypes which are adapted to specific environments (Gomez and Gomez, 1984; Pimentel-Gomes, 2009; Montgomery, 2015). Machida *et al.* (2010), investigating the combining ability of quality protein maize, reported that some of the traits were highly influenced by the environment. Therefore, selections based on multiple environments is important for release of maize varieties.

The highly significant ($p< 0.001$) genetic variation observed under maize lethal necrosis, optimum drought conditions (Table 4.3 - Table 4.5) for GY was due to the significant contribution of both general and specific combining ability effects (MSgca and MSsca, respectively) implying that both additive and non-additive gene action played a significant role in the phenotypic expression of grain yield (Falconer and Mackay, 1996; Hallauer, 2007; Acquaah, 2007; Hallauer *et al.*, 2010). The observed highly significant ($p<0.001$) GCA x environment as well the SCA x environment, suggest that wide testing in target environments is required in selecting desirable parents or hybrids. The [SSgca / SS(gca + sca)] ratio of 0.49 under optimum, almost half, suggested that additive and non-additive gene actions contributed equally to the total genetic effects sum of squares for GY under this environment. However, under drought and MLN environments, additive gene effects were more dominant than non-additive, considering the SSgca / SS(gca + sca) ratio of 0.55 and 0.65, respectively.

The combined contribution of both additive and non-additive gene action for GY in maize under non-stressed conditions has been reported by several other researchers (Gamble, 1962a,b; Eberhart and Hallauer, 1968; Stuber and Moll, 1971; Moreno-Gonzalez and Dudley, 1981; Melchinger *et al.*, 1986) and more recently (Passos *et al.*, 2010; Machida *et al.*, 2010; Zare *et al.*, 2011; De Oliveira *et al.*, 2011; Mhike *et al.*, 2011; Zare-kohan and

Heidari, 2012; Chen *et al.*, 2012; Zeinab and Helal, 2014; Abdel-Moneam *et al.*, 2014; Adebayo *et al.*, 2014; Erdal *et al*., 2015; Ertino *et al*. 2017). The prevalence of additive effects in controlling yield under drought has been reported by several researchers in tropical maize including Betrán *et al.* (2003); Adebayo *et al.* (2014); Erdal *et al*., (2015); Chauque, (2016) and Ertino *et al*. (2017).

Derera *et al.* (2008) found that the SCA effects were not significant for yield, suggesting that non- additive effects were not important in controlling yield under drought. Betrán *et al.* (2003) reported similar results, where GCA was significant under drought while SCA was not, indicating that additive genetic effects were more important than non-additive. Reports on studies on genetics of maize lethal necrosis in Africa are still limited because the disease is relatively new on the continent. Maize lethal necrosis (MLN) is a new outbreak disease in eastern Africa and has become a major threat to maize production in the region (Mahuku *et al.*, 2015a). Beyene *et al.* (2017) reported a ratio of 1:1 for GCA/SCA for yield under MLN infestation, implying the similar importance of both additive and non-additive gene effects.

The results above are in contradiction with results found in this study. From this study it was observed that additive gene effects were predominant that non-additive and also the ratio [SSgca/SStotal = 0.6] revealed that GY was mainly influenced by additive gene effects, with relatively moderate heritability estimates. Whereas MLN is a new disease in Africa, and the maize germplasm used in this study is of tropical origin, therefore, inferences drawn are still important. Significant MSgca and MSsca for the early and late MLN scores, average number of ears per plant (EPP) under MLN virus infestation, anthesis date (AD) and senescence scores (SEN) under drought suggest that both additive and non-additive genes effects controlled the inheritance of these traits. However, the anthesis-silking interval (ASI) and EPP under drought showed only a significant MSsca, suggesting that only non-additive gene effects controlled these traits in this environment, and among the inbred lines used as parents.

The traits, AD, ASI, EPP and SEN have been associated with tolerance to drought in many studies (Betrán *et al.*, 2003; Badu-Apraku, 2007; Derera *et al.*, 2008; Shira *et al.* 2010; Meseka *et al.*, 2011; Araus *et al.*, 2012; Badu-Apraku *et al.*, 2012; Mhike *et al.*, 2012; Erdal *et al*., 2015; Chauque, 2016 and Ertino *et al*. 2017). Although both additive and non-additive effects play a role in controlling the traits under MLN virus infestation, the additive gene effects are more important considering the ratio of [SSgca /SS(gca + sca)], which for all

traits was above 0.55. Beyene *et al.* (2017), concluded that additive gene action was more important than non-additive gene action for many traits including EPP and MLN scores at early and late stage and thus rapid progress for breeding for MLN can be achieved through recurrent selection.

Under drought, although AD and SEN were influenced by both additive and non-additive effects, additive effects played a major role over non-additive effects for AD [SSgca/SS(gca + sca) =0.85]. These findings agree with those reported by many researchers, including Betrán *et al.* (2003), Derera *et al.* (2008), Adebayo *et al.* (2014) and Chaúque (2016). The influence of both additive and non-additive gene effects for senescence found in this study have been reported also by Betrán *et al.* (2003), Derera *et al.* (2008), Adebayo *et al.* (2014) and Chaúque (2016). However, most studies found additive being more dominant than non-additive, while in this study both effects were equally important.

Non-additive gene effects were observed for ASI and EPP in this study contradicting most of the previous research work. Previous studies reported the influence of both additive and non-additive gene effects but with additive being predominant. However, Chaúque (2016) reported non-significant genetic effects for EPP under drought and optimum environments, while Bänziger *et al.* (2000) reported that EPP was affected mainly by severe drought stress. The fewer genotypes evaluated and the drought conditions could be the reason for this behaviour.

4.4.2. Combined drought tolerance and resistance to maize lethal necrosis

The existence of entries with good grain yield under optimum, drought and MLN virus infestation suggests that drought tolerance and MLN resistance can be selected for in the same hybrids, with low yield penalty. However, such entries were few, and these were CKSBL10011×CKDHL120918, CLDHL120918×CML494 and CKSBL10011×CML494. Under MLN where the average yield was 0.43 t/ha, these three hybrids had yield above 0.6 t/ha, the best yielding being CKSBL10011×CKDHL120918 at 2.32 t/ha. Under drought, where the average was 2.0 t/ha, these hybrids yielded higher than average, and under optimum where the average was 6.04 t/ha, these hybrids had grain yield higher than 5.5 t/ha.

Although, the single cross CKSBL10027×CKDHL120172 (entry 21) showed a negative and significance SCA for ASI and MLN score under drought and MLN, respectively, which are considered good secondary traits for selection due to their correlation with yield under respective environments (Mhike *et al.*, 2012; Beyene *et al.*, 2017). This hybrid did not show good performance under drought and MLN compared to others, most probably due to the potential yield of the lines *per se*. The three hybrids identified which showed good performance under the three environments, involved three parents, parent 2 (CKSBL10011), parent 7 (CKDHL120918) and parent 8 (CML494). This suggests that these inbred lines have high allelic frequency for grain yield. These parents showed favourable and significant effects under the three environments, meaning, although not yet reported, they should have some genes which confer some drought tolerance. CKSBL10011 showed a positive and significant GCA for yield under optimum and MLN and negative and significant GCA for MLN score under MLN environment. However, it had positive and significant GCA for ASI which is not favourable under drought. CKDHL120918 revealed negative and significant GCA for ASI and MLN score under optimum and MLN virus infested environment, which is a favourable effect. It also showed a positive and significant GCA for grain yield under MLN infestation. CML494 showed positive and significant GCA for grain yield under optimum and MLN conditions, negative and significant GCA for ASI and SEN under optimum and drought conditions. However, CML494 showed a positive and significant GCA for MLN score under MLN virus infested environment. Positive GCA is favourable for the traits which have a positive selection and negative GCA is favourable for traits which have a negative selection (Hallauer *et al.*, 2010). These parents can therefore be used in a breeding program for drought tolerance and MLN resistance. The combination of good grain yield and high levels of resistance to MLN and drought tolerance is an indication that it is possible to develop hybrids with combined drought tolerance and MLN resistance. However, since most of the drought tolerance and MLN resistance traits are mainly controlled by additive effects, the inbred lines should be developed separately under the different stresses.

4.5. Conclusion

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

1. The managed drought and MLN virus infestation conditions during the experimental growing periods of this research study were enough to discriminate the genotypes than under optimum conditions. The stressed environments were significantly different from the optimum environment and the significant genotype × environment interaction revealed that the level of performance and ranking of the hybrids depended on growing conditions.

2. General and specific combining ability effects were significant for most of the important traits under the three environments, except PH and EPO under MLN virus infestation, ASI and EPP under drought and PA under optimum, indicating importance of both additive and non-additive gene action in controlling these traits. However, additive gene action was generally predominant in most of the cases and its predominance increased from drought, optimum and MLN, in that order.
3. Improvement of tropical maize for combined drought tolerance and MLN resistance is possible in these set of parents and it can be faster when selection is conducted under combined drought and MLN, than under separate environments.

4. Three inbred lines CKSBL10011, CKDHL120918 and CML494 which are stem borer and MLN resistant were good combiners and can be good sources of resistance genes in breeding for combined drought tolerance and MLN resistance in maize.

References

Abdel-Moneam, M., Sultan, M., Sadek, S. and Shalof, M. 2014. Estimation of heterosis and genetic parameters for yield and yield components in maize using the diallel cross method. Asian Journal of Crop Science 6: 101-111.

Acquaah, G. 2007. Principles of plant genetics and breeding, United Kingdom, Oxford, Blackwell Publishing, 485 - 497.

Adams, I., Harju, V., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M., Smith, J., Fox, A. and Uzayisenga, B. 2014. First report of maize lethal necrosis disease in Rwanda. New Disease Reports 29: 22- 22.

Adebayo, M., Menkir, A., Blay, E., Gracen, V., Danquah, E. and Hearne, S. 2014. Genetic analysis of drought tolerance in adapted × exotic crosses of maize inbred lines under managed stress conditions. Euphytica 196: 261-270.

Allinne, C., Maury, P., Sarrafi, A. and Grieu, P. 2009. Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. Plant science 177: 349-359.

Aly, R., Metwali, E. and Mousa, S. 2011. Combining ability of maize (*Zea mays* L.) inbred lines for grain yield and some agronomic traits using top cross mating design. Global Journal of Molecular Sciences 6: 1-8.

Araus, J., Serret, M. and Edmeades, G. 2012. Phenotyping maize for adaptation to drought. Frontiers in physiology 3: 305-335.

Badu-Apraku, B. 2007. Genetic variances and correlations in an early tropical white maize population after three cycles of recurrent selection for Striga resistance. Maydica 52: 205-217.

Badu-Apraku, B., Akinwale, R., Franco, J. and Oyekunle, M. 2012. Assessment of reliability of secondary traits in selecting for improved grain yield in drought and low-nitrogen environments. Crop Science 52: 2050-2062.

Banziger, M. and Diallo, A. 2001. Stress tolerant maize for farmers in sub-Saharan Africa. CIMMYT Maize Research Highlights 1999–2000. Mexico D.F: CIMMYT.

Bänziger, M., Edmeades, G., Beck, D. and Bellon, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice, Mexico D.F.: CIMMYT.

Bänziger, M., Edmeades, G. and Lafitte, H. 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. Field Crops Research 75: 223-233.

Banziger, M., Vivek, B., Ayala, C. and Norgaard, J. 2012. *Fieldbook-IMIS 5*, Mexico, D.C.: CIMMYT.

Bello, O. B. and Olaoye, G. 2009. Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. African Journal of Biotechnology 8: 2518-2522.

Betrán, F., Beck, D., Benziger, M. and Edmeades, G. 2003. Secondary traits in parental inbreds and hybrids under stress and non-stress environments in tropical maize. Field crops research 83: 51-65.

Beyene, Y., Gowda, M., Suresh, M., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Tarekegne, A. and Prasanna, B. 2017. Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. Euphytica 213: 224- 236.

Beyene, Y., Mugo, S., Tefera, T., Gethi, J., Gakunga, J., Ajanga, S., Karaya, H., Musila, R., Muasya, W., Tende, R. and Njoka, S. 2012. Yield stability of stem borer resistant maize hybrids evaluated in regional trials in east Africa. African Journal of Plant Science 6: 77-83.

Bolaños, J. and Edmeades, G. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. Field Crops Research 31: 233-252.

Brandes, E. 1920. Artificial and insect transmission of sugarcane mosaic. Journal of Agricultural Research 9: 131- 138.

Cabanas, D., Watanabe, S., Higashi, C. and Bressan, A. 2013. Dissecting the mode of Maize chlorotic mottle virus transmission (*Tombusviridae: Machlomovirus*) by Frankliniella williamsi (*Thysanoptera: Thripidae*). Journal of Economic Entomology 106: 16-24.

Cakir, R. 2004. Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crops Research 89: 1-16.

Campos, H., Cooper, M., Edmeades, G., Loffler, C., Schussler, J. and Ibanez, M. 2006. Changes in drought tolerance in maize associated with fifty years of breeding for yield in the US corn belt. Maydica 51: 369 - 381.

Campos, H., Cooper, M., Habben, J., Edmeades, G. and Schussler, J. 2004. Improving drought tolerance in maize: a view from industry. Field crops research 90: 19-34.

Chaúque, P. 2016. Genetic and path coefficient analyses and heterotic orientation of maize germplasm under combined heat and drought stress in sub-tropical lowland environments. Ph.D Thesis., University of KwaZulu-Natal, Pietermaritzburg, SA.

Chen, X., Min, D., Yasir, T. and Hu, Y. 2012. Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). Field Crops Research 137, 195-201.

CIMMYT 1985. Managing trials and reporting data for CIMMYT's International maize testing program. , CIMMYT-Int., Mexico DF. 20pp.

De Oliveira, L., Miranda, G., Delima, R., De Souza, L., Galvão, J. and Dos Santos, I. 2011. Combining ability of tropical maize cultivars in organic and conventional production systems. Ciência Rural 41: 739-745.

Derera, J., Tongoona, P., Vivek, B. S. and Laing, M. D. 2008. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. Euphytica 162: 411-422.

Doupnik Jr, B. 1979. Status of corn lethal necrosis [virus diseases in the United States]: update. Proceedings of the 47th Annual Corn and Sorghum Research Conference, Chicago. American Seed Trade Association, 16-34

Eberhart, S. A. and Hallauer, A. R. 1968. Genetic Effects for Yield in Single, Three-way, and Double-Cross Maize Hybrids 1. Crop Science 8: 377-379.

Edmeades, G., Bolanos, J. and Lafitte, H. Progress in breeding for drought tolerance in maize. 1992. Proceedings of the 47th Annual Corn and Sorghum Industry Research Conference, Chicago. American Seed Trade Association, 93–111.

Erdal, S., Pamukcu, M., Ozturk, A., Aydinsakir, K. and Soylu, S. 2015. Combining abilities of grain yield and yield related traits in relation to drought tolerance in temperate maize breeding. *Turkish Journal of Field Crops* 20: 203-212.

Ertiro, B. T., Beyene, Y., Das, B., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Labuschagne, M. and Prasanna, B. M. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. *Plant breeding* 136: 197-205.

Falconer, D. and Mackay, F. 1996. Introduction to quantitative genetics, New York., Longman Scientific and Technical.

FAOSTAT 2019. FAOSTAT: Statistical databases and data sets of the Food and Agriculture Organization of the United Nations: FAOSTAT Metadata/Production/Crops. 23 March 2019 ed Rome: FAO, Rome.

Flett, B. and Mashingaidze, K. 2016. MAIZE LETHAL NECROSIS: Possible threat to local maize production [Online]. Grain SA, ARC-Grain Crops Institute, Potchefstroom. Available: https://www.grainsa.co.za/maize-lethal-necrosis:-possible-threat-to-local-maize-production [Accessed 20 June 2019].

Gamble, E. E. 1962a. Gene effects in corn (*Zea mays* L.) I. Separation and relative importance of gene effects for yield. Canadian Journal of Plant Science 42: 339-348.

Gamble, E. E. 1962b. Gene effects in corn (*Zea mays* L.) II. Relative importance of gene effects for plant height and certain component attributes of yield. Canadian Journal of Plant Science 42: 349-358.

Gomez, K. and Gomez, A. 1984. Statistical producedures for agricultural research, J. Wiley and sons, New York, USA, 690pp

Gowda, M., Das, B., Makumbi, D., Babu, R., Semagn, K., Mahuku, G., Olsen, M., Bright, J., Beyene, Y. and Prasanna, B. 2015. Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. Journal of Theoretical and applied genetics 128: 1957-1968.

Griffing, B. 1956a. Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science 9: 463-493.

Griffing, B. 1956b. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10: 31-50.

Grime, J. and Campbell, B. 1991. Growth rate, habitat productivity, and plant strategy as predictors of stress response. Response of plants to multiple stresses. Elsevier. 143-159

Hallauer, A. and Miranda, J. 1988. Quantitative genetics in maize breeding, Iowa State Univ Press, Ames, IA.

Hallauer, A. R. 2007. History, contribution, and future of quantitative genetics in plant breeding: lessons from maize. Crop Science 47: 4-19.

Hallauer, A. R., Carena, M. J. and Filho, J. B. 2010. Quantitative genetics in maize breeding New York ; London, Springer, 680pp.

Hao, Z., Li, X., Xie, C., Weng, J., Li, M., Zhang, D., Liang, X., Liu, L., Liu, S. and Zhang, S. 2011. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. Journal of integrative plant biology 53: 641-652.

Jensen, S., Wysong, D., Ball, E. and Higley, P. 1991. Seed transmission of maize chlorotic mottle virus. Plant Disease 75: 497-498.

Jiang, X., Meinke, L., Wright, R., Wilkinson, D. and Campbell, J. 1992. Maize chlorotic mottle virus in Hawaiian-grown maize: vector relations, host range and associated viruses. Crop Protection 11: 248-254.

Louie, R. 1980. Sugarcane mosaic virus in Kenya. Plant Disease 64: 944-947.

Lukanda, M., Owati, A., Ogunsanya, P., Valimunzigha, K., Katsongo, K., Ndemere, H. and Kumar, P. 2014. First report of Maize chlorotic mottle virus infecting maize in the Democratic Republic of the Congo. Plant disease 98: 1448-1448.

Machida, L., Derera, J., Tongoona, P. and MacRobert, J. 2010. Combining ability and reciprocal cross effects of elite quality protein maize inbred lines in subtropical environments. Crop science 50: 1708-1717.

Magorokosho, C., Vivek, B. and MacRobert, J. 2008. Characterization of maize germplasm grown in eastern and southern Africa: Results of the 2007 regional trials coordinated by CIMMYT. Harare, Zimbabwe, 62pp.

Mahuku, G., Lockhart, B., Wanjala, B., Jones, M., Kimunye, J., Stewart, L., Cassone, B., Sevgan, S., Nyasani, J., Kusia, E., Kumar, P., Niblett, C., Kiggundu, A., Asea, G., Pappu, H., Wangai, A., Prasanna, B. and Redinbaugh, M. 2015a. Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-Saharan Africa. Phytopathology 105: 956-965.

Mahuku, G., Wangai, A., Sadessa, K., Teklewold, A., Wegary, D., Ayalneh, D., Adams, I., Smith, J., Bottomley, E. and Bryce, S. 2015b. First report of maize chlorotic mottle virus and maize lethal necrosis on maize in Ethiopia. Plant Disease 99: 1870-1870.

Malacarne, M. F. and Vicente, F. M. 2003. Patrones Heteróticos de Líneas Tropicales Blancas de Maiz. Agronomia Tropical 53: 437-456.

Melchinger, A. E., Geiger, H. H. and Schnell, F. W. 1986. Epistasis in maize (*Zea mays* L.). Theoretical and applied genetics 72: 231-239.

Menkir, A. and Akintunde, A. 2001. Evaluation of the performance of maize hybrids-improved open-pollinated and farmers' local varieties under well-watered and drought stress conditions. Maydica 46: 227-238.

Meseka, S., Menkir, A. and Ajala, S. 2011. Genetic analysis of performance of maize inbred lines under drought stress. Journal of crop improvement 25: 521-539.

Mhike, X., Kori, P., Magorokosho, C. and Ndlela, T. 2012. Validation of the use of secondary traits and selection indices for drought tolerance in tropical maize (*Zea mays* L.). African Journal of Plant Science 6: 96-102.

Mhike, X., Lungu, D. and Vivek, B. 2011. Combining ability studies amongst AREX and CIMMYT maize (*Zea mays* L.) inbred lines under stress and non stress conditions. African Journal of Agricultural Research 6: 1952-1957.

Mir, R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125: 625-645.

Montgomery, D. 2015. Design and Analysis of Experiments (8th Edition), Arizona State University, Jhon Wiley & Sons.

Moreno-Gonzalez, J. and Dudley, J. W. 1981. Epistasis in related and unrelated maize hybrids determined by three methods. Crop Science 21: 644-651.

Nesmith, D. and Ritchie, J. 1992. Effects of soil water-deficits during tassel emergence on development and yield component of maize (*Zea mays* L.). Field Crops Research 28: 251-256.

Niblett, C. and Claflin, L. 1978. Corn lethal necrosis-a new virus disease of corn in Kansas. Plant disease reporter 62: 15-19.

Passos, A. R., Silva, S. A., Da Silva-Souza, C., De Souza, C. M. and Dos Santos Fernandes, L. 2010. Parâmetros genéticos de caracteres agronômicos em genótipos de mamoneira. Pesquisa Agropecuária Brasileira 45: 709-714.

Pimentel-Gomes, F. 2009. Curso de estatística experimental. São Paulo, Brasil, Bilbioteca de Ciências Agrárias Luz de Queiroz.

Richardson, C. 2005. The loss of property rights and the collapse of Zimbabwe. Cato Journal 25: 541 - 565.

Rosen, S. and Scott, L. 1992. Famine grips sub-Saharan Africa. . Agricultural Outlook 191, 20-24.

SAS-Institute.Inc 2013. Base SAS® 9.4 Procedures Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc.

Shiri, M., Aliyev, R. and Choukan, R. 2010. Water stress effects on combining ability and gene action of yield and genetic properties of drought tolerance indices in maize. Research Journal Environmental Science 4: 75-84.

Stuber, C. W. and Moll, R. H. 1971. Epistasis in maize (*Zea mays* L.). II: Comparison of selected with unselected populations. Genetics 67: 137-149.

Suresh, M. 2018. Phenotyping to identify Maize Lethal Necrosis resistant germplasm: Recent Advances in Phenotyping for MLN. New Maize Breeders Training Course 2018. 16-27 July 2018, Kampala, Uganda, 46 slides

Toker, C., Canci, H. and Yildirim, T. 2007. Evaluation of perennial wild Cicer species for drought resistance. Genetic Resources and Crop Evolution 54: 1781-1786.

Wangai, A., Redinbaugh, M., Kinyua, Z., Miano, D., Leley, P., Kasina, M., Mahuku, J., Scheets, J. and Jeffers, D. 2012a. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Diseaase 96: 1582- 1586.

Wangai, A., Sikinyi, E., Ochieng, J., Miyogo, S., Karanja, T., Odour, H., Kimani, E., Irungu, J., Kinyua, Z., Ngaruiya, P., Ligeyo, D. and Kipkemboi, S. 2012b. Joint assessment report: Report on status of maize lethal necrosis disease and general maize performance. Ministry of Agriculture, Kenya. Online publication. http://www.fao.org/fileadmin/user_upload/drought/docs/Maize%20Lethal%20Necrotic%20Disease%20in%20Kenya_Joint%20Assessment%20Report%20(July%202012).pdf.

Xie, L., Zhang, J., Wang, Q., Meng, C., Hong, J. and Zhou, X. 2011. Characterization of maize chlorotic mottle virus associated with maize lethal necrosis disease in China. Journal of Phytopathology 159: 191-193.

Zaidi, P., Srinivasan, G., Cordova, H. and Sanchez, C. 2004. Gains from improvement for mid-season drought tolerance in tropical maize (*Zea mays* L.). Field Crops Research 89: 135-152.

Zare-Kohan, M. and Heidari, B. 2012. Estimation of genetic parameters for maturity and grain yield in diallel crosses of five wheat cultivars using two different models. Journal of Agricultural Science 4: 74 - 85.

Zare, M., Choukan, R., Heravan, E., Bihamta, M. and Ordookhani, K. 2011. Gene action of some agronomic traits in corn (*Zea mays* L.) using diallel cross analysis. African Journal of Agricultural Research 6: 693-703.

Zeinab, E. and Helal, A. 2014. Diallel analysis and separation of genetic variance components in eight faba bean genotypes. Annals of Agricultural Sciences 59: 147-154.

Zhang, Y., Kang, M. and Lamkey, K. 2005. DIALLEL-SAS05: A comprehensive program for Griffing's and Gardner-Eberhart analysis. Agronomy Journal 97: 1097-11

5. CHAPTER FIVE

Combining ability for drought tolerance and maize weevil resistance in tropical maize germplasm

Abstract

Drought and the maize weevil are the most common stresses experienced by small scale farmers in maize grain production and storage, respectively. This study was carried out to assess gene action controlling maize grain yield and other agronomic traits under drought, and storage traits under artificial maize weevil infestation. Twenty-eight single cross maize hybrids generated from a half diallel mating design, involving eight inbred lines were evaluated during seasons 2017A and 2018A across six locations under optimum conditions, and during seasons 2017B and 2018B under managed drought across two locations in Kenya. After harvesting, the optimum sites' grain samples were evaluated for maize weevil resistance under artificial infestation in a post-harvest laboratory. Highly significant ($p \leq 0.01$) genotype and genotype × environment interaction mean squares for grain yield and days to anthesis under drought and optimum conditions were observed, revealing that there was a differential response of hybrids to changes in growing conditions. Highly significant genotypic effects were also observed on the key parameters for maize weevil resistance; Dobie's susceptibility index (dSI), living insects, weight loss and seed damage (SD). Genetic analysis resulted in highly significant mean squares ($p<0.001$) due to both general combining ability (GCA) and specific combining ability (SCA) for grain yield under drought and significant ($p<0.05$) under optimum conditions, suggesting the importance of both additive and non-additive effects. Under maize weevil infestation, highly significant mean squares ($p< 0.001$) due to both GCA and SCA for the key parameters were observed except for GCA mean squares for weight loss which was significant ($p<0.05$). Additive gene action was predominant over non-additive for dSI, SD and living insects under insect infestation and for grain yield under drought. One hybrid, CKDHL120731 × CKDHL120517 showed tolerance to drought and resistance to maize weevil, while 24 hybrids showed only tolerance to drought.

5.1. Introduction

Maize (*Zea mays* L.) is the leading cereal in global production followed by wheat and rice and is the main staple food in many African countries, including Mozambique (Ukeh *et al.*, 2010; Statista, 2019). However, its production and storage is below the demand because of yield losses caused by both field and storage pests (DeGroote, 2002). Drought and maize weevils are among the most important challenges in the maize value chain and are some of the causes of food insecurity in Africa, with drought being the most devastating constraint to crop production worldwide (Toker *et al.*, 2007; Hao *et al.*, 2011; Mir *et al.*, 2012). Drought can reduce maize grain yield by 17 to 90%, being high when the stress occurs during flowering and grain filling stages (Edmeades *et al.*, 1992; Bolaños and Edmeades, 1996; Bänziger *et al.*, 2000).

Imminent climate change which increases global warming resulting in unpredictable rainfall and natural disasters may worsen drought in the near future, subjecting maize to more drought-prone environments (Bolaños and Edmeades; 1996, Betrán *et al.*, 2003a; Campos *et al.*, 2004; Messmer *et al.*, 2009; Hao *et al.*, 2011; Mir *et al.*, 2012). Therefore, breeding of drought-tolerant maize varieties is considered vital to increase the world's maize production (Campos *et al.*, 2004; Xiong *et al.*, 2006) guaranteeing food security globally (Mir *et al.*, 2012). CIMMYT and IITA have adopted drought, as a main objective in their breeding programmes for years, aimed towards achievement of food security in sub-Saharan Africa (Monneveux *et al.*, 2006).

The maize weevil, *Sitophilus zeamais* (Motschulsky), on the other hand, is among the most damaging storage pests of maize in the tropical and subtropical regions. Small scale farmers experience widespread damages due to this pest. Worldwide, stored grain losses range from 20 to 90% have been observed in untreated maize due to *S. zeamais* infestation (Delima, 1987; Giga and Mazarura, 1991). In Mozambique, losses up to 20% have been reported under smallholder storage systems (Cugala *et al.*, 2007). Maize weevil thrives well in temperatures ranging between 15°C and 34°C and causes both quantity and quality losses (Giga *et al.*, 1991; Derera *et al.*, 2014). Infestation starts in the field especially in the early maturing, open-tipped genotypes and this can lead to ear rot and secondary pathogen infections, such as mycotoxins, resulting in production of aflatoxins and fumonisins, which are detrimental to human health (Kankolongo *et al.*, 2009; Dari *et al.*, 2010).

These infections lower the market value of the grain and also endanger the lives of the consumers of the infected grain. In many countries, including Mozambique, maize breeding programmes primarily focused on breeding for high grain yield, resistance to diseases and field pests and little focus on storage pests. It was always assumed that pesticides were the most effective and affordable method of controlling the pests (Dhliwayo and Pixley, 2003). Different synthetic insecticides, including pirimiphos-methyl and other organophosphates applied as liquid or powder have been advocated worldwide but the use in many countries, especially sub-Sahara African is very low due to cost (Dhliwayo and Pixley, 2003; Fato *et al.*, 2008; Cugala *et al.*, 2007; Golob, 2002). The use of chemicals also poses an environmental and health risk because they are indiscriminative in nature, and have side-effects both directly to the health of the farmer, and to the environment; whereas biological control methods are generally not effective although safe (Dalvie *et al.*, 2009; Meissle *et al.,* 2010).

Drought in the field and infestation of grain by maize weevils in storage can occur simultaneously during the main cropping seasons, thus reducing the harvest yield and threatening the food security in many tropical and subtropical environments. Knowledge of the inheritance of combined traits is an important pre-requisite for successful breeding aimed at developing cultivars that can cope with these challenging stresses. The type and magnitude of gene action governing the phenotypic expression of quantitative traits has been extensively studied in maize (Hallauer, 2007). Under drought, Betrán *et al.* (2003a) observed that additive gene action coupled with the severity of drought was important in tropical maize inbred lines. In a different study, Derera *et al.* (2008) also reported that additive gene action was predominant in the expression of most traits, especially grain yield, under both stressed and non-stressed conditions when they evaluated Southern Africa maize inbred lines.

However, the importance of non-additive gene action was more pronounced under non-stressed environments. This suggested that, regardless of the type of germplasm, gene action seems to change depending on the intensity of drought stress. Similar findings were reported for drought tolerance in maize by several other researchers, including Meseka *et al.* (2011) and Makumbi *et al.* (2011). Under maize weevil infestation, Derera *et al.* (1999) reported that additive gene action was more significant than non-additive gene action in defining resistance to the maize weevil, whereas Dhliwayo and Pixley (2003) reported that additive, non-additive and maternal effects were all important in controlling maize weevil

resistance in maize hybrids. Dhliwayo *et al.* (2005) and Dari *et al.* (2010) also observed that both additive and non-additive gene effects were important in the inheritance of maize weevil resistance, although additive was predominant over non-additive gene action for the main weevil resistance parameters. As a result of climate change, the level of drought and maize pests has increased in maize-growing areas and these stresses often occur together. Therefore, it is important to combine drought-tolerance and maize weevil resistance in the same variety.

This study therefore aimed at identifying maize hybrids with combined resistance to weevils and tolerant to drought stresses and determining the gene action controlling the inheritance of both drought and weevil resistance under optimum, drought and maize weevil infestation. The findings will be used for devising a strategy for breeding multiple stress tolerance/resistance in maize, thus increasing food security and improving livelihoods for the small-holder farmers in Africa.

5.2. Materials and Methods

5.2.1. Germplasm

Eight maize inbred lines from the International Maize and Wheat Improvement Center (CIMMYT) developed from different projects (Table 5.1) were crossed in a half diallel mating design in June 2016, during the 2016B season at CIMMYT´s new site at Kenya Agricultural and Livestock Research Organization (KALRO) at Kiboko. Twenty-eight single cross hybrids were generated. The procedure used to manage the crosses and pollination has been described in chapter 3 section 3.2.1.

Table 5.1. List of the inbred lines involved in the diallel-cross and their attributes.

Designation	Inbred line name	Attributes	Origin
Parent 1 (P1)	CKSBL10027	Stem borer resistant	CIMMYT- Kenya
Parent 2 (P2)	CKSBL10011	Stem borer resistant	CIMMYT- Kenya
Parent 3 (P3)	CKDHL120172	Drought tolerant	CIMMYT- Kenya
Parent 4 (P4)	CKDHL121230	Drought tolerant	CIMMYT- Kenya
Parent 5 (P5)	CKSBL10082	Stem borer resistant	CIMMYT- Kenya
Parent 6 (P6)	CKSBL10060	Stem borer resistant	CIMMYT- Kenya
Parent 7 (P7)	CKDHL120731	Storage pests resistant	CIMMYT- Kenya
Parent 8 (P8)	CKDHL120517	Storage pests resistant	CIMMYT- Kenya

5.2.2. Testing environments and field management

The agro-climatic conditions for the three sites used for evaluating the experimental hybrids are described in Table 5.2. The three sites, Kiboko, Kakamega and Embu were used to evaluate the hybrids under optimum conditions, while Kiboko alone was used to evaluate the same hybrids under managed drought in the off season (season B). At all the sites, evaluations were done over two seasons (2017A and 2018A), resulting in a total of six optimum environments and two managed droughts. The choice of sites and trial management (optimum and managed drought) have been described in Chapter 4, section 4.2.2. After harvesting of the optimum trials, the grain was prepared for post-harvest evaluation at the post-harvest laboratory situated at KALRO-Kiboko (37°42' 50.98" E, 02°13' 56.56" S; 947 masl).

Table 5.2. Description of the sites used for the maize hybrid evaluations

Site	Latitude	Longitute	Elevation m asl	Rain fall (mm)	Temperature (°C) min	Temperature (°C) max	Soil texture
KALRO- Kiboko	2°15'S	37°75'E	975	530	14.3	35.1	Sandy clay
KALRO- Kakamega	0°16'N	34°45'E	1585	1916	12.8	28.6	Sandy loam
KALRO- Embu	0° 49'S	37° 42'E	1510	1200	14.1	25.0	Clay loam

5.2.3. Experimental design and planting

The trial consisted of 28 test F1 hybrids and two commercial hybrid checks and two internal checks (within CIMMYT breeding program). For each group of the checks, one was tolerant to drought and one resistant to storage pests. The trial design was a 4 x 8 α-lattice, with two rows per plot, replicated three times. The rows were 4.5 m long, with 19 hills per row, and inter-row spacing of 0.75 m and inter-row spacing of 0.25 m, corresponding to a plant density of 53,333 plants ha^{-1}. In each hill, two seeds were planted and thinned to one plant two weeks after germination, except for border rows which maintained two plants per hill.

5.2.4. Post-harvest insect resistance screening

At harvest, clean ears from each plot were selected, and grain type was collected before shelled and then data on moisture and some grain biochemical properties such as starch, oil and protein content were measured using calibrated Infratec™ 1241 Grain Analyzer. The Infratec™ 1241 Grain Analyzer uses the infrared technology and gives instant readings of the parameters that are set (GRAINtec, 2011). Starch, oil, moisture and protein content in the grain have been related with resistance to storage pest in maize (Serratos *et al.*, 1987;

Arnason *et al*., 1997; Dhliwayo and Pixley, 2001; Garcia-Lara *et al*., 2004; Nhamucho *et al.* 2017)

After harvest, the maize cobs were sun dried for seven days and an application of Gastoxin™ (phosphine fumigant) was used to fumigate the grain in plastic drums for seven days to kill insects or eggs, which might have come from the field. Equal weights of 100±1 g of clean, undamaged grains from each plot were placed in clean 250 cm3 glass jars in the post-harvest laboratory. The jars and the acclimatization process were similar to that described in chapter 3, section 3.2.3. After the acclimatization period, 50 unsexed, adult active maize weevils aged 20 - 25 days old were randomly selected from the laboratory culture and introduced in all jars. The jars were placed in shelves in CTH room, with each treatment replicated three times using the field randomization laid out in a Completely Randomized Design (CRD).

The CRD was used because in the CTH room the environmental conditions were constant and uniform, so the observed differences on the measured parameters were attributed to the genotype effects only. The jars were left undisturbed for a period of 10 days oviposition period as recommended by Siwale *et al.* (2009) and Dari *et al.* (2010). After this period, maize weevils (the adult parents) were taken out from the jars by sieving maize using 4.7 mm and 1.0 mm sieves (Endecotts Limited, UK), separating insects, grains and powder. The grain remained on the 4.75 mm mesh, maize weevil insects went through the 4.75 mm mesh opened sieve and were collected onto the 1.00 mm mesh opening sieve while the powder went through the sieves and collected in the lower pan. During parental removal, dead and live maize weevils were counted using tweezers and a tally counter. The tweezers helped to check whether the immobile weevils were alive or dead. The number of dead parental insects was used to compute the parent mortality during the oviposition period.

5.2.5. Data collection

The data collection was done on plot basis. Recommended procedures by CIMMYT (CIMMYT, 1985) and Magorokosho *et al.* (2008) were followed for data collection in the field. The first and the last plants in each plot were taken as border plants, therefore, they were not used for the assessment of any trait. Data was collected on: days to silking (SD), days to anthesis (AD), plant height (PH) and ear height (EH), grain yield per plot, number of plants at harvest (NP), number of ears at harvest, field weight, grain weight (GW), grain moisture, ear aspect (EA) and plant aspect (PA). Anthesis-silking interval (ASI), ear position

(EPO) and ears per plant (EPP) were calculated or estimated from the collected data. At the drought sites, leaf senescence (SEN) was also assessed.

The grain yield (GY) in tonnes per hectare (tha^{-1}) from g/m^2, was calculated as follow:

$$GY\ (t\,ha^{-1}) = \frac{GW\ (g)}{1000} \times \left[\frac{100 - G.\ moisture\ (\%)}{100 - 12.5}\right] \times \frac{10}{Net\ plot\ area}$$

For the post-harvest experiments, data collection started 25 days after parental removal. The number of emerged maize weevils was counted and removed from the jars every two days until no more weevils emerged. The two-day interval of counts and removal was applied to avoid mating of the emerged insects and laying of eggs in the maize samples to produce new insects, since *S. zeamais* only mate from three days old (Walgenbach and Burkholder, 1987; Danho *et al.* 2002; Ukeh *et al.* 2010).The parental removal was done by sieving contents of the jars on 4.7 mm and 1.0 mm sieves.

The Dobie's susceptibility Index (dSI) was calculated to categorize the entries into resistant or susceptible types using formula below (Dobie, 1974):

$$dSI = 100 \times \frac{\ln\ total\ \ number\ of\ F_1\ \ adults\ emerged}{median\ \ development\ \ period\ \ of\ \ F_1}$$

Where: *ln* is the natural logarithm

After all the progeny had emerged in each jar, the progeny emergence and the median development period (MDP) were estimated per jar. Progeny emergence was considered as the total number of emerged insects in each jar while MDP as the time in days from the middle of the oviposition period (5 days) to the emergence of 50% of the progeny (Dobie, 1974). The classification of the genotypes into resistant or susceptibility was done based on the below scale (Pixley, 1997).

Dobie Index	**Classification**
≤ 4.0	Resistant
4.10 – 6.0	Moderately Resistant
6.10- 8.0	Moderately Susceptible
8.10 – 10.0	Susceptible
>10	Highly Susceptible

After determination of the progeny emergence, each glass jar was opened and the contents separated using the sieves described under 5.2.4 above. The grains were hand-sorted into damaged and undamaged. Each group was then counted and weighed. Grains with holes and/or tunnels caused by insects were considered damaged. Seed damage was calculated as a percentage by dividing the insect damaged seed with the total number of seeds in the sample, while weight loss (WL) was calculated using the Count and Weigh method developed by Boxall (1986), expressed by the formula below:

$$Weight\ loss\ (\%) = \frac{Wu * Nd - Wd * Nu}{Wu * (Nd + Nu)} \times 100$$

Where:

Wu = weight of undamaged grains, Wd = weight of insect damaged grains

Nu = number of undamaged grains Nd = number of insect damaged grains

5.2.6. Analysis of agronomic and post-harvest performance

For agronomic performance, single and combined environment analyses were carried out for the 4×8 α-lattice design (Bänziger *et al.*, 2000) in Fieldbook-IMIS5 (Banziger *et al.*, 2012) statistical software developed by CIMMYT, following the REML procedure, mixed model. Hybrid effects were considered as fixed while the effects of the rest of the sources of variation were random. For post-harvest performance, single as well as combined analyses were carried out for the complete randomized design (no block or replication effects), where the sources of variances are only the hybrids and the error and the hybrids were considered fixed effects.

The statistical models used for the single and across sites analyses for drought and optimum was similar to that described in chapter 4 section 4.2.5.

The maize weevil parental mortality (%), seed damage (%) and grain weight loss (%) were transformed with angular-transformation ($\arcsin\sqrt{proportion}$) while the data for progeny emergence were transformed with logarithm transformation base 10, ($\lg_{10}(x+1)$), where x is the observed value as recommended by Gomez and Gomez (1984). These were done

to normalize the data for analysis, but the final results were presented as back transformed means.

5.2.7. Genetic analysis

Genetic analysis was carried out following the Griffings' Method IV (which excluded the parents and the reciprocal crosses) and model I (fixed) (Griffing, 1956a, Griffing, 1956b). The procedure was similar to that described in chapter 4 section 4.2.6. The genetic analysis was done using only the 28 hybrids that were generated for the half diallel mating design.

5.3. Results

5.3.1. Performance and combining ability estimates

Combined analysis of variance for each field environment and post-harvest performance are shown in Tables 5.3 to 5.5. Statistical models significantly explained the total variation observed for all traits in all individual and across environments analyses and the coefficients of determination (R^2) for grain yield (GY) ranged from 0.90 under drought to 0.92 under optimum while the R^2 for post-harvest Dobie's susceptibility index (dSI) was 0.81. Highly significant ($p<0.001$) differences were observed for the genotypes for the different measured traits, except anthesis-silking interval (ASI) under drought, number of ears per plant (EPP) under optimum, which were significant ($p<0.05$) and median development period (MDP) and grain oil content (OIL) under post-harvest evaluation which were non-significant.

Environmental effects and genotype × environment (GxE) interaction effects were also significant for most traits except EPP, ear position (EPO) and leaf senescence (SEN) under drought and MDP, weight loss (WL) and OIL under post-harvest evaluation where GxE was non-significant. Means for GY were 2.65 t/ha under drought and 5.37 t/ha under optimum. The Dobie's Susceptibility Index (dSI) ranged from 3.73 to 10.01.

5.3.1.1. Performance of the hybrids under managed drought stress conditions

The climatic data of the two seasons during the trial growth are presented in chapter 4 section 4.3.1 on graphics 4.3.1 and 4.3.2 for 2017A and 2018A. The temperatures were slightly higher in 2017 compared to 2018A and the relative humidity was relatively lower in 2017 compared to 2018, which affected negatively some of the collected traits. The trial performance was greater in 2018 than 2017, where the grain yield ranged from 0.50 to 5.74 t/ha with a mean of 3.86 t/ha while in 2017 the grain yield ranged from 0.23 to 2.05 t/ha with a trial mean of 1.43 t/ha. In 2017, only one entry (4%) yielded 2.00 t/ha and the rest were lower, while in 2018 around 24 entries (86%) yielded 3.0 t/ha and higher. In 2017, the trial days to anthesis (AD) varied from 62 to 70 days with anthesis-silk interval (ASI) ranging from -1 to 8 days while in 2018A it ranged from 70 to 96 days, with ASI ranging from 0 to 3 days (Appendix 5-1).

The general combining ability mean squares (MSgca) were highly significant ($p<0.01$) for all parameters except for the plant aspect (PA) and ASI which were significant ($p<0.05$) and for AD which were not significant. The specific combining ability mean squares (MSsca) were highly significant ($p<0.001$) for grain yield (GY), plant height (PH) and senescence, significant ($p<0.05$) for EPP, AD, PA and EA and non-significant for ASI and EPO. The ratios for sum of squares [(SSgca/ SS(gca + sca)], ranged from 0.29 for AD to 0.88 for EPO. The senescence, plant aspect and ear position showed the highest values of the proportion of GCA effects to the total observed genetic variability [(SSgca/SStotal)], 0.3, 0.33 and 0.42, respectively which can suggest good heritability while EPP, AD and ASI showed the lowest proportion of 0.05, 0.05 and 0.08, respectively. In this environment the coefficients of determination (R^2) varied from 0.62 to 0.95, having the lowest for PA and the highest for AD. The grain yield showed the coefficients of determination of 0.90 (Table 5.3).

Table 5.3. Mean squares for grain yield and other traits of the 28 diallel cross hybrids evaluated under managed drought during 2017A and 2018A in Kenya

Source	DF	GY	EPP	AD	ASI	PH	EPO	PA	EA	SEN
ENV	1	247.71***	4.13***	2572.89***	58.82*	33688.34***	0.05***	-	18.6***	10.01**
REP(ENV)	4	21.02***	0.78***	16.83*	66.35***	4087.27***	0.00***	0.76ns	6.77***	33.41***
Cross	27	3.23***	0.06**	66.86***	23.02*	1460.49***	0.00***	1.80***	1.81***	10.40***
Cross x ENV	27	1.65***	0.03ns	129.91***	27.45**	146.51*	0.00ns	-	1.21**	1.16ns
GCA	7	8.34***	0.09**	58.97ns	27.76*	3941.91***	0.02***	3.77*	4.23**	25.06**
SCA	20	1.43***	0.05*	51.49*	12.40ns	591.99***	0.00ns	1.11*	0.94*	5.27***
GCA*ENV	7	4.77***	0.03ns	24.94ns	1.83ns	348.04***	0.00ns	-	2.84***	3.01*
SCA*ENV	20	0.56ns	0.02ns	48.82ns	9.13ns	75.97ns	0.00ns	-	0.64ns	0.52ns
Error	108	0.49	0.03	5.42	12.10	90.18	0.00	0.56	0.52	1.13
R^2		0.90	0.77	0.95	0.63	0.91	0.80	0.62	0.69	0.79
CV		26.51	21.66	3.38	94.34	5.02	4.11	33.04	26.47	18.94
Trial mean		2.65	0.75	70.25	3.03	189.07	0.54	2.27	2.73	5.61
Min		0.37	0.35	66.19	-1.00	149.67	0.49	1.00	2.17	3.75
Max		3.55	0.35	79.57	8.00	210.33	0.58	4.33	4.50	9.00
SSgca/SS(gca + sca)		0.67	0.38	0.29	0.44	0.70	0.88	0.54	0.61	0.62
SSgca/Ssentry		0.67	0.38	0.23	0.31	0.70	0.88	0.54	0.61	0.62
SSsca/Ssentry		0.33	0.62	0.57	0.40	0.30	0.12	0.46	0.39	0.38
SSgca/SStotal		0.11	0.05	0.05	0.08	0.27	0.42	0.33	0.16	0.30

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

GY = grain yield; EPP = ears/plant, AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position; PA = plant aspect; EA = ear aspect; SEN= senascence

5.3.1.2. Performance of the hybrids under optimum conditions

The genotypes under optimum conditions were significantly different and high yielding compared to the drought stress environments. Environmental effects and genotype × environment (GxE) interaction effects were significant for all the measured parameters. The grain yield varied from 2.99 to 6.65 t/ha with an average of 5.37 t/ha. The ASI was lower varying from - 0.17 to 2.0 compared to the ASI under drought conditions which showed an average of 0.79 days, while the anthesis days varied from 65 to 71 days (Table 5.4). All traits showed medium to high coefficients of determination (R^2) ranging from 0.60 (for plant aspect (PA)) to 0.99 for days to anthesis, except number of ears per plant (EPP) which showed a coefficient of determination of 0.50. The coefficient of variation was lower than 20 in most of the traits except for ASI which was high due to the nature of the trait. Out of the 28 evaluated hybrids 13 (46.4%) had across environments yield higher than 5.5t/ha (Appendix 5-2).

The general combining ability mean squares (MSgca) were significant at $p<0.05$ for all traits except ear aspect (EA) while the mean squares due to specific (MSsca) combining ability were highly significant ($p<0.01$) for GY, AD, PH, EPO and EA and non-significant for EPP, ASI and PA. MSgca were highly influenced by the environment compared to the MSsca. MSsca for EPP, ASI, and EPO were not influenced by the environment. The ratios for sum of squares [(SSgca /SS(gca + sca)], ranged from 0.41 for EA to 0.84 for AD. The lowest proportion of GCA effects to the total observed genetic variability [(SSgca / SStotal)] was 0.02 for AD and the maximum was 0.17 for EPO.

Table 5.4. Mean squares for grain yield and other traits of the 28 diallel cross hybrids evaluated under optimum conditions during 2017A and 2018A in Kenya

Source	DF	GY	EPP	AD	ASI	PH	EPO	PA	EA
ENV	5	357.61***	0.20***	10146.89***	44.76***	57885.22**	0.18***	2.32***	22.35***
REP(ENV)	12	5.36***	0.02*	12.97***	2.60**	357.34**	0.00***	0.30ns	1.08***
Cross	27	11.12***	0.02*	57.22***	5.12***	3389.32***	0.01***	0.59**	2.58***
Cross x ENV	135	2.68***	0.02*	7.56***	1.61**	533.11***	0.00***	0.86***	0.66***
GCA	7	19.44*	0.04*	185.18***	15.00***	7121.86*	0.034*	1.06*	4.10ns
SCA	20	7.70**	0.01ns	12.43***	1.66ns	2082.93***	0.00***	0.42ns	2.05***
GCA*ENV	35	5.80**	0.02**	19.43***	2.13**	924.12***	0.00***	1.63***	1.28***
SCA*ENV	100	1.58**	0.01ns	3.41**	1.42ns	252.31***	0.00ns	0.59***	0.44***
Error	324	0.69	0.01	2.35	1.15	125.59	0.00	0.30	0.24
R^2		0.92	0.50	0.99	0.62	0.92	0.90	0.60	0.79
CV		15.52	11.62	2.24	135.49	4.94	4.58	20.21	18.02
Trial mean		5.37	0.93	68.56	0.79	226.89	0.50	2.71	2.71
Min		2.99	0.85	65.06	-0.17	189.27	0.43	2.22	1.94
Max		6.65	1.02	71.44	2.00	252.67	0.54	3.00	3.94
SSgca/SS(gca + sca)		0.47	0.55	0.84	0.76	0.54	0.87	0.47	0.41
SSgca/Ssentry		0.45	0.55	0.84	0.76	0.54	0.87	0.47	0.41
SSsca/Ssentry		0.51	0.45	0.16	0.24	0.46	0.13	0.53	0.59
SSgca/Sstotal		0.05	0.04	0.02	0.11	0.12	0.17	0.03	0.08

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

GY = grain yield; EPP = ears/plant; AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position

PA= plant aspect; EA= Plant aspect

5.3.1.3. Performance of the hybrids under maize weevil infestation

The results showed differences in maize weevil resistant reactions among the evaluated hybrids. The Dobie's susceptibility index (dSI) was used to group the genotypes into different resistance categories ranging from 3.73 to 10.01, with a mean of 8.25. It was observed that out of the 28 hybrids evaluated, only one (0.4%) was classified as resistant, 12 (42.9%) susceptible and 15 (53.6%) highly susceptible. The number of parent mortality and the number of final living insects had great influence on weight loss and seed damage. Only seven entries (25%) showed less than 50 living insects and only two entries (0.07%) showed parent mortality more than 30%. The grain weight loss ranged from 1.42 to 7.99% with only five hybrids (17.9%) with less than 2.5% weight loss. Seed damage was higher than the grain weight loss, ranging from 11.64 to 34.12%, with 19 hybrids (67.9%) having seed damage higher than 20% (Appendix 5-3).

Among the evaluated hybrids, 14% were flint, 35.7% semi flint, 21.4% semi flint/semi dent and 28.6% semi-flint. The protein content among the hybrids varied from 9.09% to 11.40%, while the starch ranged from 68.45% to 70.26%. The oil content and the insect medium development period was not significantly different among the hybrids. In this environment, the coefficients of determination (R^2) varied from 0.42 to 0.95, with the lowest being for oil content which was not significant. The Dobie's susceptibility index (dSI), number of living insects (living insects), grain texture (TEX), grain starch (STARCH) and grain moisture (MOI) showed R^2 above 0.8 (Table 5.5).

The general combining ability mean squares (MSgca) were highly significant ($p<0.01$) for all traits except for the weight loss (WL) and grain moisture (MOI) which were significant at 5%. ($p<0.05$). The specific combining ability mean squares (MSsca) were also highly significant ($p<0.001$) for most of the collected traits, excluding seed damage (SD) which was significant at 5% ($p<0.05$) and weight loss and parental mortality which were non-significant. The grain texture and the protein content showed the highest values of the proportion of GCA effects to the total observed genetic variability [(SSgca/SStotal)], 0.38 and 0.30, while grain moisture and insect parent mortality showed the lowest values with 0.02 and 0.04 respectively. The ratios for sum of squares [(SSgca/SS(gca + sca)] for all traits were above 0.5, where the highest was observed for grain protein content (0.89) and the lowest observed on grain weight (0.51).

Table 5.5. Mean squares for post-harvest insect pest parameters due to infestation of maize weevil (*S. zeamais*) conditions of the 28 diallel cross hybrids evaluated during 2017A and 2018A in Kenya

Source	DF	SI	MDP	living MW	WL	SD	PM	OIL	PROTEIN	MOI	STARCH	TEX
ENV	5	217.19***	1591.14***	9.37225***	0.00072***	0.00401***	0.02524***	28.67***	34.39***	364.71**	146.35***	19.85***
REP(ENV)	12	2.67ns	8.68ns	0.05691ns	0.00017*	0.00018*	0.00028*	3.19ns	0.32ns	0.62*	0.84*	0.92ns
Cross	27	28.18***	27.56ns	0.82353***	0.00019***	0.00068***	0.00045***	4.41ns	7.29***	3.31***	4.30***	17.08***
Cross x ENV	135	3.40***	17.06ns	0.12079***	0.00009ns	0.00015***	0.00020ns	3.42ns	0.61***	1.22***	0.71***	0.94***
GCA	7	72.73***	-	2.14515***	0.00036*	0.00214***	0.0011**	-	24.73***	7.34*	11.90***	43.34***
SCA	20	12.60***	-	0.36096***	0.00012ns	0.00016*	0.0002ns	-	1.08***	2.18***	1.63***	7.55***
GCA*ENV	35	2.57*	-	0.15276***	0.00010ns	0.00019***	0.00035***	-	1.17***	2.29***	1.06***	1.16***
SCA*ENV	100	3.69***	-	0.1096***	0.00009ns	0.00014**	0.00014ns	-	0.44ns	0.78***	0.59*	0.47ns
Error	324	1.66	21.61	0.05684	0.00008	0.00009	0.00016	3.38	0.38	0.32	0.45	0.56
R^2		0.81	0.61	0.82	0.46	0.67	0.77	0.42	0.79	0.95	0.87	0.82
CV		15.60	10.21	14.59	54.21	20.83	28.48	34.97	5.92	4.37	0.97	28.77
Trial mean		8.25	45.52	64.55	3.82	23.06	24.32	5.26	10.38	12.91	69.51	2.61
Min		3.73	43.94	9.11	1.42	11.64	15.90	4.79	9.09	12.44	68.45	1.00
Max		10.01	50.33	99.11	7.99	34.12	43.33	7.53	11.40	14.40	70.26	4.40
SSgca/SS(gca + sca)		0.67	-	0.68	0.51	0.82	0.64	-	0.89	0.54	0.72	0.67
SSgca/Ssentry		0.67	-	0.68	0.51	0.82	0.64	-	0.88	0.58	0.72	0.66
SSsca/Ssentry		0.33	-	0.32	0.49	0.18	0.36	-	0.11	0.49	0.28	0.33
SSgca/Sstotal		0.18	-	0.14	0.05	0.17	0.04	-	0.30	0.02	0.08	0.38

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (p > 5%).

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

5.3.2. Mean performance for yield, drought and post-harvest parameters

The highest yield under optimum conditions was 6.65 t/ha, observed on CKSBL10011 × CKDHL120172 and the trial mean of 5.38 t/ha, while highest grain yield under drought was 5.44 t/ha observed on CKSBL10011 × CKDHL121230 and trial mean of 2.65 t/ha. The single crosses CKSBL10011 × CKDHL120172, CKDHL120172 × CKSBL10060 and CKDHL120172 × CKDHL121230 produced high yields both under optimum and drought stress conditions. The single cross CKSBL10011 × CKDHL120172 had 6.65 t/ha under optimum and 3.55 t/ha under drought, CKDHL120172 × CKSBL10060 yielded 6.44 t/ha under optimum and 3.49 t/ha under drought while CKDHL120172 × CKDHL121230 yielded 6.22 t/ha under optimum and 3.31 t/ha under drought (Table 5.6). CKSBL10011 × CKDHL120172 also showed the lowest anthesis and silk interval (ASI). The single cross CKSBL10011 × CKSBL10060 had the lowest grain yield under optimum (2.99 t/ha), under drought (0.37 t/ha) conditions and the highest score of leaf senescence of 9.0.

The lowest Dobie's susceptibility index (dSI) was 3.87 (resistant) and observed on CKDHL120731 × CKDHL120517. The mean dSI was 8.25 indicating entries were susceptible. The single cross CKDHL120731 × CKDHL120517, apart from having the lowest dSI score, also had the highest parental mortality of 43.33%, highest protein content of 11.40%, lowest number of living insects of 9.0, lowest seed damage of 11.64% and flint grain texture. The parent mortality ranged from 15.90% (CKDHL120172 × CKDHL120731) to 43.33% with a mean of 24.32%, protein content ranged from 9.09% (CKSBL10011 × CKDHL120172) to 11.40% with a mean of 10.38%, the number of living insects ranged from 9 to 99 (CKSBL10027 × CKDHL120172) while the seed damage ranged from 11.64 to 34.12% (CKDHL120172 × CKDHL120731) with a mean of 23.06%.

The CKDHL120172 × CKDHL120731 also showed the lowest parental mortality. The average grain texture of the hybrids was semi flint/semi-dent. The single cross CKSBL10027 × CKDHL120172), showed the highest dSI of 10.01, high number of living insects (99) and the highest starch content of 70.26 and lowest parental mortality of 17.44%. Out of the 28 evaluated hybrids, 24 entries (86%) had good performance under both optimum and drought conditions but were susceptible under post-harvest infestation. Only one hybrid (4%), CKDHL120731 × CKDHL120517, showed good performance under the two field conditions and maize weevil infestation.

Table 5.6. Mean of yield of the 28 test hybrids evaluated under optimum, drought and post-harvest resistance parameters

Entry	Cross	Optimum	Drought			Post harvest- Maize weevil infestation							
		GY opt	GY drght	ASI	SEN	SI	living MW (#)	WL (%)	SD (%)	PM	Protein	Starch	TEX
1	CKSBL10011 × CKDHL121230	5.44	3.55	0.12	4.58	9.21	74	4.21	29.18	20.37	9.49	69.68	3.60
2	CKSBL10011 × CKSBL10082	4.90	2.59	1.27	6.58	8.09	58	6.19	18.71	24.22	10.49	69.08	2.67
3	CKSBL10011 × CKSBL10060	2.99	0.37	2.17	9.00	9.42	90	4.03	25.37	25.08	9.99	69.99	2.07
4	CKSBL10027 × CKSBL10011	5.27	1.30	2.52	8.25	8.62	66	3.69	24.33	22.56	10.27	69.82	2.13
5	CKSBL10011 × CKDHL120172	6.65	3.26	4.44	4.42	9.21	78	3.85	28.31	19.78	9.09	69.83	4.33
6	CKSBL10011 × CKDHL120731	6.01	2.11	4.00	5.67	8.57	58	3.11	21.66	25.50	10.55	68.88	3.33
7	CKSBL10011 × CKDHL120517	6.39	2.87	2.50	5.00	6.61	36	2.09	15.69	29.68	11.21	68.78	4.20
8	CKDHL121230 × CKSBL10082	5.14	2.97	0.67	5.50	8.75	69	4.02	27.57	24.33	10.37	69.93	2.00
9	CKDHL121230 × CKSBL10060	5.59	2.65	1.67	5.08	8.02	49	2.27	20.04	25.02	10.17	69.33	4.04
10	CKSBL10027 × CKDHL121230	5.55	3.04	9.05	4.92	8.96	81	3.44	24.84	20.89	10.46	69.75	1.07
11	CKDHL120172 × CKDHL121230	6.22	3.31	10.18	4.75	9.47	91	5.09	32.45	21.36	9.62	70.24	3.67
12	CKDHL121230 × CKDHL120731	5.50	3.38	3.10	4.58	7.28	61	2.60	22.53	22.11	10.97	69.03	1.20
13	CKDHL121230 × CKDHL120517	5.46	3.37	2.00	5.17	8.23	62	3.42	21.32	26.56	11.09	70.04	2.53
14	CKSBL10082 × CKSBL10060	4.78	2.39	0.35	6.00	8.41	54	4.20	19.03	25.69	10.27	69.59	3.07
15	CKSBL10027 × CKSBL10082	3.76	1.85	2.07	5.83	8.66	80	3.25	21.26	25.11	10.81	69.67	1.53
16	CKDHL120172 × CKSBL10082	5.95	2.92	2.45	4.83	9.05	70	2.98	27.63	19.34	9.52	69.75	1.93
17	CKSBL10082 × CKDHL120731	5.35	2.79	1.60	4.83	8.67	74	3.78	24.73	24.22	11.02	68.64	1.67
18	CKSBL10082 × CKDHL120517	5.33	2.78	2.90	6.00	6.98	31	2.66	15.52	27.78	11.09	69.31	2.73
19	CKSBL10027 × CKSBL10060	4.32	1.40	1.77	8.75	8.29	77	2.86	21.27	25.00	10.25	69.68	1.87
20	CKDHL120172 × CKSBL10060	6.44	3.49	2.49	4.25	8.55	67	3.18	27.79	21.44	9.16	69.86	4.40
21	CKSBL10060 × CKDHL120731	6.09	2.33	1.67	6.83	7.37	50	2.19	18.75	23.56	10.71	68.45	3.27
22	CKSBL10060 × CKDHL120517	5.70	2.24	2.17	6.67	6.53	36	1.42	14.20	34.49	11.09	68.93	4.07
23	CKSBL10027 × CKDHL120172	5.80	2.76	4.13	4.25	10.01	99	7.15	31.41	17.44	9.90	70.26	2.27
24	CKSBL10027 × CKDHL120731	5.23	2.62	1.67	5.33	8.17	67	7.99	24.41	20.92	10.76	69.03	1.04
25	CKSBL10027 × CKDHL120517	5.01	2.66	0.46	5.42	7.68	48	7.21	16.66	27.09	11.06	69.59	2.13
26	CKDHL120172 × CKDHL120731	5.03	3.03	3.57	3.75	9.73	98	5.45	34.12	15.90	10.20	69.58	1.67
27	CKDHL120172 × CKDHL120517	5.65	3.49	4.00	5.00	8.85	74	2.96	25.20	22.32	9.73	70.15	3.67
28	CKDHL120731 × CKDHL120517	5.00	2.66	2.83	5.92	3.73	9	1.66	11.64	43.33	11.40	69.24	1.00
Min		**2.99**	**0.37**	**0.12**	**3.75**	**3.73**	**9**	**1.42**	**11.64**	**15.90**	**9.09**	**68.45**	**1.00**
Max		**6.65**	**3.55**	**10.18**	**9.00**	**10.01**	**99**	**7.99**	**34.12**	**43.33**	**11.40**	**70.26**	**4.40**
Mean		**5.38**	**2.65**	**2.78**	**5.61**	**8.25**	**65**	**3.82**	**23.06**	**24.32**	**10.38**	**69.51**	**2.61**
StError		**0.00**	**0.14**	**0.42**	**0.25**	**0.24**	**4**	**0.32**	**1.07**	**1.00**	**0.12**	**0.09**	**0.21**

GY opt= Grain Yield under optimu conditions, GY drght= Grain Yield under drought conditions, ASI = anthesis-silking interval, SEN= senascence SI= Dobie's Susceptibility Index;
living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage; PM = Parent mortality; Protein= grain Protein content; Starch=grain Starch content, TEX= grain texture

5.4. Discussion

5.4.1. Gene action

The highly significant environmental mean squares observed under drought, optimum and maize weevil artificial infestation indicate that the experimental conditions were different, with each environment unique and suitable for evaluating the test hybrids, while significance of test hybrids suggests that the genetic make-up of these hybrids varied (Allinne *et al.*, 2009; Aly *et al.*, 2011). Significant environmental effects and genotype × environment interactions observed in most recorded traits suggest that results should be described separately for each environment to determine which genotypes are adapted to specific environments (Appendixes 5.1- 5.6). However, across analysis summary can also be done to assess the stability of the genotypes (Gomez and Gomez, 1984; Pimentel-Gomes, 2009; Montgomery, 2015). Evaluating hybrids in multiple environments helps to get unbiased and accurate phenotypic information. The observed significant ($p<0.05$) GCA x environment as well the SCA x environment in some traits, suggests that wide testing in is required for better selection for the parents or or hybrids.

The genetic variation observed for grain yield under drought and optimum conditions was due to the significant contribution of both general and specific combining ability effects (MSgca and MSsca, respectively) suggesting that both additive and non-additive gene action played a significant role in the phenotypic expression of this trait (Falconer and Mackay, 1996; Hallauer, 2007, Acquaah, 2007; Hallauer *et al.*, 2010). Additive and non-additive gene effects had almost equal contributions under optimum conditions, where the ratio [SSgca/SS(gca + sca)], was almost half (0.47), while under drought conditions, the additive gene effects were higher than the non-additive effects with a ratio 0.67. The effect of both additive and non-additive gene action for GY in maize under optimum conditions has been reported by various researchers (Zeinab and Helal, 2014; Abdel-Moneam *et al.*, 2014; Erdal *et al*., 2015; Chauque, 2016; Ertino *et al*., 2017). The significance and prevalence of additive effects in governing yield in tropical maize under drought has also been observed by several investigators (Betrán *et al.*, 2003b; Derera *et al.*, 2008; Oyekunle and Badu-Apraku, 2014). Betrán *et al.* (2003b) and Derera *et al.* (2008) reported that SCA effects for grain yield under drought were less important compared to additive genetic effects.

Significant MSgca and MSsca for number of ears per plant (EPP), plant height (PH), plant aspect (PA), ear aspect (EA) and senescence score (SEN) under drought and anthesis days (AD), plant height (PH) and ear position (EPO) under optimum conditions indicate that they were all under both additive and non-additive genes effects. However, in this study based on the ratio [SSgca/SS(gca+sca)] it was observed that PH, EA, senescence under drought and AD, EPO under optimum were influenced more by additive genes affects while EPP under drought was under non-additive gene effects. The anthesis-silking interval (ASI) and EPO under drought and EPP, ASI and PA under optimum were influenced only by additive gene effects in this environment, while ear aspect (EA) under optimum and anthesis days (AD) under drought were under non-additive gene effects only.

These findings agree with reports by many researchers including Betrán *et al.* (2003b); Derera *et al.* (2008); Adebayo *et al.* (2014) and Chaúque (2016). Non-additive effects for ear aspect (EA) under drought have also been reported by Derera *et al.* (2008). Chaúque (2016) observed highly significant additive gene effects under optimum conditions for PH and AD, and both additive and non- additive gene effects for PH under drought conditions. On the other hand, Betrán *et al.* (2003b) observed additive effects on the inheritance of ASI, senescence and EPP under drought conditions. The EPP has been associated with tolerance to drought in many studies since stress leads to barrenness when maize plants are affected by drought in the period from just before tassel emergence to the beginning of grain filling (Edmeades, 1996).

The genetic variation observed for post-harvest traits under maize weevil infestation were mainly due to the significant contribution of both general and specific combining ability effects (MSgca and MSsca, respectively) suggesting that both additive and non-additive gene action played a significant role in resistance. Comparable findings have been reported by several investigators (Schoonhoven *et al.*, 1975, Widstrom *et al.*, 1975; Widstrom *et al.*, 1983; Tipping *et al.*, 1989; Derera *et al.*, 1999; Derera *et al.*, 2001a; Derera *et al.*, 2001b; Dhliwayo *et al.*, 2005; Dari *et al.*, 2010). Dari *et al.* (2010) also reported that both additive and non-additive gene actions were important for weevil resistance. Although both additive and non-additive gene effects were important, based on the ratio of [SSgca /SS(gca + sca)], it was observed that additive gene effects were predominant over non-additive. Grain weight loss and parent mortality were under the influence of additive gene effects only.

Derera *et al.* (1999) reported that additive gene action was preponderant over non-additive gene action in governing resistance to the maize weevil, whereas Dhliwayo and Pixley (2003) reported that additive, non- additive and maternal effects were all important. Maternal effects have been reported to be vital for weevil resistance of F1 seed (Dhliwayo, 2002), while farmers store F2 grain, where maternal effects have generally dissipated (Dhliwayo *et al.*, 2005). These results suggest the significance of both additive and non-additive gene action for weevil resistance in F2 grain and are similar with reports by Derera *et al.* (1999) and Dhliwayo and Pixley (2003). Therefore, the most effective breeding strategy for maize weevil resistance would be through selection during line development followed by evaluation of specific hybrid combinations among the best identified inbred lines.

The number of living insects, the Dobie's susceptibility index (dSI) and the seed damage revealed high significance of additive gene effects and high values for the ratio [SSgca /SStotal], which can be equated to narrow-sense heritability. These findings imply selections can be effective in breeding for insect resistance through recurrent selection and pedigree methods. The presence of non-additive gene action suggests the importance of dominance effects. Similar findings were reported by Tende (2016) for combined stem borer and storage insect pest resistance in maize hybrids.

5.4.2. Combining drought and maize weevil resistance in one genotype

The reduction in yield due to drought was about 51% which is classified as moderate drought. Bänziger *et al.* (2000) and Betrán *et al.* (2003b) classified experiments with yield reduction of about 50% as moderate drought stress. To be classified as severe drought stress environment, grain yield must be reduced to between 15 – 20% of the yield under well-watered environments at the same site and same season (Bolaños and Edmeades, 1996; Bänziger *et al.*, 2000). Therefore, the drought stress experiment under discussion falls on the moderate drought stress side. The existence of entries with good grain yield under optimum, drought and good maize weevil resistance suggest that drought tolerance and maize weevil resistance can be obtained in maize hybrids with a low yield penalty. However, only one (CKDHL120731×CKDHL120517) out of the 28 single cross hybrids can be included in this category. This hybrid yielded 2.65 t/ha under drought where the trial mean was 2.65 t/ha and 5.0 t/ha under optimum condition where the average yield was 5.37

t/ha and had the lowest Dobie's susceptibility index (dSI) under maize weevil infestation, thus making it resistant based on the SI categories, and also showed less WL, SD, living insects and high parental mortality. Various entries showed good performance under optimum and drought but reacted negatively to maize weevil infestation. This agrees with various studies on the maize weevil resistance. Dari *et al.* (2010) and Derera *et al.* (2014) reported that to develop weevil resistance in a hybrid both parents should be resistant. This result also supports the previous studies on availability of sources of maize weevil resistant (Mwololo *et al.*, 2012; Matewele, 2014; Tende, 2016). These resistance sources can be explored for combined resistance.

Nine hybrids out of the 28, showed yield higher than 3t/ha under drought and above 5t/ha under optimum conditions, also supporting the previous reports about availability of drought tolerant sources in tropical germplasm. All those nine hybrids showed good leaf senescence (score < 5) but with varied ASI, suggesting stronger linkage between drought and leaf senescence compared to drought with ASI. However, this is not in line with Bänziger *et al.* (2000) who suggest equal weight for both traits. This could be attributed to reduced number of hybrids evaluated. All of the top nine hybrid yields under drought share at least one described drought tolerant line, suggesting that the existence of one drought tolerant inbred line in a single cross can make the hybrid tolerant to drought, which is not applicable for the maize weevil resistance in this experiment. This is an agreement with results observed by Derera *et al.* (2008).

5.5. Conclusion

The stressed environments were significantly different from the unstressed environments and the significant genotype × environment interaction revealed that the level of performance of the hybrids depended on the growing conditions.

General and specific combining ability effects were significant for grain yield and most of the other field and storage traits, indicating importance of both additive and non-additive gene action in controlling these traits. However, additive gene action was generally predominant in most of the cases.

Tolerant to drought was observed in many crosses with only one recognized drought tolerant inbred line, while resistance to maize weevil requires a cross with both parents recognized as maize weevil resistant inbred lines.

Combination of drought tolerance and maize weevil resistance was obtained in one single cross (CKDHL120731 × CKDHL120517). Development of tropical maize for combined drought stress tolerance and maize weevil resistance is possible from these sets of parents and it can be faster when the inbred lines are developed for combined drought tolerance and maize weevil resistance conditions.

References

Abdel-Moneam, M., Sultan, M., Sadek, S. and Shalof, M. 2014. Estimation of heterosis and genetic parameters for yield and yield components in maize using the diallel cross method. Asian Journal of Crop Science 6: 101-111.

Acquaah, G. 2007. Principles of plant genetics and breeding, United Kingdom, Oxford, Blackwell Publishing, 485-497.

Adebayo, M., Menkir, A., Blay, E., Gracen, V., Danquah, E. and Hearne, S. 2014. Genetic analysis of drought tolerance in adapted× exotic crosses of maize inbred lines under managed stress conditions. Euphytica 196: 261-270.

Allinne, C., Maury, P., Sarrafi, A. and Grieu, P. 2009. Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions. Plant science 177: 349-359.

Aly, R., Metwali, E. and Mousa, S. 2011. Combining ability of maize (*Zea mays* L.) inbred lines for grain yield and some agronomic traits using top cross mating design. Global Journal of Molecular Sciences 6: 1-8.

Arnason, J., Conilh De Beyssac, B., Philogene, B., Bergvinson, D., Serratos, J. and Mihm, J. 1997. Mechanisms of resistance in maize grain to the maize weevil and the larger grain borer. In: MIHM, J. (ed.) pp 91-95. Insect Resistant Maize.- Recent Advances and Utilization. A Proceedign of an International Symposium. Mexico City, 27 Nov.–3 Dec. 1997: CIMMYT.

Bänziger, M., Edmeades, G., Beck, D. and Bellon, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice, Mexico D.F.: CIMMYT.

Banziger, M., Vivek, B., Ayala, C. and Norgaard, J. 2012. *Fieldbook-IMIS 5*, Mexico, D.C.: CIMMYT.

Betrán, F., Beck, D., Benziger, M. and Edmeades, G. 2003a. Secondary traits in parental inbreds and hybrids under stress and non-stress environments in tropical maize. Field crops research 83: 51-65.

Betrán, F., Ribaut, J., Beck, D. and De Leon, D. 2003b. Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Science 43: 797-806.

Bolaños, J. and Edmeades, G. 1996. The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. Field Crops Research 48: 65-80.

Boxall, R. 1986. A critical review of the methodology for assessing farm level grain losses after harvest. Report of the TDR G191.

Campos, H., Cooper, M., Habben, J., Edmeades, G. and Schussler, J. 2004. Improving drought tolerance in maize: a view from industry. Field crops research 90: 19-34.

Chaúque, P. 2016. Genetic and path coefficient analyses and heterotic orientation of maize germplasm under combined heat and drought stress in sub-tropical lowland environments. PhD Thesis, University of KwaZulu-Natal, SA.

CIMMYT. 1985. Managing trials and reporting data for CIMMYT's International maize testing program. , CIMMYT-Int., Mexico DF. 20pp.

Cugala, D., Sidumo, A., Santos, L. and Givá, N. 2007. Uso do método de controlo biológico contra a broca maior do grão do milho armazenado, *Prostephanus truncatus* (horn) (*Coleoptera*: *Bostrichidae*) nos celeiros das famílias rurais em Moçambique. Moçambique- Maputo: Universidade Eduardo Mondlane.

Dalvie, M., Africa, A. and London, L. 2009. Change in the quantity and acute toxicity of pesticides sold in South African crop sectors, 1994–1999. Environment international 35: 683-687.

Danho, M., Gaspar, C. and Haubruge, E. 2002. The impact of grain quality on the biology of *Sitophilus zeamais* Motsch. (*Coleoptera*:Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio. Journal of Stored Products Research 38: 259-266.

Dari, S., Pixley, K. and Setimela, P. 2010. Resistance of early generation maize inbred lines and their hybrids to maize weevil [*Sitophilus zeamais* (Motschulsky)]. Crop Science Society of America 50: 1310-1317.

Degroote, H. 2002. Maize yield losses from stemborers in Kenya. Insect Science and its Application 22: 89-96.

Delima, C. 1987. Insect pests and post harvest problems in the tropics. Insect Science and its Applications 8: 673-676.

Derera, J., Pixley, K. and Giga, D. 1999. Inheritance of maize weevil resistance in maize hybrids among maize lines from Southern Africa, Mexico and CIMMYT Zimbabwe.

Maize Production Technology for the Future: Challenges and Opportunities. Proceedings of the Eastern and Southern Africa Regional Maize Conference 6; 21-25 Sep 1998, CIMMYT EARO, Addis Ababa (Ethiopia).

Derera, J., Pixley, K. and Giga, D. 2001a. Resistance of maize to the maize weevil. I. Antibiosis. African Crop Science Journal 9: 431-440

Derera, J., Pixley, K. and Giga, D. 2001b. Resistance of maize to the maize weevil: II. non-preference. African Crop Science Journal 9: 441-450.

Derera, J., Pixley, K. and Giga, D. 2010. Appraisal of protocol for the rapid screening of maize genotypes for maize weevil resistance African Entomology 18: 8-16.

Derera, J., Pixley, K., Giga, D. and Makanda, I. 2014. Resistance of maize to the maize weevil: III. Grain weight loss assessment and implications for breeding. Journal of Stored Products Research 59: 24–35.

Derera, J., Tongoona, P., Vivek, B. S. and Laing, M. D. 2008. Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. Euphytica 162: 411-422.

Dhliwayo, T. 2002. Breeding investigations for resistance to the maize weevil (*Sitophilus zeamais*, Motsch.) in maize (*Zea mays* L.). Mphil Thesis, University of Zimbabwe, Zimbabwe.

Dhliwayo, T. and Pixley, K. 2001. Breeding for resistance to maize weevil (Sitophilus zeamais Motsch.): Is it feasible? Seventh Eastern and Southern Africa Regional Maize Conference, 11- 15th February, pp. 134-138.

Dhliwayo, T. and Pixley, K. 2003. Divergent Selection for Resistance to Maize Weevil in Six Maize Populations. Crop Science Society of America 43: 2043-2049.

Dhliwayo, T., Pixley, K. and Kazembe, V. 2005. Combining Ability for Resistance to Maize Weevil among 14 Southern African Maize Inbred Lines. Crop Science Society of America 45: 662-667.

Dobie, P. 1974. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by *Sitophilus zeamais*. Journal of Stored Products Research 10: 183-197.

Edmeades, G. 1996. Developing Drought and Low N-tolerant Maize: Proceedings of a Symposium, March 25-29, 1996, CIMMYT, El Batán, Mexico, CIMMYT.

Edmeades, G., Bolanos, J. and Lafitte, H. 1992. Progress in breeding for drought tolerance in maize. Proceedings of the 47th Annual Corn and Sorghum Industry Research Conference, 1992 Chicago (USA). American Seed Trade Association, 93–111.

Erdal, S., Pamukcu, M., Ozturk, A., Aydinsakir, K. and Soylu, S. 2015. Combining abilities of grain yield and yield related traits in relation to drought tolerance in temperate maize breeding. Turkish Journal of Field Crops 20: 203-212.

Ertiro, B. T., Beyene, Y., Das, B., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Labuschagne, M. and Prasanna, B. M. 2017. Combining ability and testcross performance of drought-tolerant maize inbred lines under stress and non-stress environments in Kenya. Plant breeding 136: 197-205.

Falconer, D. and Mackay, F. 1996. Introduction to quantitative genetics, New York., Longman Scientific and Technical.

Fato, P., Chaúque, P., Ecole, C. and Cugala, D. 2008. The Status of Development of Maize Resistant to Field and Storage Pests in Mozambique. In: Mugo, S., Gethi, J., Ouma, J., Murenga, G., Mulaa, M., Likhayo, P., Gichuki, V., Kega, V., DeGroote, H. & Chavangi, A. (eds.) Book of abstract of the Insect Resistant Maize for Africa (IRMA) .(2008) " Consolidating Experiences from IRMA I and II: Achievements, Prospects and Lessons", IRMA project ect End-of-Phase II Conference, 28-30 October. Nairobi- Kenya KARI and CIMMYT.

Garcia-Lara, S., Bergvinson, D., Burt, A., Ramputh, A., Diaz-Pontones, D. and Arnason, J. 2004. The role of pericarp cell wall components in maize weevil resistance. Crop Science Society of America, 44, 1546 - 1552.

Giga, D. and Mazarura, U. 1991. Levels of resistance to the maize weevil, *Sitophilus zeamais* (Motsch.) in exotic, local open-pollinated and hybrid maize germplasm. Insect Science and its Applications 12: 159-169.

Giga, D., Mutemerewa, S., Moyo, G. and Neeley, D. 1991. Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. Crop protection 10: 287-292.

Golob, P. 2002. Chemical, physical and cultural of *Prostephanus truncatus*. Integrated Pest Management Reviews 7: 245–277.

Gomez, K. and Gomez, A. 1984. Statistical procedures for agricultural research, J. Wiley and sons, New York, USA.

Graintec. 2011. Foss Infratec 1241- Grain Analyser [Online]. Available: <http://www.graintec.com.au/foss-infratec-1241---grain-analyser.html> [Accessed 11th June 2011].

Griffing, B. 1956a. Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science 9: 463-493.

Griffing, B. 1956b. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10: 31-50.

Hallauer, A. R. 2007. History, contribution, and future of quantitative genetics in plant breeding: lessons from maize. Crop Science 47: 4-19.

Hallauer, A. R., Carena, M. J. and Filho, J. B. 2010. Quantitative genetics in maize breeding New York ; London, Springer.

Hao, Z., Li, X., Xie, C., Weng, J., Li, M., Zhang, D., Liang, X., Liu, L., Liu, S. and Zhang, S. 2011. Identification of functional genetic variations underlying drought tolerance in maize using SNP markers. Journal of integrative plant biology 53: 641-652.

Kankolongo, M., Hell, K. and Nawa, I. 2009. Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. Journal of Food Science and Agriculture 89: 1366-1375.

Magorokosho, C., Vivek, B. and Macrobert, J. 2008. Characterization of maize germplasm grown in eastern and southern Africa: Results of the 2007 regional trials coordinated by CIMMYT. Harare, Zimbabwe.

Makumbi, D., Betrán, J., Bänziger, M. and Ribaut, J. 2011. Combining ability, heterosis and genetic diversity in tropical maize (*Zea mays* L.) under stress and non-stress conditions. Euphytica 180: 143-162.

Matewele, M. 2014. Diversity Analysis and Breeding for Maize Weevil (*Sitophilus zeamais* Motschulsky) and Larger Grain Borer (*Prostephanus truncatus* Horn) Resistance in Productive Maize Germplasm in Malawi. PhD Thesis, University of KwaZulu-Natal, SA.

Meissle, M., Mouron, P., Musa, T., Bigler, F., Pons, X., Vasileiadis, V., Otto, S., Antichi, D., Kiss, J. and Pálinkás, Z. 2010. Pests, pesticide use and alternative options in

European maize production: current status and future prospects. Journal of Applied Entomology 134: 357-375.

Meseka, S., Menkir, A. and Ajala, S. 2011. Genetic analysis of performance of maize inbred lines under drought stress. Journal of crop improvement 25: 521-539.

Messmer, R., Fracheboud, Y., Bänziger, M., Vargas, M., Stamp, P. and Ribaut, J. 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theoretical and Applied Genetics 119: 913-930.

Mir, R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R. and Varshney, R. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125: 625-645.

Monneveux, P., Sanchez, C., Beck, D. and Edmeades, G. 2006. Drought tolerance improvement in tropical maize source populations. Crop Science 46: 180-191.

Montgomery, D. 2015. Design and Analysis of Experiments (8th Edition), Arizona State University, Jhon Wiley & Sons.

Mwololo, J. K., Mugo, S. N., Tefera, T., Okori, P., Munyiri, S. W., Semagn, K., Otim, M. and Beyene, Y. 2012. Resistance of tropical maize genotypes to the larger grain borer. Journal of Pest Science 85: 267-275.

Nhamucho, E., Mugo, S., Gohole, L., Tefera, T., Kinyua, M. and Mulima, E. 2017. Resistance of selected Mozambican local and improved maize genotypes to maize weevil, *Sitophilus zeamais* (Motschulsky). Journal of Stored Products Research 73: 115-124.

Oyekunle, M. and Badu-Apraku, B. 2014. Genetic Analysis of Grain Yield and Other Traits of Early-Maturing Maize Inbreds under Drought and Well-Watered Conditions. Journal of agronomy and crop science 200: 92-107.

Pimentel-Gomes, F. 2009. Curso de estatística experimental. São Paulo, Brasil, Bilbioteca de Ciências Agrárias Luz de Queiroz.

Pixley, K. 1997. CIMMYT Mid-altitude Maize breeding programme. In: CIMMYT-Zimbabwe Annual Research Report 1996/1997, 7-13. Harare, Zimbabwe.

Statista. 2019. Worldwide production of grain in 2018/19, by type (in million metric tons) [Online]. Available: https://www.statista.com/statistics/263977/world-grain-production-by-type/ [Accessed 10 October 2019].

Schoonhoven, A., Horber, E., Wassom, C. and Mills, R. 1975. Selection for resistance to the maize weevil in kernels of maize. Euphytica 24: 639-644.

Serratos, A., Arnason, J., Nozzolillo, C., Lambert, J., Philogiène, B., Fulcher, G., Davidson, K., Peacock, L., Atkinson, J. and Morand, P. 1987. Factors contributing to resistance of exoticmaize populations to maize weevil, *Sitophilus zeamais*. Journal of Chemical Ecology, 13, 751-762.

Siwale, J., Macrobert, J. and Lungu, D. 2009. Comparative resistance of improved maize genotypes and landraces to maize weevil African Crop Science Journal 17: 1-16.

Tende, R. 2016. Combining Chilo partellus Swinhoe and *Sitophilus zeamais* Motschulsky insect pest resistance in early maturing maize hybrids. PhD Thesis, University of KwaZulu-Natal, SA.

Tefera, T., Mugo, S., Likhayo, P. and Beyene, Y. 2011. Resistance of three-way cross experimental maize hybrids to post-harvest insect pests, the large grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*). International Journal of Tropical Insect Science 31: 3-12.

Tipping, P. W., Cornelius, P. L. and Legg, D. E. 1989. Inheritance of resistance in whole kernel maize to oviposition by the maize weevil (*Coleoptera*: Curculionidae). . Journal of Economic Entomology 82: 1466-1469.

Toker, C., Canci, H. and Yildirim, T. 2007. Evaluation of perennial wild Cicer species for drought resistance. Genetic Resources and Crop Evolution 54: 1781-1786.

Tuberosa, R. and Salvi, S. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends in plant science 11: 405-412.

Ukeh, D., Birkett, M., Bruce, T., Allan, E., Pickett, J. and Luntz, A. 2010. Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. Pest Management Science 66: 44-50.

Walgenbach, C. and Burkholder, W. 1987. Mating behaviour of the maize weevil *Sitophilus zeamais* (*Coleoptera*: Curculionidae). Annal of the Entomological Society of America 80: 578-583.

Widstrom, N. W., Hanson, W. D. and Redlinger, L. M. 1975. Inheritance of maize weevil resistance in maize. Crop Science 15: 467- 470.

Widstrom, N. W., Mcmillian, W. W., Redlinger, L. M. and Wiser, W. J. 1983. Dent corn inbred sources of resistance to the maize weevil (*Coleoptera*: Curculionidae). Journal of Economic Entomology 76: 31-33.

Xiong, L., Wang, R., Mao, G. and Koczan, J. 2006. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. Plant physiology 142: 1065-1074.

Zeinab, E. and Helal, A. 2014. Diallel analysis and separation of genetic variance components in eight faba bean genotypes. Annals of Agricultural Sciences 59: 147-154.

6. CHAPTER SIX

Gene action controlling maize lethal necrosis disease and maize weevil resistance in tropical maize germplasm

Abstract

Maize lethal necrosis (MLN) disease and maize weevils (MW) are currently among the main maize constraints limiting maize production under small-scale farming systems in African tropical environments. The MLN disease can devastate maize in the field, while MW infestations occur in storage and both lead to food and seed insecurity. In this study, eight maize inbred lines with varying levels of tolerance to MLN disease and MW infestation were crossed in a half-diallel design. The resultant 28 F1 crosses and 4 commercial checks were evaluated using an alpha lattice design with three replications, under optimum and artificial MLN conditions in Kenya during 2016 and 2017. Grain samples from the optimum sites were evaluated for MW resistance under artificial infestation. Highly significant ($p<0.001$) genotype and genotype × environment interaction effects were observed for MLN scores under artificial MLN infestation; grain yield (GY), plant (PA) and ear (EA) aspects under optimum conditions; parent mortality (PM), Dobie's susceptibility index (dSI), number of living insects, weight loss (WL), seed damage (SD), grain texture (TEX) and protein content under MW infestation. Highly significant ($p<0.001$) genotype differences were observed for field weight (FW) and GY under MLN infestation. Genetic analysis using Griffing's Method 4, detected significant mean squares ($p<0.01$) due to general combining ability (GCA) only for FW and GY under MLN and WL under maize weevil infestation and highly significant mean squares ($p<0.001$) due to both GCA and specific combining ability (SCA) for MLN scores under MLN infestation and GY, PA and EA under optimum growing conditions; PM, dSI, number of living insects, SD, TEX and protein content under weevil infestation suggesting the importance of both additive and non-additive effects. For other traits under the three evaluation conditions, additive gene action was predominant. Three hybrids, CKDHL120918×CKSBL10060, CKSBL10060×CKDHL120731 and CML494×CKDHL120731 showed good performance under the three evaluation conditions and four, CML494×CKSBL10082, CML494×CKSBL10060, CML442×CML494 and CML494×CKDHL120517 showed good performance only under optimum and weevil infestation. The results suggest that breeding for combined MLN and maize weevil resistant can be archived. However, the inbred line development should be developed under, the three conditions for better selection.

6.1. Introduction

Several factors including drought, low soil fertility and socio-economic factors contribute to low productivity of maize in Africa. From 2011, maize lethal necrosis (MLN) disease has become one of the constraints for maize production in Eastern Africa (Mahuku *et al.*, 2015a). The MLN is a foliar disease resulting from co-infection by two viruses, which include maize chlorotic mottle virus (MCMV) and any other virus from the Potyviridae family, such as maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV) or wheat streak mosaic virus (WSMV). In Africa, this disease was first observed in the Rift Valley region (Kenya) in 2011 and then it spread to different maize agro-ecological zones, where it has caused considerable losses (Wangai *et al.*, 2012). Currently, MLN has also been observed in Rwanda, Democratic Republic of Congo, Uganda, Tanzania, Ethiopia and South Sudan (Wangai *et al.*, 2012; Adams *et al.*, 2014; Lukanda *et al.*, 2014; Mahuku *et al.*, 2015b; Flett and Mashingaidze, 2016). It can affect maize plants from seedling to near maturity and when the infestation occurs at an early stage, complete yield loss may be experienced (Uyemoto, 1983; Wangai *et al.*, 2012).

Maize weevil *Sitophilus zeamais* (Motschulsky), on the other hand, is one of the most damaging storage pests of maize causing grain losses of 15 to 30% in tropical and subtropical regions worldwide (Bergvinson, 2001). This seriously compromises the net yield and the next planting season if grain or seed is stored without adequate protection. Control methods have focused mainly on the use of chemicals. However, pesticides alone are not always practical due to various concerns, including, inadequate financial sources among small scale farmers to purchase the chemicals, environmental concerns, restricted accessibility of appropriate formulations in rural areas and the development of maize weevil insecticide-resistance (Guedes *et al.*, 1995; Ribeiro *et al.*, 2003; Oliveira *et al.*, 2007).

Resistance to insecticides has been reported by several researchers including Kljajić and Perić (2006) who observed resistance of granary weevil *Sitophilus granarius* (L.) to a wide variety of insecticides in Serbia and Montenegro. These insecticides included chlorpyrifosmethyl, cypermethrin, deltamethrin, dichlorvos, malathion and pirimphos-methyl. Some of these are among the widely used insecticides globally, including in sub-Saharan Africa to control most of the storage pests including the maize weevil. It is, therefore, important to incorporate an integrated pest management (IPM) approach, which includes development of resistant varieties and complementary techniques that are safe for

human use with less pollution to the environment and are easily accessible to small-scale farmers in the rural areas of developing countries (Derera *et al.*, 2010).

Breeding maize varieties with both resistance to maize lethal necrosis and maize weevils is, thus, a favourable strategy to guarantee high maize yields and less grain losses in storage in tropical countries. Limited MLN studies and numerous laboratory-based storage insect pests' research have shown the availability of genetic variation for these two traits under artificial infestation in tropical maize germplasm. Reports on combining ability estimates for MLN and maize weevil resistance suggest a predominance of additive gene action over non-additive gene action, implying that rapid breeding progress can be archived from recurrent selection (Derera *et al.*, 1999; Dhliwayo *et al.*, 2005; Dari *et al.*, 2010; Beyene *et al.*, 2017).

Dhliwayo and Pixley (2003) also reported significant maternal effects in inheritance of resistance to maize weevil resistance among maize hybrids. The importance of MLN and the maize weevil has increased in maize-growing areas, calling for the development of maize genotypes that are high yielding, and possessing both MLN and maize weevil resistance. This study, therefore, aimed at breeding for combined resistance to maize lethal necrosis disease and maize weevil pest in single cross hybrids and determining the gene action controlling this resistance. The findings would be used to devise a strategy for multiple stress tolerance/resistance breeding, thus increasing food security and improving livelihoods for the small-holder farmers in Africa.

6.2. Materials and Methods

6.2.1. Germplasm

Eight tropical maize inbred lines from the International Maize and Wheat Improvement Center (CIMMYT) developed from different projects, were crossed in a half diallel mating design generating 28 F1 hybrids. The selected parental inbred lines had different levels of resistance or tolerance reactions to storage pests, stem borers, viral diseases, and abiotic stresses (Table 6.1).

Table 6.1. Description and origin of the maize inbred lines used in the diallel-cross to generate the 28 F1 single cross hybrids

Designation	Inbred line name	Attributes	Origin
Parent 1 (P1)	CML395	Resistant to MSV	CIMMYT- Kenya
Parent 2 (P2)	CML442	Drought and Low N tolerant	CIMMYT- Kenya
Parent 3 (P3)	CKDHL120918	Maize lethal Necrosis (MLN) resistant	CIMMYT- Kenya
Parent 4 (P4)	CML494	Maize lethal Necrosis (MLN) resistant	CIMMYT- Kenya
Parent 5 (P5)	CKSBL10082	Stem borer resistant	CIMMYT- Kenya
Parent 6 (P6)	CKSBL10060	Stem borer resistant	CIMMYT- Kenya
Parent 7 (P7)	CKDHL120731	Storage pests resistant	CIMMYT- Kenya
Parent 8 (P8)	CKDHL120517	Storage pests resistant	CIMMYT- Kenya

6.2.2. Testing environments and field management

Crosses were made in June 2016, during the 2016B season at Kenya Agricultural and Livestock Research Organization (KALRO), Kiboko (2°15' 50.24" S, 37°75' 30.11" E, 975 masl). The resultant 28 F1 hybrids developed were then evaluated in four different sites in Kenya, namely Kiboko, Kakamega, Embu and Naivasha (Table 6.2). Kakamega, Kiboko and Embu were used as optimum evaluation sites and the grain samples harvested from these sites were evaluated for maize weevil resistance, while Naivasha was the MLN disease site. At all sites, the evaluation was done in two main seasons, 2017A and 2018A, respectively, resulting in six optimum environments and two MLN disease environments. The choice of these sites and the trial management were as described in chapter 4 section 4.2.2 for optimum and artificial infestation of MLN and chapter 5, section 5.2.4 for post-harvest insect resistance screening. The agro-climatic conditions for the four sites are described in Table 6.2.

Table 6.2. Agro-climatic description of the sites where the diallel-cross hybrids were evaluated

Site	Latitude	Longitute	Elevation m asl	Rain fall (mm)	Temperature (°C) min	Temperature (°C) max	Soil texture
KALRO- Kiboko	2°15'S	37°75'E	975	530	14.3	35.1	Sandy clay
KALRO- Kakamega	0°16'N	34°45'E	1585	1916	12.8	28.6	Sandy loam
KALRO- Embu	0° 49'S	37° 42'E	1510	1200	14.1	25.0	Clay loam
KALRO/CIMMYT- Naivasha	0° 41'S	36° 23'E	1904	131	8.4	27.6	Clay loam

6.2.3. Experimental design and planting

The trial consisted of 28 test F1 hybrids, two commercial hybrid checks and two internal checks (within CIMMYT breeding program). The trial design was a 4 x 8 α-lattice, with two rows per plot, replicated three times, except under MLN infestation were only 1 row plots were used. The rows were 4.5 m long, with 19 hills per row, and inter-row spacing of 0.75 m and intra-row spacing of 0.25 m, corresponding to a plant density of 53,333 plants ha^{-1}. In each hill, two seeds were planted and thinned to one plant two weeks after germination, except for the border rows which maintained two plants per hill.

6.2.4. Data collection

Data were collected on a per plot basis using recommended procedures by CIMMYT (CIMMYT, 1985) and Magorokosho *et al.* (2008), excluding the first and the last plants in each plot, as they were considered as border plants. Under optimum conditions, data was collected on: days to anthesis (AD), days to silking (SD), plant height (PH) and ear height (EH), grain weight (GW) per plot, number of plants at harvest (NP), number of ears at harvest, ear aspect (EA) and plant aspect (PA). Ear position (EPO) and ears per plant (EPP) were calculated or estimated from the collected data while under MLN evaluation sites, four (4) scores of MLN disease reaction for early to late stage, field weight (FW), grain yield (GY), plant and ear aspects were collected.

The grain yield (GY) in tonnes per hectare (t ha^{-1}) were calculated as follows:

$$GY\ (t\,ha^{-1}) = \frac{GW\ (g)}{1000} \times \left[\frac{100 - G.\ moisture\ (\%)}{100 - 12.5}\right] \times \frac{10}{Net\ plot\ area}$$

The MLN disease assessment was done using rating scores collected four times during the growing period at two-week intervals. Score 1 and 2 were considered as MLN-early while the score 3 and 4 were considered MLN-late. Based on these scores, resistant hybrids were the ones with scores 1- 3, tolerant hybrids had scores 4-5 and susceptible ones had scores higher than 5.

After harvesting of the optimum trials, the grain was prepared for post-harvest evaluation at the post-harvest laboratory situated at KALRO-Kiboko (37°42' 50.98" E, 02°13' 56.56" S; 947 masl). Data collection for post-harvest experiments started 24 days after removal of the parental insects as described in chapter 5 section 5.2.5 and data was collected on Dobie's

susceptibility index (dSI), weight loss (WL), seed damage (SD), number of living insects and some seed properties including protein content, starch and oil. The classification of the genotypes into resistant and susceptible categories, was done using Dobie's susceptibility index (dSI) developed by Pixley (1997), where materials with SI below or equal to 4.0 were classified as resistant, 4.1 to 6.0 as moderately resistant, 6.1 to 8.0 as moderately susceptible, 8.1 to 10.0 as susceptible and above 10 classified as highly susceptible.

6.2.5. Analysis of agronomic and post-harvest performance

For agronomic performance, single and combined environment analyses were carried out for the 4×8 α-lattice design (Bänziger *et al.*, 2000) in Fieldbook-IMIS5 (Banziger *et al.*, 2012) statistical software developed by CIMMYT, following the REML procedure, mixed model. Hybrid effects were considered as fixed while the effects of the rest of the sources of variation were random. For post-harvest performance, single as well as combined analyses were carried out for the complete randomized design (no block or replication effects), where the sources of variances are only the hybrids and the error and the hybrids were considered fixed effects.

The statistical models used for the single and across sites analyses for drought and optimum was similar to that described in chapter 4 section 4.2.5.

The maize weevil parental mortality (%), seed damage (%) and grain weight loss (%) were transformed by angular-transformation ($\arcsin\sqrt{proportion}$) while the data for progeny emergence by logarithm transformation base 10, ($\lg_{10}(x+1)$), where x is the observed value, recommended by Gomez and Gomez (1984). These were done to normalize the data for analysis but the final results are presented as back transformed means.

6.2.6. Genetic analysis

Genetic analyses were carried out following the Griffings' Method IV (which excluded the parents and the reciprocal crosses) and model I (fixed) (Griffing, 1956a, Griffing, 1956b). The analysis was similar with analysis described in chapter 4 section 4.2.6. The genetic analysis was done using only the 28 hybrids that were generated for the half diallel mating design.

6.3. Results

6.3.1. Performance and combining ability estimates

Analysis of variance models significantly ($p<0.001$) explained the total variation observed for all traits in all individual and across environments, except for number of ears per plant (EPP) under optimum environment, ear aspect (EA) under MLN infested environments and grain starch under maize weevil infestation, which were non-significant. Analysis of variance for each environmental condition and post-harvest evaluation (Tables 6.3- 6.5) showed highly significant differences among the genotypes in respect of the different traits, excluding for EPP under optimum environment, EA under MLN infested environments and grain starch under maize weevil infestation.

Environmental (E) and genotype × environment interaction (GxE) effects were also significant for most of the traits except for EPP, MLN score 1 and grain starch where GxE were not significant under optimum, MLN and maize weevil infested conditions, respectively. The coefficients of determination (R^2) for grain yield (GY) ranged from 0.62 under MLN to 0.87 under optimum. Grand means for GY were 6.03 t/ha under optimum and 0.32 t/ha under MLN.

6.3.1.1. Performance under maize lethal necrosis environments

Due to the severe MLN infestation in the season 2017A, the trials did not reach harvest maturity, so the data for field weight (FW), grain yield (GY) and ear aspect (EA) were analysed only from the season 2018A. Using an average MLN score from early to late-stage, it was observed that out of the 28 hybrids evaluated, two (7.1%) were resistant, 16 (57.1%) were tolerant and 10 (35.7%) were susceptible. For the MLN early stage, the score ranged from 2.17 to 5.5 while at the late stage the scores were high ranging from 2.92 to 7.42. The field weight varied from 0 to 1.44 t/ha with a trial mean of 0.47 while the grain yield ranged from 0 to 1.07 t/ha with a trial mean of 0.32 t/ha. Only three hybrids showed grain yield above 0.5 t/ha (Appendix 6-1). The ear aspect ranged from 3.3 to 5.0 with a trial mean of 4.06. Higher scores for ear aspect meant bad quality since score 1.0 represented the best and score 5.0 represented the worst.

The general combining ability mean squares (MSgca) were highly significant for all traits while the specific combining ability mean squares (MSsca) were significant for all other parameters excluding FW and GY (Table 6.3). The GCA and SCA effects were significant for all MLN scores. In this environment the coefficients of determination (R^2) varied from

0.38 to 0.85, having the lowest for EA and the highest for MLN score 4. The ratios for sum of squares [(SSgca/ SS(gca + sca)], ranged from 0.66 for GY to 0.86 for MLN score 2. The lowest proportion of GCA effects to the total observed genetic variability [(SSgca/SStotal)] was 0.38 for MLN score 3 and the maximum was 0.53 for MLN score 2.

6.3.1.2. Performance of maize hybrids under managed optimum conditions

The yields varied from 3.81 to 7.41 t/ha with a trial average of 6.03 t/ha. All significant traits showed medium to high coefficients of determination (R^2) which ranged from 0.57 to 0.89, with PA having the lowest and PH the highest values. Fifteen out of the 28 evaluated hybrids (53.6%), showed yield higher than 6.10 t/ha (Appendix 6-2). The plants were generally tall averaging 2.30 m but had very good ear position (EPO) at almost half of the plant height (Table 6.4)

The general and specific combining ability mean squares (MSgca and MSsca), were significant ($p<0.01$) for all parameters. MSgca was influenced more by the environment compared to the MSsca. The interaction effects of GCA and environment (GCAxE) were significant for all recorded traits, while the interaction effects of SCA and environment (SCAxE) were significant only for PA. The ratios of sum of squares [(SSgca / SS(gca + sca)], ranged from 0.57 for GY to 0.86 for EPO. The lowest proportion of GCA effects to the total observed genetic variability [(SSgca / SStotal)] was 0.06 for GY and the maximum was 0.22 for EPO.

Table 6.3. Mean squares for grain yield and other traits of 28 diallel cross hybrids evaluated under MLN artificial infestation during 2017A and 2018A in Kenya

Source	DF	FW	GY	EA	MLN1	MLN2	MLN3	MLN4
ENV	1	-	-	-	3.43***	3.72**	18.67***	8.60***
REP(ENV)	4	0.06ns	0.03ns	0.07ns	0.40ns	2.18***	1.97**	3.38***
Cross	27	0.30***	0.15***	0.53ns	1.76***	3.97***	3.89***	5.86***
Cross x ENV	27	-	-	-	0.23ns	0.57*	0.95**	0.83***
GCA	7	0.87***	0.39**	-	5.62***	13.13***	10.94***	16.78***
SCA	20	0.11ns	0.07ns	-	0.42*	0.76**	1.43***	2.04***
GCA*ENV	7	-	-	-	0.26ns	0.80*	1.02*	1.09**
SCA*ENV	20	-	-	-	0.22ns	0.49ns	0.93**	0.74**
Error	108	0.09	0.05	0.46	0.21	0.35	0.43	0.34
R2		0.64	0.62	0.38	0.72	0.78	0.77	0.85
CV		63.66	69.01	16.67	14.64	13.35	12.39	9.81
Trial mean		0.47	0.32	4.06	3.15	4.40	5.27	5.98
Min		0.00	0.00	3.33	2.00	2.33	2.83	3.00
Max		1.44	1.07	5.00	4.50	6.50	6.83	8.00
SSgca/SS(gca + sca)		0.74	0.66	-	0.83	0.86	0.73	0.74
SSgca/Ssentry		0.74	0.66	-	0.83	0.86	0.73	0.74
SSsca/Ssentry		0.26	0.34	-	0.17	0.14	0.27	0.26
Ssgca/Sstotal		0.47	0.40	-	0.48	0.53	0.38	0.49

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (P > 5%)

FW= Field weight, GY= Grain Yield, EA= ear aspect, MLN1 - MLN4 = MLN scores

Table 6.4. Mean squares for grain yield and other traits of 28 diallel cross hybrids evaluated under optimum conditions during 2017A and 2018A in Kenya

Source	DF	GY	EPP	PH	EPO	PA	EA
ENV	5	383.46***	1.87**	69253.01***	0.223***	3.400***	22.324***
REP(ENV)	12	5.44***	0.22ns	616.29***	0.002*	0.679*	0.950***
Cross	27	11.88***	0.33ns	2431.68***	0.022***	1.512***	2.020***
Cross x ENV	135	2.35***	0.41ns	295.94***	0.002**	0.598***	0.789***
GCA	7	26.23**	-	6002.66**	0.073***	3.677**	5.153**
SCA	20	6.86***	-	1181.84***	0.004***	0.754**	0.918***
GCA*ENV	35	3.94***	-	543.62***	0.003***	0.987***	2.013***
SCA*ENV	100	1.79*	-	209.25ns	0.001ns	0.462*	0.358ns
Error	324	1.26	0.41	172.06	0.00	0.34	0.28
R2		0.87	0.37	0.89	0.85	0.57	0.76
CV		18.61	66.07	5.73	6.91	21.87	20.68
Trial mean		6.03	0.96	228.94	0.47	2.65	2.57
Min		3.81	0.83	199.67	0.39	2.22	1.89
Max		7.40	1.53	251.00	0.54	3.17	3.39
SSgca/SS(gca + sca)		0.57	-	0.64	0.86	0.63	0.66
SSgca/Ssentry		0.57	-	0.64	0.86	0.63	0.66
SSsca/Ssentry		0.43	-	0.36	0.14	0.37	0.34
SSgca/Sstotal		0.06	-	0.08	0.22	0.10	0.10

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (P > 5%)

GY= Grain yield, EPP= ears per plant, PH= Plant height, EPO= ear position, PA= Plant aspect, EA= Ear aspect

6.3.1.3. Performance of maize hybrids under maize weevil infestation

The Dobie's susceptibility index (dSI) was used to group the genotypes into different resistance categories. This index ranged from 3.63 to 9.31, with a trial mean of 7.74, revealing differences in maize weevil resistance reaction among the evaluated hybrids. Out of the 28 hybrids evaluated, only one (3.6%) was classified as resistant, 18 (64.3%) moderately susceptible and nine (32.1%) susceptible. The resistant hybrid, showed relatively lower seed damage, lower weight loss and higher parent mortality. The percent seed damage (SD) was higher than the percent of grain weight loss (WL), and ranging from 12.06 to 34.94%, with a mean of 22.25%, while WL ranged from 1.89 to 6.5% with a mean of 3.28% (Appendix 6-3). The grain protein content among the hybrids varied from 9.71 to 12.42%, with a mean of 11.00%, while the grain oil content ranged from 4.62 to 6.08% with a mean of 5.25% (Table 6.5).

The evaluated hybrids were composed of 18% flint, 39% semi-flint, 14% semi flint/semi-dent, 25% semi dent and 4% dent. The coefficients of determination (R^2) varied from 0.41 to 0.79, with the lowest and non-significant observed for starch and the highest observed for the number of living insects.

The general combining ability mean squares (MSgca) were highly significant ($p<0.01$) for all traits except for parent mortality (PM) which was significant ($p<0.05$). The specific combining ability mean squares (MSsca) were highly significant ($p<0.001$) for most of the recorded parameters, excluding median development period (MDP), weight loss (WL), grain oil content and grain protein content which were non-significant. The environment had a significant influence on GCA for all recorded traits and had a significant influence on SCA only for SI, the number of living insects, weight loss and parent mortality.

The ratios of the sum of squares [(SSgca/SS(gca + sca)] for all traits were above 0.5, where the highest was observed for grain texture (0.91) and the lowest observed for parent mortality (0.53). The grain texture and seed damage showed high values of the proportion of GCA effects to the total observed genetic variability [(SSgca/SStotal)], 0.47 and 0.24, respectively, while median development period, insect parent mortality and grain oil content showed the lowest values with 0.04 and 0.07, respectively.

Table 6.5. Mean squares for post-harvest insect pest parameters of the 28 diallel cross hybrids infested with maize weevil (*S. zeamais*) conditions during 2017A and 2018A in Kenya

Source	DF	SI	MDP	living MW	WL	SD	PM	OIL	PROTEIN	STARCH	TEX
ENV	5	88.19***	998.25***	4.99***	0.048***	0.152***	2.701***	22.55***	34.51***	135.24***	0.20ns
REP(ENV)	12	4.27***	2.92ns	0.00	0.009*	0.036***	0.159***	0.31ns	1.28ns	20.26ns	0.87ns
Cross	27	23.57***	18.21***	0.83***	0.015***	0.121***	0.180***	1.97***	8.78***	22.24ns	14.34***
Cross x ENV	135	2.76***	8.00*	0.10***	0.008***	0.022***	0.052***	0.73ns	1.59***	15.36ns	2.20***
GCA	7	58.02**	52.27***	1.89**	0.046***	0.381***	0.367*	4.81**	29.44***	-	50.40***
SCA	20	11.51***	6.28ns	0.45***	0.003ns	0.030***	0.115***	0.87ns	1.38ns	-	1.72**
GCA*ENV	35	3.66***	13.33***	0.14***	0.007*	0.022**	0.088***	1.23**	3.71***	-	2.95***
SCA*ENV	100	2.44***	6.14ns	0.09***	0.008***	0.022***	0.040**	0.53ns	0.88ns	-	0.68ns
Error	324	1.48	6.30	0.05	0.00	0.01	0.03	0.69	0.89	16.30	0.85
R2		0.76	0.76	0.79	0.54	0.66	0.77	0.55	0.70	0.41	0.76
CV		15.74	5.10	13.72	40.91	22.69	32.75	15.69	8.58	5.87	35.93
Trial mean		7.74	49.16	65	3.28	22.25	25.02	5.28	11.00	68.78	2.57
Min		3.63	47.17	7	1.89	12.06	14.72	4.62	9.71	65.29	1.17
Max		9.31	52.00	106	6.50	34.94	57.06	6.08	12.42	70.35	4.56
SSgca/SS(gca + sca)		0.64	0.74	0.59	0.83	0.82	0.53	0.66	0.88	-	0.91
SSgca/Ssentry		0.64	0.74	0.59	0.82	0.82	0.53	0.63	0.87	-	0.91
SSsca/Ssentry		0.36	0.26	0.41	0.17	0.18	0.47	0.33	0.12	-	0.09
SSgca/Sstotal		0.20	0.04	0.17	0.10	0.24	0.07	0.07	0.22	-	0.47

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%; ns = not statistically significant (P > 5%)

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage; PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; Starch=grain Starch content, TEX= grain texture

6.3.2. Mean performance for yield, MLN scores and post-harvest parameters

The highest grain yield under optimum conditions was 7.40 t/ha observed for entry 14 (CML395×CML494), while under MLN infestation the highest grain yield was 1.07 t/ha for entry 13 (CKDHL120918×CKSBL10060). The trial mean grain yield under optimum was 6.03 t/ha and 15 entries (53.6%) yielded higher than this, while under MLN infestation the trial mean grain yield was 0.32 t/ha and 12 entries (42.9%) yielded above the trial mean. Two entries (7.1%), CKDHL120918×CKSBL10060 and CKDHL120918×CKSBL10082, showed resistance to MLN, while 15 (53.6%) were susceptible. Under maize weevil infestation, only one entry (3.6%) entry 26 (CKDHL12073×CKDHL120517) showed resistance and 16 entries (57.1%) were susceptible.

A few entries showed good performance under the three evaluation conditions and included entry 13 (CKDHL120918×CKSBL10060), entry 11 (CKSBL10060×CKDHL120731) and entry 16 (CML494×CKDHL120731). These yielded above the average under optimum conditions (above 6.03 t/ha), yielded at least 0.5 t/ha under MLN artificial infestation and being classified as tolerant or resistant and they were classified as moderately susceptible under maize weevil infestation. Entries 11 and 13 also showed less weight loss under maize weevil infestation, while entry 16 showed high parent mortality.

Entries 2 (CML494×CKSBL10082), 8 (CML494×CKSBL10060), 15 (CML442×CML494) and 17 (CML494×CKDHL120517) showed good performance under optimum and under maize weevil infestation. They yielded higher than the trial mean (6.03 t/ha) and were classified as moderately susceptible under maize weevil infestation. No single entry showed good performance under optimum and MLN infestation (Table 6.6).

Table 6.6. Mean of yield of 28 diallel cross maize hybrids under optimum, MLN and post-harvest maize weevil infestation

Entry	Cross	Pedigree	Optimum	MLN environment				Post-harvest- Maize weevil infestation								
			GY opt	GY MLN	MLN early stage	MLN late stage	MLN score avarage	SI	MDP (d)	living MW	WL (%)	SD (%)	PM (%)	OIL	PROTEIN	TEX
1	5×6	CKSBL10082 × CKSBL10060	4.96	0.43	3.50	5.08	4.29	6.99	49.00	**39**	**2.22**	**15.89**	30.61	4.97	10.44	2.42
2	4×5	CML494 × CKSBL10082	6.28	0.43	4.00	5.92	4.96	7.64	48.33	54	**2.39**	**15.67**	23.89	4.97	11.36	1.83
3	1×5	CML395 × CKSBL10082	*5.90*	0.12	3.67	6.08	4.88	9.31	47.17	104	4.06	32.83	20.89	5.31	10.34	1.54
4	2×5	CML442 × CKSBL10082	*5.76*	0.30	4.25	5.92	5.08	7.95	48.83	73	3.67	20.94	22.67	5.19	10.38	4.33
5	5×7	CKSBL10082 × CKDHL120731	*5.76*	0.47	3.75	5.58	4.67	8.04	48.67	70	**2.61**	23.44	22.56	5.33	11.22	1.75
6	5×8	CKSBL10082 × CKDHL120517	*5.75*	0.50	3.50	4.92	4.21	6.87	50.11	**42**	**2.94**	19.06	26.22	4.99	11.32	2.67
7	3×5	CKDHL120918 × CKSBL10082	5.40	0.72	2.75	3.92	**3.33**	7.29	49.17	**47**	**2.56**	17.94	22.56	5.61	11.23	1.42
8	4×6	CML494 × CKSBL10060	**7.26**	0.23	4.00	5.83	4.92	7.48	49.50	60	**3.00**	21.39	29.78	5.06	10.18	3.83
9	1×6	CML395 × CKSBL10060	**7.23**	0.12	3.58	6.42	5.00	8.44	47.61	86	4.50	27.89	21.28	5.30	9.71	3.50
10	2×6	CML442 × CKSBL10060	**6.95**	0.13	4.08	6.33	5.21	8.21	49.00	68	3.17	23.00	22.78	5.94	9.84	4.56
11	6×7	CKSBL10060 × CKDHL120731	6.23	0.49	3.42	5.00	4.21	7.41	49.67	50	**2.17**	20.06	28.89	5.54	10.69	2.42
12	6×8	CKSBL10060 × CKDHL120517	**6.61**	0.28	3.75	6.08	4.92	6.95	49.83	**44**	**2.61**	**14.94**	35.83	5.16	11.24	3.17
13	3×6	CKDHL120918 × CKSBL10060	6.14	**1.07**	2.17	2.92	**2.54**	7.27	50.00	**49**	**2.44**	19.89	22.44	5.51	11.34	2.08
14	1×4	CML395 × CML494	**7.40**	0.16	3.58	6.33	4.96	9.27	47.83	106	4.06	34.94	16.67	5.20	10.48	2.00
15	2×4	CML442 × CML494	**6.84**	0.12	4.92	6.58	5.75	6.81	49.33	**49**	**2.06**	**14.39**	26.67	5.14	11.00	4.25
16	4×7	CML494 × CKDHL120731	6.39	0.45	3.75	5.67	4.71	7.03	49.00	**43**	3.56	18.06	**36.44**	5.17	11.71	1.67
17	4×8	CML494 × CKDHL120517	**6.79**	0.18	4.25	6.08	5.17	7.07	49.00	57	**2.28**	18.06	26.44	4.80	11.47	3.20
18	3×4	CKDHL120918 × CML494	5.75	0.42	3.50	5.33	4.42	7.23	49.50	51	**2.67**	18.22	23.56	5.57	**12.11**	1.67
19	1×2	CML395 × CML442	6.25	0.00	4.25	6.58	5.42	9.26	48.33	106	5.50	34.56	20.67	5.02	9.71	4.08
20	1×7	CML395 × CKDHL120731	6.22	0.44	3.83	6.25	5.04	8.99	49.17	104	5.00	32.56	19.33	5.21	10.66	1.24
21	1×8	CML395 × CKDHL120517	6.13	0.07	4.17	5.83	5.00	8.90	48.67	96	4.17	30.89	17.56	4.89	10.21	2.00
22	1×3	CML395 × CKDHL120918	5.41	0.23	2.75	5.17	3.96	9.27	47.83	99	6.50	30.78	14.72	5.42	11.12	1.33
23	2×7	CML442 × CKDHL120731	**6.50**	0.30	4.58	5.83	5.21	8.37	49.33	75	3.56	23.39	20.33	6.08	11.58	3.92
24	2×8	CML442 × CKDHL120517	3.81	0.00	5.50	7.42	6.46	7.77	48.83	53	2.83	15.56	22.61	4.62	11.19	3.86
25	2×3	CML442 × CKDHL120918	5.85	0.31	3.58	5.50	4.54	8.01	49.33	64	3.78	23.00	24.67	5.50	11.52	3.17
26	7×8	CKDHL120731 × CKDHL120517	4.71	0.18	4.25	5.17	4.71	**3.63**	52.00	**7**	**2.28**	**12.06**	**57.06**	5.01	11.63	1.67
27	3×7	CKDHL120918 × CKDHL120731	5.16	0.38	3.25	4.75	4.00	7.85	50.67	67	3.39	25.56	19.33	5.80	**12.42**	1.17
28	3×8	CKDHL120918 × CKDHL120517	5.44	0.34	3.17	5.00	4.08	7.35	50.67	46	**1.89**	18.06	24.06	5.40	11.77	1.25
Min			**3.81**	**0.00**	**2.17**	**2.92**	**2.54**	**3.63**	**47.17**	**7**	**1.89**	**12.06**	**14.72**	**4.62**	**9.71**	**1.17**
Max			**7.40**	**1.07**	**5.50**	**7.42**	**6.46**	**9.31**	**52.00**	**106**	**6.50**	**34.94**	**57.06**	**6.08**	**12.42**	**4.56**
Mean			**6.03**	**0.32**	**3.78**	**5.62**	**4.70**	**7.74**	**49.16**	**65**	**3.28**	**22.25**	**25.02**	**5.28**	**11.00**	**2.57**
StError			**0.15**	**0.04**	**0.13**	**0.17**	**0.14**	**0.22**	**0.19**	**5**	**0.21**	**1.25**	**1.52**	**0.06**	**0.13**	**0.21**

traits values: **Bold** = best entries (1st selection), underlines= good (2nd selection) and *italic*= moderate (3rd selection)

6.4. Discussion

6.4.1. Environmental influence and gene action for MLN resistance and grain yield

The observed highly significant environmental mean squares and genotype × environment interactions under different evaluation conditions indicate that the experimental growing conditions or storage conditions were different from season to season or location to location. This suggests that results should be described separately for detailed information for each environment to determine which genotypes are adapted to a specific environment. However, across environment analysis is useful to assess the stability of the genotypes (Gomez and Gomez, 1984; Pimentel-Gomes, 2009; Montgomery, 2015). Evaluating hybrid in multiple environments helps to get unbiased and accurate phenotypic information. The significant interaction of GCA x environment as well the SCA x environment observed, suggests that wide testing in target environments is essential in selecting desirable parents or hybrids. The Appendixes 6.1 to 6.6 show individual and across environment information of the traits means, GCA and SCA.

The MLN resistance assessed by leaf scores at early and late stages of the maize growth revealed the influence of both additive and non-additive gene effects. Although both additive and non-additive gene effects play a role in controlling the traits, the additive is most important considering the ratio [SSgca / SS(gca + sca)] which for all traits was above 0.70. The importance of additive over non-additive gene effects on the MLN severity have been reported by Beyene *et al.* (2017) who observed GCA:SCA ratio of 3.5 at early stages, and 2.5 at late stage. At the early stages, the hybrids tend to show resistance which is broken up with time destroying the old leaves and affecting the new leaves. Therefore, rapid progress for MLN disease breeding can be feasible from recurrent selection methods.

The genetic variation observed for field (unshelled grain) and grain yield under maize lethal necrosis infestation was due to the significant contribution of the mean squares due to general combining ability (MSgca) suggesting that only additive gene action played an important role in the phenotypic expression of these traits, while under optimum conditions variation was due to the significant contribution of both MSgca and MSsca suggesting that both additive and non-additive gene action played a significant role (Falconer and Mackay, 1996; Hallauer, 2007; Acquaah, 2007; Hallauer *et al.*, 2010). Additive and non-additive

gene effects were equally important in their contributions under optimum conditions, where the ratio [SSgca/SS(gca + sca)], was 0.57, while under MLN conditions, the additive gene effects were predominant over non-additive gene effects with ratios [SSgca/SS(gca + sca)] of 0.74 for field weight and 0.66 for grain weight.

The combined contribution of both additive and non-additive gene action for GY in maize under optimum conditions has been reported by various other researchers (Gamble, 1962; Stuber and Moll, 1971; Moreno-Gonzalez and Dudley, 1981; Mhike *et al.*, 2011; Chaúque, 2016). Studies on the genetics of maize lethal necrosis in tropical maize germplasm are still very few as the disease in Africa it is relatively new. Beyene *et al.* (2017) reported a ratio of 1:1 for GCA/SCA for grain yield under MLN infestation, revealing equal significance of both additive and non-additive gene effects in a study on genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. This is contrary to the observations made in this study, where additive gene effects were predominant over non-additive gene effects.

6.4.2. Gene action involved in important post-harvest and seed biochemical parameters

The genetic variation observed for Dobie's susceptibility index (dSI), number of living insects, seed damage, parent mortality and grain texture under maize weevil infestation were due to the significant contribution of both mean squares of general (MSgca) and specific (MSsca) combining, suggesting that both additive and non-additive gene action played a major role while the genetic variation observed for median development period, weight loss, grain oil and protein content were only due to contribution of general combining ability mean squares indicating that only additive gene action played a role. Although in some above-mentioned parameters both additive and non-additive gene effects were important, based on the ratio of [SSgca /SS(gca + sca)] it was observed that additive gene effects were predominant with the ratio above 0.70 observed. Only the number of living insects and parent mortality showed equal influence of both additive and non-additive gene effects, having the ratios 0.59 and 0.53, respectively.

The influence of both additive and non-additive gene effects in maize weevil resistance has been reported by various researchers including Derera *et al.* (1999), Dhliwayo and Pixley (2003) and Dari *et al.* (2010). Derera *et al.* (1999) reported also that additive gene action was more predominant over non-additive gene action in governing resistance to maize

weevil while Dhliwayo and Pixley (2003) reported also influence of maternal effects on F1 seed. However, farmers harvest and store F2 grain, in which maternal effects mostly dissipated (Dhliwayo, 2002; Dhliwayo *et al.*, 2005). The presence of non-additive gene action emphasizes the need to consider dominance effects. Similar findings were reported by Tende (2016) on the genetic analysis of combined stem borer and storage insect pest resistance in maize hybrids. Effective breeding for maize weevil resistance can therefore be made through the recurrent selection and pedigree methods. This suggests that selection should be done during inbred line development followed by evaluating hybrids combinations among the superior lines.

6.4.3. Combining maize lethal necrosis and maize weevil resistance in same genotype

Maize lethal necrosis disease is a new and serious threat in Africa. The yield reduction from the optimum to MLN in this study was around 95%. Yield loss of around 60% due to this disease under small scale farmers in Kenya have been reported (DeGroote *et al.*, 2016). In commercial varieties, yield losses of 30 to 100% depending on the stage of the disease onset and severity have also been reported (Mahuku *et al.*, 2015a). The existence of genotypes with good grain yield under optimum and MLN infestation with acceptable levels of resistance under maize weevil infestation identified in this study suggest that resistance/tolerance to maize lethal necrosis and maize weevil resistance can be obtained in maize hybrids without or with low yield penalty. Although very few hybrids were observed in this group, this indicates that a breeding programme targeting the combined traits in a single hybrid can be achieved.

The maize weevil resistant genotypes performed poorly under optimum and MLN infestation while the MLN resistant genotypes, in spite of showing moderate Dobie's susceptibility indices (dSI), had lower weight loss and seed damage. The existence of a few maize weevil resistant genotypes in this study emphasises the need for both parents to be resistant *per se,* which was found only in inbred lines CKDHL120731 and CKDHL120517. This is in line with various research work on maize weevil resistance, which observed that to accomplish the highest weevil resistance in a hybrid both parents should be resistant (Dari *et al.*, 2010; Derera *et al.*, 2014). Various hybrids showed good performance under optimum growing

conditions and were moderately susceptible to the maize weevil but with less weight loss and seed damage, supporting the idea that high resistance can be achieved with breeding.

Availability of resistant sources of maize weevil resistance have been reported by several researchers, including Mwololo *et al.* (2012), Matewele (2014) and Tende (2016). However, availability of MLN resistance is still limited, no single hybrid showed good performance under MLN infestation besides the two CKDHL120918×CKSBL10060 and CKDHL120918×CKSBL10082, mentioned above, which also showed acceptable performance under maize weevil infestation with low weight loss, seed damage and number of living insects. The limitation of MLN resistance source germplasm in Africa has been reported. According to Semagn *et al.* (2015), since 2013, more than 95,000 maize germplasm materials, containing CIMMYT Maize lines (CMLs), elite inbred lines, experimental single cross and three-way hybrids from CIMMYT and International Institute of Tropical Agriculture (IITA) and commercial varieties in eastern and southern Africa, maize inbred lines with expired Plant Variety Protection certificates (off-PVP) from USA have been screened under MLN artificial inoculation at Naivasha, Kenya and high levels of susceptibility have been observed, especially on commercial hybrids from East and Southern Africa (ESA).

6.5. Conclusion

General and specific combining ability effects were significant for grain yield and most other field and storage traits, indicating the importance of both additive and non-additive gene action in controlling these traits. However, additive gene action was generally predominant in most of the cases except for grain yield under optimum conditions, where both additive and non-additive were equally important.

Inbred lines CKSBL10060 and CKDHL120731, were involved in the crosses which showed good performance under the three evaluation conditions while CML494 was involved in all the four crosses which performed well under optimum and maize weevil infestation.

Breeding for combined MLN and maize weevil resistant is possible in these sets of parents. However, the inbred line development should be done under, the three conditions for better selection.

References

Acquaah, G. 2007. Principles of plant genetics and breeding, United Kingdom, Oxford, Blackwell Publishing 485-497.

Adams, I., Harju, V., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M., Smith, J., Fox, A. and Uzayisenga, B. 2014. First report of maize lethal necrosis disease in Rwanda. New Disease Reports 29: 22- 22.

Bänziger, M., Edmeades, G., Beck, D. and Bellon, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: from theory to practice, Mexico D.F.: CIMMYT.

Banziger, M., Vivek, B., Ayala, C. and Norgaard, J. 2012. *Fieldbook-IMIS 5*, Mexico, D.C.: CIMMYT.

Bergvinson, D. 2001. Storage Pest Resistance pp 32- 39. In: CIMMYT, 2001. Maize Research Highlights. Mexico City.

Beyene, Y., Gowda, M., Suresh, M., Mugo, S., Olsen, M., Oikeh, S., Juma, C., Tarekegne, A. and Prasanna, B. 2017. Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. Euphytica 213: 224 - 236.

Chaúque, P. 2016. Genetic and path coefficient analyses and heterotic orientation of maize germplasm under combined heat and drought stress in sub-tropical lowland environments. PhD Thesis, University of KwaZulu-Natal, SA.

CIMMYT 1985. Managing trials and reporting data for CIMMYT's International maize testing program. , CIMMYT-Int., Mexico DF. 20pp.

Dari, S., Pixley, K. and Setimela, P. 2010. Resistance of early generation maize inbred lines and their hybrids to maize weevil [*Sitophilus zeamais* (Motschulsky)]. Crop Science Society of America 50: 1310-1317.

Degroote, H., Oloo, F., Tongruksawattana, S. and Das, B. 2016. Community-survey based assessment of the geographic distribution and impact of maize lethal necrosis (MLN) disease in Kenya. Crop protection 82: 30-35.

Derera, J., Pixley, K. and Giga, D. 1999. Inheritance of maize weevil resistance in maize hybrids among maize lines from Southern Africa, Mexico and CIMMYT Zimbabwe. Maize Production Technology for the Future: Challenges and Opportunities. Proceedings of the Eastern and Southern Africa Regional Maize Conference 6; 21-25 Sep 1998, CIMMYT EARO, Addis Ababa (Ethiopia).

Derera, J., Pixley, K. and Giga, D. 2010. Appraisal of protocol for the rapid screening of maize genotypes for maize weevil resistance African Entomology 18: 8-16.

Derera, J., Pixley, K., Giga, D. and Makanda, I. 2014. Resistance of maize to the maize weevil: III. Grain weight loss assessment and implications for breeding. Journal of Stored Products Research 59: 24–35.

Dhliwayo, T. 2002. Breeding investigations for resistance to the maize weevil (*Sitophilus zeamais*, Motsch.) in maize (*Zea mays* L.). Mphil Thesis, University of Zimbabwe, Zimbabwe.

Dhliwayo, T. and Pixley, K. 2003. Divergent Selection for Resistance to Maize Weevil in Six Maize Populations. Crop Science Society of America 43: 2043-2049.

Dhliwayo, T., Pixley, K. and Kazembe, V. 2005. Combining Ability for Resistance to Maize Weevil among 14 Southern African Maize Inbred Lines. Crop Science Society of America 45: 662-667.

Falconer, D. and Mackay, F. 1996. Introduction to quantitative genetics, New York. Longman Scientific and Technical.

FAOSTAT 2019. FAOSTAT: Statistical databases and data sets of the Food and Agriculture Organization of the United Nations: FAOSTAT Metadata/Production/Crops. 23 March 2019 ed Rome: FAO, Rome.

Flett, B. and Mashingaidze, K. 2016. Maize Lethal Necrosis: Possible threat to local maize production [Online]. Grain SA, ARC-Grain Crops Institute, Potchefstroom. Available: https://www.grainsa.co.za/maize-lethal-necrosis:-possible-threat-to-local-maize-production [Accessed 20 June 2019].

Gamble, E. E. 1962. Gene effects in corn (*Zea mays* L.) I. Separation and relative importance of gene effects for yield. Canadian Journal of Plant Science 42: 339-348.

Gomez, K. and Gomez, A. 1984. Statistical producedures for agricultural research, J. Wiley and sons, New York, USA.

Griffing, B. 1956a. Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Science 9: 463-493.

Griffing, B. 1956b. A generalized treatment of the use of diallel crosses in quantitative inheritance. Heredity 10: 31-50.

Guedes, R., Lima, J., Santos, J. and Cruz, C. 1995. Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch.(*Coleoptera*: Curculionidae). Journal of Stored Products Research 31: 145-150.

Hallauer, A. R. 2007. History, contribution, and future of quantitative genetics in plant breeding: lessons from maize. Crop Science 47: 4-19.

Hallauer, A. R., Carena, M. J. and Filho, J. B. 2010. Quantitative genetics in maize breeding New York ; London, Springer.

Kim, S. and Kossou, D. 2003. Responses and genetics of maize germplasm resistant to the maize weevil *Sitophilus zeamais* Motschulsky in West Africa. Journal of Stored products Research 39: 489-505.

Kljajić, P. and Perić, I. 2006. Susceptibility to contact insecticides of granary weevil Sitophilus granarius (L.)(*Coleoptera*: Curculionidae) originating from different locations in the former Yugoslavia. Journal of Stored Products Research 42: 149-161.

Lukanda, M., Owati, A., Ogunsanya, P., Valimunzigha, K., Katsongo, K., Ndemere, H. and Kumar, P. 2014. First report of Maize chlorotic mottle virus infecting maize in the Democratic Republic of the Congo. Plant disease 98: 1448.

Magorokosho, C., Vivek, B. and Macrobert, J. 2008. Characterization of maize germplasm grown in eastern and southern Africa: Results of the 2007 regional trials coordinated by CIMMYT. Harare, Zimbabwe.

Mahuku, G., Lockhart, B., Wanjala, B., Jones, M., Kimunye, J., Stewart, L., Cassone, B., Sevgan, S., Nyasani, J., Kusia, E., Kumar, P., Niblett, C., Kiggundu, A., Asea, G., Pappu, H., Wangai, A., Prasanna, B. and Redinbaugh, M. 2015a. Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-Saharan Africa. Phytopathology 105: 956-965.

Mahuku, G., Wangai, A., Sadessa, K., Teklewold, A., Wegary, D., Ayalneh, D., Adams, I., Smith, J., Bottomley, E. and Bryce, S. 2015b. First report of maize chlorotic mottle virus and maize lethal necrosis on maize in Ethiopia. Plant Disease 99: 1870-1870.

Mason, L. and Zuber, M. 1976. Diallel Analysis of Maize for Leaf Angle, Leaf Area, Yield, and Yield Components 1. Crop Science 16: 693-696.

Matewele, M. 2014. Diversity Analysis and Breeding for Maize Weevil (*Sitophilus zeamais* Motschulsky) and Larger Grain Borer (*Prostephanus truncatus* Horn) Resistance in Productive Maize Germplasm in Malawi. PhD Thesis, University of KwaZulu-Natal, SA.

Mhike, X., Lungu, D. and Vivek, B. 2011. Combining ability studies amongst AREX and CIMMYT maize (*Zea mays* L.) inbred lines under stress and non stress conditions. African Journal of Agricultural Research 6: 1952-1957.

Montgomery, D. 2015. Design and Analysis of Experiments (8th Edition), Arizona State University, Jhon Wiley & Sons.

Moreno-Gonzalez, J. and Dudley, J. W. 1981. Epistasis in related and unrelated maize hybrids determined by three methods. Crop Science 21: 644-651.

Murtadha, M., Ariyo, O. and Alghamdi, S. 2018. Analysis of combining ability over environments in diallel crosses of maize (*Zea mays* L.). Journal of the Saudi Society of Agricultural Sciences 17: 69-78.

Mwololo, J. K., Mugo, S. N., Tefera, T., Okori, P., Munyiri, S. W., Semagn, K., Otim, M. and Beyene, Y. 2012. Resistance of tropical maize genotypes to the larger grain borer. Journal of Pest Science 85: 267-275.

Oliveira, E., Guedes, R., Tótola, M. and De Marco Jr, P. 2007. Competition between insecticide-susceptible and-resistant populations of the maize weevil, *Sitophilus zeamais*. Chemosphere 69: 17-24.

Pimentel-Gomes, F. 2009. Curso de estatística experimental. São Paulo, Brasil, Bilbioteca de Ciências Agrárias Luz de Queiroz.

Pixley, K. 1997. CIMMYT Mid-altitude Maize breeding programme. In: CIMMYT-ZIMBABWE (ed.) Annual Research Report 1996/1997, 7-13. Harare, Zimbabwe.

Ribeiro, B., Guedes, R., Oliveira, E. and Santos, J. 2003. Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (*Coleoptera*: Curculionidae). Journal of Stored Products Research 39: 21-31.

Salazar, F., Pandey, S., Narro, L., Perez, J., Ceballos, H., Parentoni, S. and Filho, B. 1997. Diallel analysis of acid-soil tolerant and intolerant tropical maize populations. Crop science 37: 1457-1462.

Semagn, K., Beyene, Y., Babu, R., Nair, S., Gowda, M., Das, B., Tarekegne, A., Mugo, S., Mahuku, G. and Worku, M. 2015. Quantitative trait loci mapping and molecular breeding for developing stress resilient maize for sub-Saharan Africa. Crop Science 55: 1449-1459.

Stuber, C. and Moll, H. 1971. Epistasis in maize (*Zea mays* L.). II: Comparison of selected with unselected populations. Genetics 67: 137-149.

Tende, R. 2016. Combining Chilo partellus Swinhoe and *Sitophilus zeamais* Motschulsky insect pest resistance in early maturing maize hybrids. PhD Thesis, University of KwaZulu-Natal, SA.

Tefera, T., Mugo, S., Likhayo, P. and Beyene, Y. 2011. Resistance of three-way cross experimental maize hybrids to post-harvest insect pests, the large grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*). International Journal of Tropical Insect Science 31: 3-12.

Uyemoto, J. 1983. Biology and control of maize chlorotic mottle virus. Plant Disease 67: 7-10.

Wangai, A., Redinbaugh, M., Kinyua, Z., Miano, D., Leley, P., Kasina, M., Mahuku, J., Scheets, J. and Jeffers, D. 2012. First Report of Maize chlorotic mottle virus and Maize Lethal Necrosis in Kenya. Plant Diseaase 96: 1582- 1586.

7. CHAPTER SEVEN

General overview

7.1. Introduction

This chapter provides a synopsis of the study on “Inheritance of post-harvest pest resistance and genetic analysis of combining drought tolerance, maize lethal necrosis and maize weevil resistance in tropical maize germplasm”. The investigation was accomplished through four experiments, each of which was addressing an objective. The synopsis reports the key objectives and highlights the main findings and their implications for future breeding activities for the target stresses.

7.2. Major findings

7.2.1. Heritability and gene effects controlling post-harvest maize weevil and larger grain borer resistance in tropical maize germplasm

This study aimed at determining the genetic effects influencing the inheritance of resistance to *S. zeamais* and *P. truncatus* in two crosses from divergent inbred lines for resistance to storage pests using generation mean analysis method involving six generations namely; two parents (P1 and P2), F1, F2 and backcross generations (BCP1 and BCP2). The results showed differences in the storage pest resistance levels in the different generations of the two crosses. Additive, dominance and epistasis gene effects were observed for *S. zeamais* resistance in maize, whereas, *P. truncatus* resistance was under the influence of only additive and dominance gene action. Further, moderate narrow-sense heritability estimates were observed confirming the involvement of additive and non-additive gene effects in the expression of resistance to both insect pests. The additive and pooled dominance × dominance effects did not have significant effects in both crosses for all traits under *S. zeamais* infestation while the interaction effects of additive × dominance had significant effects. For *P. truncatus* resistance, the cross which did not fit on the simple additive-dominance model, did not show significance for additive gene effects but showed significance of dominance, interaction of additive × dominance and pooled additive ×

additive in all traits. For all susceptibility parameters recorded P1 and P2 showed contrasting reactions while the other generations showed continuous variations.

7.2.2. Genetic analyses and potential of combining drought tolerance and maize lethal necrosis (MLN) resistance in tropical maize germplasm

Diallel analysis involving eight inbred lines with varying levels of drought tolerance and maize lethal necrosis resistance revealed that the two traits can be combined in hybrids. Genotypes and environments were significantly different resulting in significant genotype × environment interaction effects for grain yield under stress and optimum conditions. The yield reduction due to MLN was 93% of the optimum (6.04 t/ha), while reduction due to drought was 67%. Out of the 28 evaluated hybrids, 42.8% were resistant to MLN at the early stage and 57.2% tolerant, while at late stages only 3.6% were resistant and 35.7% tolerant, suggesting that the breeding should focus more on the late stage MLN scores. Under drought, out of 28 hybrids evaluated in the two seasons 67.9% yielded above 2 t/ha, 42.9% showed an anthesis-silking interval (ASI) below 4 days and 42.9% had low leaf senescence scores, indicating the existence of drought-tolerant hybrids in the set.

The highest yield under optimum conditions was 7.35 t/ha with trial mean of 6.04 t/ha, under MLN infestation the highest grain yield was 2.32 t/ha with the trial mean of 0.43 t/ha while under drought conditions the highest was 2.67 t/ha with a trial mean of 1.99 t/ha. The single cross CKSBL10011×CKDHL120918 showed the highest yield under drought of 2.01 t/ha, the lowest late MLN score under the MLN environment and 5.50 t/ha under optimum conditions while CKSBL10011×CML494 was the only entry which showed good performance across the three environments. Mean squares due to both general combining ability (GCA) and specific combining ability (SCA) were significant for most of the recorded traits, including grain yield under all environments, suggesting the importance of both additive and non-additive gene effects. However, additive gene action was predominant across all evaluation conditions.

7.2.3. Combining ability for drought tolerance and maize weevil resistance in tropical maize germplasm

This study involved eight parental inbred lines with varied reaction to drought and the maize weevil used to generate 28 single cross hybrids in a half diallel mating design and evaluated across six sites under optimum conditions and over two sites under managed drought

stress. The grain samples harvested from the optimum sites were evaluated for maize weevil resistance under artificial infestation in a post-harvest laboratory. Genotype and genotype × environment interaction mean squares for important traits under drought (grain yield, days to anthesis and leaf senescence), optimum conditions (grain yield, days to anthesis) and maize weevil infestation (Dobie's susceptibility index, living insects, weight loss and seed damage) were significant.

Out of the 28 evaluated hybrids, 24 entries (86%) showed good performance under optimum and drought conditions but had susceptible reactions under post-harvest insect pest infestation. Only one hybrid (4%), CKDHL120731 × CKDHL120517, performed well under the two field conditions and under maize weevil infestation. Significant mean squares due to both general combining ability (GCA) and specific combining ability (SCA) for grain yield under drought and optimum conditions and for the key resistance parameters under maize weevil infestation were observed indicating the importance of both additive and non-additive gene action in controlling these traits. Additive gene action was generally predominant in most of the cases. For grain yield under drought, Dobie's susceptibility index, seed damage and living insects under maize weevil infestation, additive gene action was predominant. Resistance to drought was observed in many crosses with at least one drought-tolerant inbred line parent, while resistance to maize weevil requires a cross with both maize weevil resistant inbred line parents.

7.2.4. Gene action controlling important traits under optimum, maize lethal necrosis and maize weevil infestation in tropical maize germplasm

Eight maize inbred lines with varying levels of tolerance to MLN and maize weevil resistance were crossed in a half-diallel design generating 28 F1 hybrids. The hybrids were evaluated in the field under optimum and artificial MLN conditions in Kenya and after harvesting, grain samples from optimum sites were evaluated for maize weevil resistance under artificial infestation. Grain yield under MLN infestation was not influenced by the environment.

Highly significant genotype and genotype × environment interaction effects were observed for MLN scores under artificial MLN infestation; grain yield, plant and ear aspects under optimum and the key parameters to access maize weevil resistance, namely, parent mortality, Dobie's Susceptibility Index, number of living insects, weight loss, seed damage, grain texture and protein content under weevil infestation revealing that the hybrids differed and their performance changed significantly with change in growth and storage conditions.

Significant mean squares due to only general combining ability (GCA) for grain yield under MLN and weight loss under maize weevil infestation, and due to both GCA and specific combining ability (SCA) for MLN scores under MLN infestation and grain yield, plant and ear aspects under optimum growing conditions and parent mortality, Dobie's Susceptibility Index, number of living insects, seed damage, grain texture and protein content under weevil infestation were observed suggesting the importance of both additive and non-additive effects. Additive gene action was predominant for most of the traits under the three evaluation conditions. Three hybrids, CKDHL120918 × CKSBL10060, CKSBL10060 × CKDHL120731 and CML494 × CKDHL120731 showed good performance under the three evaluation conditions and four, CML494 × CKSBL10082, CML494 × CKSBL10060, CML442 × CML494 and CML494 × CKDHL120517 showed good performance only under optimum and weevil infestation

7.3. The implication of the findings in the practical breeding programs

Drought, maize lethal necrosis and maize weevil infestation can occur simultaneously during the main cropping seasons in many tropical environments, causing frequent crop failures and grain loss, causing food insecurity among small-scale farmers in Africa. This has raised many concerns among farmers and public leadership and triggered exciting debates among scientists during recent years. In the past, breeding programs focused on improving tolerance or resistance to a single stress in a genotype. However, from a crop improvement perspective, breeding varieties with increased resilience to multiple stresses is important to meet the food demands for the increasing population in the tropical and subtropical hot and water-limited environments.

The observed genetic variability for combined traits in tropical maize germplasm in this study, is important information for breeders since effective selection for a particular trait can only be successful when there is genetic variation in the available germplasm. The identified superior genotypes across environments can be used immediately in breeding programs, especially in sub-Saharan Africa. In addition, the results indicated that the improvement of tropical maize for combined stresses is possible and it can be faster when the inbred lines and hybrids are developed and evaluated under combined stress environments, than under a single stress.

The importance of both additive and non-additive gene action, with increased prevalence of additive gene action rather than non-additive gene action, especially under stressful environments, is an indicator of the feasibility of breeding for tolerance/ resistance to combined stresses, and suggesting that recurrent selection can be applied for rapid breeding progress. Inbred lines with favourable alleles for grain yield under drought tolerance and MLN infestation in the field and with maize weevil resistance in the storage were identified. Three inbred lines CKSBL10011, CKDHL120918, CML494, CKDHL120731 and CKDHL120517 were good combiners and can be good sources of resistance genes in breeding for combined drought tolerance, MLN and maize weevil resistance in maize.

The findings of this research are important for multiple stresses breeding in maize, and will act as baseline studies for future research when breeding for combined stresses, especially, drought, maize lethal necrosis and post-harvest pests.

APPENDICES

Appendix 4-1. Means of the GY and other traits under across analysis for MLN virus infested environments

Entry	Cross	Pedigree	MLN1	MLN2	MLN3	MLN4	GY	EPP	NP	PH	EPO
1	2×4	CKSBL10011 × CKDHL121230	2.50	3.67	4.67	5.33	0.51	0.77	9.67	138.00	0.65
2	2×8	CKSBL10011 × CML494	3.17	4.83	5.17	5.50	0.59	0.55	9.67	126.67	0.61
3	1×2	CKSBL10027 × CKSBL10011	3.00	3.83	5.00	5.33	0.81	0.97	9.67	156.67	0.62
4	2×5	CKSBL10011 × CML395	3.17	3.83	4.50	5.67	0.45	0.51	7.00	141.83	0.58
5	2×6	CKSBL10011 × CML442	3.33	4.50	5.83	6.50	0.71	0.96	7.67	130.28	0.64
6	2×3	CKSBL10011 × CKDHL120172	2.50	3.67	4.83	5.67	0.54	0.73	9.67	149.67	0.66
7	2×7	CKSBL10011 × CKDHL120918	2.17	2.33	2.83	2.83	2.32	1.52	10.00	175.67	0.57
8	4×8	CKDHL121230 × CML494	3.00	4.67	5.50	6.33	0.78	0.82	10.00	125.00	0.60
9	1×4	CKSBL10027 × CKDHL121230	3.00	4.67	5.50	6.50	0.00	-	9.00	122.50	0.69
10	4×5	CKDHL121230 × CML395	3.00	3.83	5.00	6.17	0.00	-	7.00	116.25	0.64
11	4×6	CKDHL121230 × CML442	3.67	4.83	5.33	6.83	0.00	-	8.33	107.08	0.62
12	3×4	CKDHL120172 × CKDHL121230	2.67	4.00	5.00	6.00	0.00	-	9.33	124.50	0.63
13	4×7	CKDHL121230 × CKDHL120918	2.50	4.00	4.67	5.83	0.00	-	10.00	128.67	0.57
14	1×8	CKSBL10027 × CML494	3.67	4.83	5.50	6.33	0.00	-	8.67	129.58	0.60
15	5×8	CML395 × CML494	2.83	4.17	5.50	6.33	0.80	0.54	10.00	154.33	0.57
16	6×8	CML442 × CML494	4.17	5.50	5.83	7.00	0.00	-	5.67	127.50	0.71
17	3×8	CKDHL120172 × CML494	3.33	4.67	5.33	6.33	0.90	0.51	9.33	136.89	0.62
18	7×8	CKDHL120918 × CML494	2.67	4.00	5.00	6.17	0.88	1.05	9.67	131.33	0.62
19	1×5	CKSBL10027 × CML395	3.17	4.00	5.50	6.83	0.00	-	9.33	156.56	0.67
20	1×6	CKSBL10027 × CML442	3.67	5.00	5.67	6.50	0.00	-	10.33	139.37	0.59
21	1×3	CKSBL10027 × CKDHL120172	2.33	3.67	4.67	5.67	0.16	0.72	9.33	160.67	0.65
22	1×7	CKSBL10027 × CKDHL120918	2.50	3.67	4.67	5.17	0.63	1.06	8.33	145.67	0.64
23	5×6	CML395 × CML442	3.50	5.00	6.00	7.67	0.00	-	3.33	159.67	0.62
24	3×5	CKDHL120172 × CML395	3.50	5.17	6.67	8.50	0.00	-	2.33	163.67	0.62
25	5×7	CML395 × CKDHL120918	2.33	3.00	4.00	5.17	0.93	0.50	10.33	159.67	0.61
26	3×6	CKDHL120172 × CML442	3.33	5.17	6.00	6.67	0.00	-	6.00	121.67	0.57
27	6×7	CML442 × CKDHL120918	3.17	4.67	5.50	6.33	0.52	0.62	9.33	132.00	0.65
28	3×7	CKDHL120172 × CKDHL120918	2.50	3.83	4.83	5.67	0.57	0.72	9.67	141.25	0.51
29	WE6109	WE6109	2.00	3.00	4.17	4.67	0.86	0.91	11.00	172.00	0.91
30	CZH1258	CZH1258	3.33	4.83	5.50	6.83	0.00	-	6.67	133.33	0.66
31	WE1101	WE1101	3.50	5.00	5.83	6.67	0.00	-	5.00	118.57	0.31
32	PHB3253	PHB3253	3.83	5.33	6.50	7.50	0.00	-	-	-	-
	Min		**2.00**	**2.33**	**2.83**	**2.83**	**0.00**	**0.50**	**2.33**	**107.08**	**0.31**
	Max		**4.17**	**5.50**	**6.67**	**8.50**	**2.32**	**1.52**	**11.00**	**175.67**	**0.91**
	Mean		**3.03**	**4.29**	**5.20**	**6.14**	**0.40**	**0.79**	**8.43**	**139.56**	**0.62**
	StError		**0.09**	**0.13**	**0.13**	**0.18**	**0.09**	**0.05**	**0.37**	**3.06**	**0.02**

MLN1- MLN4= MLN scores, GY = grain yield; EPP = ears/plant, NP= number of plants, PH = plant height; EPO = ear position

Appendix 4-2. Means of the GY and other traits under across analysis for drought environments

Entry	Cross	Pedigree	GY	EPP	EA	AD	ASI	PH	EPO	SEN	PA
1	2×4	CKSBL10011 × CKDHL121230	2.40	0.73	2.67	65.50	2.33	168.75	0.47	4.33	2.67
2	2×8	CKSBL10011 × CML494	2.36	0.66	2.67	67.67	1.17	193.08	0.48	4.33	2.00
3	1×2	CKSBL10027 × CKSBL10011	0.62	0.45	4.17	67.17	5.00	169.75	0.58	9.00	3.33
4	2×5	CKSBL10011 × CML395	1.84	0.59	3.00	68.33	5.50	192.67	0.56	5.83	2.33
5	2×6	CKSBL10011 × CML442	2.19	0.63	3.00	70.50	4.00	188.75	0.54	4.83	2.00
6	2×3	CKSBL10011 × CKDHL120172	2.12	0.57	2.67	69.50	7.00	195.75	0.55	4.50	2.00
7	2×7	CKSBL10011 × CKDHL120918	2.01	0.64	2.67	67.50	3.00	178.25	0.51	6.17	2.33
8	4×8	CKDHL121230 × CML494	2.21	0.63	2.83	68.17	2.17	177.00	0.44	4.17	1.00
9	1×4	CKSBL10027 × CKDHL121230	2.27	0.67	2.67	66.67	3.17	170.58	0.50	4.17	1.67
10	4×5	CKDHL121230 × CML395	2.07	0.63	3.00	68.17	4.67	178.00	0.51	4.50	2.00
11	4×6	CKDHL121230 × CML442	2.25	0.55	2.67	68.00	6.33	168.08	0.48	3.83	1.33
12	3×4	CKDHL120172 × CKDHL121230	2.29	0.69	2.67	67.83	5.17	173.08	0.49	4.50	1.67
13	4×7	CKDHL121230 × CKDHL120918	2.26	0.69	2.83	67.67	5.00	166.08	0.49	4.67	2.33
14	1×8	CKSBL10027 × CML494	2.42	0.80	3.00	69.00	2.83	177.42	0.49	4.17	1.33
15	5×8	CML395 × CML494	2.18	0.64	2.67	70.17	5.50	198.33	0.45	4.50	2.00
16	6×8	CML442 × CML494	2.67	0.57	2.67	72.33	4.67	177.08	0.47	4.17	2.00
17	3×8	CKDHL120172 × CML494	2.06	0.54	3.00	70.83	4.33	192.75	0.47	4.50	1.67
18	7×8	CKDHL120918 × CML494	2.26	0.64	2.17	69.33	3.50	183.00	0.46	4.33	2.33
19	1×5	CKSBL10027 × CML395	1.23	0.50	3.67	68.67	7.33	174.50	0.54	5.33	2.67
20	1×6	CKSBL10027 × CML442	1.84	0.75	3.17	70.67	4.83	182.92	0.55	6.50	2.67
21	1×3	CKSBL10027 × CKDHL120172	1.79	0.49	3.00	68.33	4.83	179.67	0.55	3.83	2.00
22	1×7	CKSBL10027 × CKDHL120918	1.90	0.61	2.83	69.50	2.83	174.25	0.50	5.33	2.00
23	5×6	CML395 × CML442	2.22	0.61	2.83	71.33	5.17	187.08	0.64	4.33	2.67
24	3×5	CKDHL120172 × CML395	0.74	0.43	3.83	73.00	5.00	159.33	0.54	4.67	3.00
25	5×7	CML395 × CKDHL120918	1.72	0.50	3.50	69.17	6.83	189.17	0.53	5.17	2.33
26	3×6	CKDHL120172 × CML442	2.08	0.47	3.00	71.67	3.83	190.00	0.59	4.17	1.67
27	6×7	CML442 × CKDHL120918	2.17	0.50	3.00	73.00	2.17	185.08	0.55	4.17	2.00
28	3×7	CKDHL120172 × CKDHL120918	1.78	0.52	3.00	70.50	3.00	187.42	0.53	5.33	2.67
29	WE6109	WE6109	1.95	0.67	3.00	67.83	2.17	187.08	0.50	6.17	1.67
30	CZH1258	CZH1258	1.58	0.53	3.17	69.33	1.83	170.92	0.50	6.00	2.67
31	WE1101	WE1101	1.85	0.53	3.25	72.83	3.20	174.92	0.55	5.17	2.33
32	PHB3253	PHB3253	1.76	0.57	2.79	71.67	4.32	188.00	0.55	5.67	1.67
Min			**0.62**	**0.43**	**2.17**	**65.50**	**1.17**	**159.33**	**0.44**	**3.83**	**1.00**
Max			**2.67**	**0.80**	**4.17**	**73.00**	**7.33**	**198.33**	**0.64**	**9.00**	**3.33**
Mean			**1.97**	**0.59**	**2.97**	**69.43**	**4.15**	**180.59**	**0.52**	**4.95**	**2.13**
StError			**0.08**	**0.02**	**0.07**	**0.34**	**0.28**	**1.72**	**0.01**	**0.18**	**0.09**

GY = grain yield; EPP = ears/plant, EA = ear aspect, AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height
EPO = ear position; SEN= senascence, PA= Plant aspect,

Appendix 4-3. Means of the GY and other traits under across analysis for optimum environments

Entry	Cross	Pedigree	GY	EPP	EA	AD	ASI	PH	EPO	PA	HC	TEX
1	2×4	CKSBL10011 × CKDHL121230	6.43	1.00	2.67	65.06	0.83	222.56	0.46	2.39	10.06	3.33
2	2×8	CKSBL10011 × CML494	6.90	0.97	2.33	67.61	-0.39	238.11	0.48	2.33	4.71	2.67
3	1×2	CKSBL10027 × CKSBL10011	5.37	0.97	2.78	67.56	1.89	225.72	0.52	2.83	2.67	2.00
4	2×5	CKSBL10011 × CML395	6.84	1.07	2.33	69.67	1.67	249.11	0.53	3.06	5.60	2.67
5	2×6	CKSBL10011 × CML442	6.49	0.97	2.78	70.17	1.50	242.47	0.51	2.50	5.92	5.00
6	2×3	CKSBL10011 × CKDHL120172	6.72	1.00	2.22	69.61	1.50	255.17	0.53	3.17	5.97	4.67
7	2×7	CKSBL10011 × CKDHL120918	5.50	0.99	2.89	68.83	-0.44	224.53	0.48	2.83	15.91	2.00
8	4×8	CKDHL121230 × CML494	6.87	1.00	2.56	66.83	-0.78	228.14	0.42	1.78	8.33	2.00
9	1×4	CKSBL10027 × CKDHL121230	5.89	0.98	2.72	65.67	1.44	215.58	0.46	2.39	5.52	1.00
10	4×5	CKDHL121230 × CML395	6.20	1.02	2.28	68.17	1.61	230.94	0.48	2.78	17.46	1.33
11	4×6	CKDHL121230 × CML442	5.81	0.96	2.78	66.50	1.00	211.19	0.46	2.22	7.34	5.00
12	3×4	CKDHL120172 × CKDHL121230	6.69	0.96	2.78	66.56	1.50	229.11	0.48	2.56	13.70	3.67
13	4×7	CKDHL121230 × CKDHL120918	5.29	0.97	2.94	66.67	1.11	204.00	0.45	2.50	17.80	4.33
14	1×8	CKSBL10027 × CML494	6.66	0.98	2.44	69.94	0.28	229.58	0.46	2.22	1.21	1.67
15	5×8	CML395 × CML494	7.05	0.98	2.28	71.50	0.22	245.42	0.46	2.67	11.83	1.67
16	6×8	CML442 × CML494	6.62	1.48	2.78	71.50	0.61	235.31	0.46	2.50	8.39	5.00
17	3×8	CKDHL120172 × CML494	7.35	1.02	2.11	71.44	0.11	249.69	0.46	2.33	10.30	4.67
18	7×8	CKDHL120918 × CML494	6.05	0.96	2.28	70.72	-1.61	222.33	0.43	2.39	8.16	2.00
19	1×5	CKSBL10027 × CML395	5.79	0.98	2.50	69.22	2.61	226.11	0.51	3.06	9.28	1.00
20	1×6	CKSBL10027 × CML442	5.70	1.00	2.44	69.06	2.11	226.36	0.49	2.67	3.38	2.67
21	1×3	CKSBL10027 × CKDHL120172	5.75	0.99	2.39	69.06	2.28	237.33	0.51	2.94	3.49	1.33
22	1×7	CKSBL10027 × CKDHL120918	5.22	0.98	2.56	69.50	0.50	222.47	0.48	2.89	10.92	1.00
23	5×6	CML395 × CML442	6.23	0.97	2.50	71.72	2.44	227.50	0.52	2.94	0.60	5.00
24	3×5	CKDHL120172 × CML395	2.91	0.85	3.89	74.72	3.67	207.72	0.53	3.33	9.04	3.33
25	5×7	CML395 × CKDHL120918	5.68	0.99	2.61	69.94	0.33	225.35	0.51	2.94	13.91	1.00
26	3×6	CKDHL120172 × CML442	6.30	0.96	2.67	71.56	3.00	242.69	0.52	2.89	4.64	5.00
27	6×7	CML442 × CKDHL120918	5.67	0.96	2.50	70.83	0.78	235.42	0.50	2.83	23.65	4.00
28	3×7	CKDHL120172 × CKDHL120918	5.24	0.95	2.89	70.39	0.72	234.42	0.52	3.11	16.16	2.00
29	WE6109	WE6109	6.11	1.50	2.83	65.50	-1.94	224.17	0.47	2.67	29.70	3.33
30	CZH1258	CZH1258	6.38	0.99	2.72	67.39	0.72	226.86	0.47	2.61	12.50	4.67
31	WE1101	WE1101	5.16	0.88	2.94	72.72	1.89	225.33	0.50	2.72	14.22	3.67
32	PHB3253	PHB3253	6.66	0.93	2.89	68.83	2.50	245.61	0.48	2.28	6.35	3.33
Min			2.91	0.85	2.11	65.06	-1.94	204.00	0.42	1.78	0.60	1.00
Max			**7.35**	**1.50**	**3.89**	**74.72**	**3.67**	**255.17**	**0.53**	**3.33**	**29.70**	**5.00**
Mean			**6.05**	**1.01**	**2.63**	**69.20**	**1.05**	**230.20**	**0.49**	**2.67**	**9.96**	**3.00**
StError			**0.15**	**0.02**	**0.06**	**0.40**	**0.22**	**2.12**	**0.01**	**0.06**	**1.14**	**0.25**

GY = grain yield; EPP = ears/plant; EA = ear aspect; AD = days to anthesis; ASI = anthesis-silking interval; PH = plant height;
EPO = ear position; PA= plant aspect; HC= bad husk cover; TEX= grain texture

Appendix 4-4. Across and individual site of general combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under maize lethal necrosis infestation at Naivasha, Kenya 2017A and 2018A

	Genotype	MLN1	MLN2	MLN3	MLN4	GY	EPP	NP	PH
	CKSBL10027	0.04	-0.01	0.06	-0.06	-	-	-	-
Across	CKSBL10011	-0.21*	-0.51***	-0.55***	-0.98***	-	-	-	-
	CKDHL120172	-0.15	0.07	0.20	0.30**	-	-	-	-
	CKDHL121230	-0.13	-0.01	-0.07	0.05	-	-	-	-
	CML395	0.07	-0.13	0.17	0.60***	-	-	-	-
	CML442	0.63***	0.82***	0.67***	0.80***	-	-	-	-
	CKDHL120918	-0.54***	-0.71***	-0.77***	-0.92***	-	-	-	-
	CML494	0.29***	0.47***	0.28*	0.22	-	-	-	-
Site 5	CKSBL10027	0.17	-0.13	0.01	-0.24	-	-	-	-
	CKSBL10011	-0.33*	-0.63***	-0.54***	-0.96***	-	-	-	-
	CKDHL120172	-0.06	0.26	0.35**	0.15	-	-	-	-
	CKDHL121230	-0.17	-0.01	-0.10	0.15	-	-	-	-
	CML395	0.06	0.10	0.46***	0.88***	-	-	-	-
	CML442	0.67***	0.71***	0.51***	0.76***	-	-	-	-
	CKDHL120918	-0.56***	-0.63***	-0.88***	-0.85***	-	-	-	-
	CML494	0.22*	0.32*	0.18	0.10	-	-	-	-
Site 10	CKSBL10027	-0.08	0.10	0.11	0.11	-0.24***	-0.06	0.83*	5.89
	CKSBL10011	-0.08	-0.40***	-0.56***	-1.0***	0.48***	0.25***	0.61	7.19*
	CKDHL120172	-0.25***	-0.13	0.06	0.44**	-0.14**	-0.08	-0.67*	3.78
	CKDHL121230	-0.08	-0.01	-0.06	-0.06	-0.29***	-0.10	0.61	-18.94***
	CML395	0.08	-0.35***	-0.11	0.33*	-0.14**	-0.12*	-1.72***	12.72***
	CML442	0.58***	0.93***	0.83***	0.83***	-0.3***	-0.19***	-1.5***	-9.68**
	CKDHL120918	-0.53***	-0.79***	-0.67***	-1.0***	0.47***	0.29***	1.28***	6.43
	CML494	0.36***	0.65***	0.39*	0.33*	0.15**	-0.01	0.56	-7.39*

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

MLN1- MLN4= MLN scores 1 at early stage and 4 at late stage; GY= Grain Yield,EPP = ears/plant; NP= number of plants ; PH= Plant Height

Appendix 4-5. Across and individual site of general combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under drought conditions in Kenya 2017A and 2018A

	Genotype	GY	EA	AD	PH	EPO	SEN
Across	CKSBL10027	-0.32*	0.30*	-0.84*	-5.90	0.01*	-
	CKSBL10011	-0.08	0.02	-1.48***	3.76	0.01	-
	CKDHL120172	-0.19	0.08	1.10**	2.26	0.02*	-
	CKDHL121230	0.29	-0.23	-2.17***	-10.48*	-0.04***	-
	CML395	-0.33	0.30*	0.63	2.44	0.03***	-
	CML442	0.24*	-0.06	2.08***	2.42	0.03***	-
	CKDHL120918	0.02	-0.12	0.27	-0.20	-0.01	-
	CML494	0.36**	-0.28*	0.41	5.70	-0.06***	-
Site 4	CKSBL10027	0.05	0.04	-0.97**	-2.03	0.02***	-
	CKSBL10011	0.05	-0.13	-1.36***	2.42	0.01*	-
	CKDHL120172	-0.19*	0.21	0.97**	4.17	0.03***	-
	CKDHL121230	0.16*	-0.35*	-2.25***	-5.83*	-0.04***	-
	CML395	-0.05	0.21	0.36	2.94	0.03***	-
	CML442	-0.05	0.10	2.47***	-2.86	0.03***	-
	CKDHL120918	-0.08	0.10	0.14	-2.03	-0.02**	-
	CML494	0.10	-0.18	0.64*	3.22	-0.07***	-
Site 9	CKSBL10027	-0.69***	0.56***	-0.71**	-9.76***	0.01	0.75***
	CKSBL10011	-0.20	0.17	-1.60***	5.10**	0.01	0.86***
	CKDHL120172	-0.19	-0.06	1.24***	0.35	0.01	-0.39*
	CKDHL121230	0.43**	-0.11	-2.10***	-15.13***	-0.04**	-0.61**
	CML395	-0.61***	0.39*	0.90***	1.93	0.03*	0.08
	CML442	0.53***	-0.22	1.68***	7.71***	0.04**	-0.31
	CKDHL120918	0.11	-0.33*	0.40	1.63	0.00	0.22
	CML494	0.62***	-0.39**	0.18	8.18***	-0.05***	-0.61**
	*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%						

GY = grain yield; EA = ear aspect; AD = days to anthesis; PH = plant height; EPO = ear position; SEN= senascence

Appendix 4-6. Across and individual site of general combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

	Genotypes	GY	AD	ASI	PH	EPO	PA
across	CKSBL10027	-0.32	-0.83	0.58***	-4.65*	0.00	0.04
	CKSBL10011	0.32	-1.08	-0.18	7.76***	0.02***	0.06
	CKDHL120172	-0.23	1.39	0.86***	7.51**	0.02***	0.26***
	CKDHL121230	0.15	-3.26***	-0.15	-11.59***	-0.03***	-0.36***
	CML395	-0.27	1.66*	0.82***	0.18	0.02***	0.34***
	CML442	0.09	1.06	0.64***	1.64	0.01*	-0.03
	CKDHL120918	-0.61***	0.31	-1.04***	-7.10**	-0.01	0.12*
	CML494	0.87***	0.76	-1.53***	6.25**	-0.04***	-0.42***
Site 1	CKSBL10027	-0.04	-0.83	0.75	3.91	0.00	0.29**
	CKSBL10011	0.23	-0.61	-0.47	13.74***	0.02***	-0.32***
	CKDHL120172	-0.79**	1.39*	2.36***	4.94	0.02**	0.46***
	CKDHL121230	1.35***	-2.78***	-1.14**	-14.84***	-0.03***	-0.36***
	CML395	-1.57***	1.83**	1.53***	-3.09	0.01**	0.40***
	CML442	-0.38	0.11	1.64***	-2.84	0.01	0.13
	CKDHL120918	0.41	-0.39	-2.08***	-8.42**	-0.01	-0.04
	CML494	0.80**	1.28*	-2.58***	6.60**	-0.01	-0.54***
Site 2	CKSBL10027	-0.44**	-0.58***	0.71**	-9.89***	0.01**	-0.01
	CKSBL10011	0.29*	-1.47***	0.38	10.69***	0.01**	0.43***
	CKDHL120172	-0.08	0.47**	0.54*	8.28***	0.02***	0.43***
	CKDHL121230	0.49**	-2.19***	0.26	-12.39***	-0.02***	-0.57***
	CML395	0.10	0.25	0.65**	2.67	0.02**	0.38**
	CML442	-0.01	1.69***	0.82***	0.28	-0.01*	-0.18
	CKDHL120918	-0.79***	0.92***	-1.54***	-3.47*	0.00	-0.07
	CML494	0.44**	0.92***	-1.85***	3.83*	-0.04***	-0.40***
Site 3	CKSBL10027	0.05	-1.71***	-0.03	-4.14	0.01	0.03
	CKSBL10011	-0.07	-2.15***	0.03	2.02	0.01*	0.25
	CKDHL120172	-0.62**	3.13***	-0.42	1.52	0.03***	0.03
	CKDHL121230	-0.57*	-3.71***	-0.25	0.36	-0.04***	-0.03
	CML395	0.18	2.85***	0.36	-2.96	0.04***	0.08
	CML442	0.55*	1.51***	0.19	4.44	0.03***	-0.03
	CKDHL120918	-0.48*	0.01	0.42	1.98	-0.02***	-0.03
	CML494	0.97***	0.07*	-0.31	-3.23	-0.06***	-0.31
	*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%						
	GY= grain yield, AD= anthesis date, ASI= anthesis-silking interval,						
	PH= plant Height, EPO = ear position, PA= plant aspect						

Continuation Appendix 4-6

	Genotypes	GY	AD	ASI	PH	EPO	PA
Site 6	CKSBL10027	-0.80**	-0.72	0.92***	-3.14	0.01	-0.01
	CKSBL10011	0.15	-0.39	0.08	8.75***	0.03***	-0.13
	CKDHL120172	0.26	1.17**	0.64*	7.94***	0.02***	0.47***
	CKDHL121230	0.24	-3.28***	-0.19	-17.31***	-0.04***	-0.74***
	CML395	0.51*	2.39***	0.03	7.78***	0.04**	0.43***
	CML442	-0.42	0.28	0.69**	1.78	0.02**	-0.13
	CKDHL120918	-1.09***	-0.17	-0.86**	-12.78***	0.00	0.65***
	CML494	1.16***	0.72*	-1.31***	6.97**	-0.04***	-0.57***
Site 7	CKSBL10027	-0.72***	-0.75***	0.92***	-12.32***	0.01*	-0.07
	CKSBL10011	0.62***	-0.92***	-0.31*	7.38**	0.02***	0.04
	CKDHL120172	0.13	0.42**	0.97***	7.38**	0.03***	-0.07
	CKDHL121230	0.11	-2.25***	-0.19	-10.60***	-0.03***	-0.24
	CML395	-0.44**	0.36*	1.14***	-2.40	0.03***	0.26
	CML442	0.33*	1.36***	0.69***	4.32	0.00	0.04
	CKDHL120918	-0.58***	0.97***	-1.58***	-2.38	0.00	0.15
	CML494	0.55**	0.81***	-1.64***	8.63***	-0.05***	-0.13
Site 8	CKSBL10027	0.02	-0.40	0.22	-2.34	-0.01	0.01
	CKSBL10011	0.73**	-0.96***	-0.78***	3.99	0.02**	0.07
	CKDHL120172	-0.26	1.76***	1.06***	14.99***	0.02**	0.24*
	CKDHL121230	-0.73**	-5.35***	0.61**	-14.78***	-0.03***	-0.21
	CML395	-0.39	2.26***	1.22***	-0.92	0.03***	0.46***
	CML442	0.45	1.38***	-0.22	1.88	0.02*	-0.04
	CKDHL120918	-1.10***	0.54*	-0.61***	-17.51***	-0.01	0.07
	CML494	1.28***	0.76**	-1.5***	14.69***	-0.04***	-0.60***
	*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%						
	GY= grain yield, AD= anthesis date, ASI= anthesis-silking interval,						
	PH= plant Height, EPO = ear position, PA= plant aspect						

Appendix 4-7. Across specific combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under MLN virus infested conditions in Naivasha, Kenya 2017A and 2018A

Single cross	Pedigree	MLN1	MLN2	MLN3	MLN4	GY	EPP	NP	EPO
S12	CKSBL10027 × CKSBL10011	0.15	0.11	0.33	0.27	0.13	0.14	-0.30	-0.02
S13	CKSBL10027 × CKDHL120172	-0.57*	-0.64**	-0.76**	-0.67**	0.11	0.22	0.64	0.02
S14	CKSBL10027 × CKDHL121230	0.07	0.44*	0.35	0.41	0.09	-0.25*	-0.97	0.04
S15	CKSBL10027 × CML395	0.04	-0.11	0.10	0.19	-0.05	-0.09	1.70*	0.03
S16	CKSBL10027 × CML442	-0.01	-0.06	-0.23	-0.34	0.10	-0.15	2.48**	-0.06*
S17	CKSBL10027 × CKDHL120918	-0.01	0.14	0.21	0.05	-0.04	0.18	-2.30**	0.03
S18	CKSBL10027 × CML494	0.32	0.11	0.35	0.08	-0.61	-0.04	-1.25	-0.04
S23	CKSBL10011 × CKDHL120172	-0.15	-0.14	-0.01	0.25	-0.35***	-0.09	1.20	0.05*
S24	CKSBL10011 × CKDHL121230	-0.18	-0.06	0.02	0.16	-0.24*	-0.03	-0.08	0.02
S25	CKSBL10011 × CML395	0.29	0.22	0.13	-0.06	-0.12	-0.26*	-0.41	-0.04
S26	CKSBL10011 × CML442	-0.10	-0.06	-0.29	0.58*	-0.32**	0.25*	0.03	0.01
S27	CKSBL10011 × CKDHL120918	-0.10	-0.69**	0.55*	-1.37***	0.09	0.33**	-0.41	-0.02
S28	CKSBL10011 × CML494	0.07	0.61**	0.13	0.16	0.48***	-0.34**	-0.02	0.00
S34	CKDHL120172 × CKDHL121230	-0.07	-0.31	-1.01***	-0.45	0.93***	0.03	0.87	0.01
S35	CKDHL120172 × CML395	0.57*	0.97***	0.27	1.50***	-0.48***	0.20	-3.80***	0.02
S36	CKDHL120172 × CML442	-0.15	0.03	-0.29	-0.53*	0.00	-0.19	-0.36	-0.05
S37	CKDHL120172 × CKDHL120918	0.18	0.22	1.13***	0.19	-0.15	-0.13	0.53	-0.07**
S38	CKDHL120172 × CML494	0.18	-0.14	-0.01	-0.28	0.01	-0.04	0.92	0.01
S45	CKDHL121230 × CML395	0.04	-0.28	-0.04	-0.59*	0.01	0.11	-0.41	0.01
S46	CKDHL121230 × CML442	0.15	-0.22	0.24	-0.12	-0.19	-0.08	0.70	-0.02
S47	CKDHL121230 × CKDHL120918	0.15	0.47*	-0.31	0.61*	0.46***	-0.07	-0.41	-0.03
S48	CKDHL121230 × CML494	-0.18	-0.06	-0.56*	-0.03	0.17	0.29*	0.31	-0.03
S56	CML395 × CML442	-0.21	0.06	-0.26	0.16	0.00	0.31**	-1.97**	-0.01
S57	CML395 × CKDHL120918	-0.21	-0.42*	-0.42*	-0.62*	0.16	-0.31*	2.25**	0.02
S58	CML395 × CML494	-0.54*	-0.44*	-0.12	-0.59*	0.36***	0.03	2.64***	-0.05
S67	CML442 × CKDHL120918	0.07	0.31	-0.44*	0.36	-0.08	-0.13	1.03	0.04
S68	CML442 × CML494	0.24	-0.06	-0.29	-0.12	-0.29**	-0.02	-1.91*	0.08**
S78	CKDHL120918 × CML494	-0.10	-0.03	0.33	0.77**	-0.18	0.13	-0.69	0.03

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

MLN1- MLN4= MLN scores 1 at early stage and 4 at late stage; GY= Grain Yield, EPP = ears/plant; NP= number of plants ; EPO = ear position

Appendix 4-8. Across specific combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under drought conditions in Kenya 2017A and 2018A

Single cross	Pedigree	GY	EPP	EA	AD	ASI	PH	SEN
S12	CKSBL10027 × CKSBL10011	-0.99	-0.18*	0.89*	0.19	0.96	-8.75	2.56***
S13	CKSBL10027 × CKDHL120172	0.30	-0.04	-0.33	-1.22	-0.06	2.67	-1.36**
S14	CKSBL10027 × CKDHL121230	0.30	-0.01	-0.36	0.39	-1.01	6.32	-0.81*
S15	CKSBL10027 × CML395	-0.11	-0.07	0.11	-0.42	1.30	-2.68	-0.33
S16	CKSBL10027 × CML442	-0.08	0.15	-0.03	0.14	0.30	5.75	1.22**
S17	CKSBL10027 × CKDHL120918	0.20	0.01	-0.31	0.78	-0.92	-0.29	-0.47
S18	CKSBL10027 × CML494	0.38	0.14*	0.03	0.14	-0.56	-3.03	-0.81*
S23	CKSBL10011 × CKDHL120172	0.39	0.04	-0.39	0.58	2.58*	9.10	-0.81*
S24	CKSBL10011 × CKDHL121230	0.18	0.05	-0.08	-0.14	-1.37	-5.16	-0.75*
S25	CKSBL10011 × CML395	0.24	0.03	-0.28	-0.11	-0.06	5.84	0.06
S26	CKSBL10011 × CML442	0.03	0.04	0.08	0.61	-0.06	1.93	-0.56
S27	CKSBL10011 × CKDHL120918	0.07	0.04	-0.19	-0.58	-0.29	-5.94	0.25
S28	CKSBL10011 × CML494	0.07	-0.01	-0.03	-0.56	-1.76	2.99	-0.75*
S34	CKDHL120172 × CKDHL121230	0.18	0.10	-0.14	-0.39	0.60	0.67	0.67
S35	CKDHL120172 × CML395	-0.74	-0.04	0.50*	1.97*	-1.42	-26.00**	0.14
S36	CKDHL120172 × CML442	0.03	-0.04	0.03	-0.81	-1.09	4.68	0.03
S37	CKDHL120172 × CKDHL120918	-0.05	0.02	0.08	-0.17	-1.15	4.72	0.67
S38	CKDHL120172 × CML494	-0.11	-0.03	0.25	0.03	0.55	4.15	0.67
S45	CKDHL121230 × CML395	0.11	0.01	-0.03	0.42	-1.04	5.40	0.19
S46	CKDHL121230 × CML442	-0.28	-0.10	0.00	-1.19	2.13	-4.50	-0.08
S47	CKDHL121230 × CKDHL120918	-0.05	0.04	0.22	0.28	1.58	-3.87	0.22
S48	CKDHL121230 × CML494	-0.44	-0.09	0.39	0.64	-0.90	1.14	0.56
S56	CML395 × CML442	0.31	0.07	-0.36	-0.67	-0.90	1.59	-0.28
S57	CML395 × CKDHL120918	0.03	-0.04	0.36	-1.03	1.55	6.29	0.03
S58	CML395 × CML494	0.15	0.04	-0.31	-0.17	0.58	9.56	0.19
S67	CML442 × CKDHL120918	-0.08	-0.07	0.22	1.36	-1.62	2.22	-0.58
S68	CML442 × CML494	0.07	-0.06	0.06	0.56	1.24	-11.68*	0.25
S78	CKDHL120918 × CML494	-0.12	0.01	-0.39	-0.64	0.85	-3.14	-0.11

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP= number of ears/plant; EA = ear aspect; AD = days to anthesis;
AD = days to anthesis; PH = plant height; SEN= senascence

Appendix 4-9. Across specific combining ability effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

Single cross	Pedigree	GY	EA	AD	ASI	PH	EPO	PA	HC	TEX
S12	CKSBL10027 × CKSBL10011	-0.68*	0.28*	0.19	0.40	-7.54	0.01	0.06	0.31	0.36
S13	CKSBL10027 × CKDHL120172	0.25	-0.27*	-0.79	-0.25	4.32	-0.01	-0.04	-0.95	-0.70*
S14	CKSBL10027 × CKDHL121230	0.03	0.10	0.47	-0.08	1.67	0.00	0.03	-1.74	-0.37
S15	CKSBL10027 × CML395	0.33	-0.07	-0.89	0.12	0.43	0.00	0.00	4.11	0.41
S16	CKSBL10027 × CML442	-0.11	-0.13	-0.45	-0.20	-0.78	-0.01	-0.02	0.50	-0.53*
S17	CKSBL10027 × CKDHL120918	0.11	-0.06	0.73*	-0.13	4.07	-0.01	0.05	-0.72	0.36
S18	CKSBL10027 × CML494	0.07	0.15	0.73*	0.14	-2.17	0.01	-0.07	-1.50	0.47
S23	CKSBL10011 × CKDHL120172	0.58	-0.46**	0.02	-0.27	9.74*	0.00	0.17	-0.86	0.69*
S24	CKSBL10011 × CKDHL121230	-0.08	0.02	0.11	0.07	-3.77	-0.01	0.01	0.41	0.02
S25	CKSBL10011 × CML395	0.74*	-0.26*	-0.19	-0.07	11.01*	0.01	-0.02	-1.98	0.13
S26	CKSBL10011 × CML442	0.03	0.17	0.91*	-0.05	2.91	-0.01	-0.20	0.65	-0.14
S27	CKSBL10011 × CKDHL120918	-0.26	0.25	0.32	-0.32	-6.30	-0.02*	-0.03	1.88	-0.59*
S28	CKSBL10011 × CML494	-0.33	0.01	-1.35	0.23	-6.06	0.01	0.02	-0.40	-0.48
S34	CKDHL120172 × CKDHL121230	0.72*	-0.03	-0.86	-0.30	3.04	0.00	-0.03	1.97	-0.03
S35	CKDHL120172 × CML395	-2.64***	1.14***	2.39*	0.90**	-30.12***	0.00	0.06	-0.61	0.41
S36	CKDHL120172 × CML442	0.40	-0.09	-0.17	0.42	3.39	0.00	-0.02	-2.71	-0.53*
S37	CKDHL120172 × CKDHL120918	0.03	0.09	-0.60	-0.19	3.85	0.01	0.05	0.05	-0.98**
S38	CKDHL120172 × CML494	0.66*	-0.37*	0.01	-0.31	5.78	-0.01	-0.19	3.12	1.13***
S45	CKDHL121230 × CML395	0.28	-0.44**	0.48	-0.15	12.20*	0.00	0.12	5.00*	-0.92**
S46	CKDHL121230 × CML442	-0.47	0.05	-0.58	-0.58*	-9.01*	0.00	-0.06	-2.83	0.13
S47	CKDHL121230 × CKDHL120918	-0.29	0.18	0.33	1.21***	-7.47	0.00	0.06	-1.13	2.02***
S48	CKDHL121230 × CML494	-0.19	0.11	0.05	-0.19	3.33	0.00	-0.12	-1.67	-0.87**
S56	CML395 × CML442	0.37	-0.17	-0.28	-0.10	-4.48	0.00	-0.04	-7.49**	0.91**
S57	CML395 × CKDHL120918	0.51	-0.09	-1.31	-0.54	2.11	0.01	-0.19	-2.94	-0.53*
S58	CML395 × CML494	0.41	-0.11	-0.20	-0.16	8.83*	-0.01	0.07	3.91	-0.42
S67	CML442 × CKDHL120918	0.15	-0.21	0.18	0.09	10.71*	0.01	0.06	9.10***	-0.14
S68	CML442 × CML494	-0.38	0.38*	0.40	0.42	-2.74	0.00	0.28*	2.77	0.30
S78	CKDHL120918 × CML494	-0.25	-0.16	0.36	-0.13	-6.98	-0.01	0.01	-6.23*	-0.14

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY= grain yield, EA= ear aspect, AD= anthesis date, ASI= anthesis-silking interval, PH= plant Height,
EPO = ear position, PA= plant aspect, HC= bad husk cover, TEX= grain texture

Appendix 5-1. Means of the Grain yield and other traits under across analysis for drought environments

Entry	Cross	Pedigree	GY	EPP	AD	ASI	PH	EPO	PA	EA	SEN
1	2×4	CKSBL10011 × CKDHL121230	3.55	0.89	67.17	0.12	190.75	0.49	1.33	2.33	4.58
2	2×5	CKSBL10011 × CKSBL10082	2.59	0.79	79.17	1.27	171.25	0.54	3.33	2.83	6.58
3	2×6	CKSBL10011 × CKSBL10060	0.37	0.35	67.83	2.17	149.67	0.54	4.33	4.50	9.00
4	1×2	CKSBL10027 × CKSBL10011	1.30	0.73	66.89	2.52	184.33	0.57	2.00	3.83	8.25
5	2×3	CKSBL10011 × CKDHL120172	3.26	0.68	68.98	4.44	207.58	0.56	2.67	2.50	4.42
6	2×7	CKSBL10011 × CKDHL120731	2.11	0.66	68.48	4.00	210.33	0.52	1.33	3.00	5.67
7	2×8	CKSBL10011 × CKDHL120517	2.87	0.81	68.83	2.50	208.58	0.57	2.00	2.50	5.00
8	4×5	CKDHL121230 × CKSBL10082	2.97	0.81	79.57	0.67	168.08	0.53	2.67	2.50	5.50
9	4×6	CKDHL121230 × CKSBL10060	2.65	0.81	78.35	1.67	177.67	0.51	1.67	3.00	5.08
10	1×4	CKSBL10027 × CKDHL121230	3.04	0.73	66.19	9.05	175.92	0.51	1.67	3.00	4.92
11	3×4	CKDHL120172 × CKDHL121230	3.31	0.76	73.56	10.18	196.58	0.50	1.67	2.67	4.75
12	4×7	CKDHL121230 × CKDHL120731	3.38	0.80	67.37	3.10	192.25	0.49	1.67	2.33	4.58
13	4×8	CKDHL121230 × CKDHL120517	3.37	0.83	67.17	2.00	182.50	0.53	1.33	2.33	5.17
14	5×6	CKSBL10082 × CKSBL10060	2.39	0.76	79.38	0.35	176.83	0.58	3.00	3.17	6.00
15	1×5	CKSBL10027 × CKSBL10082	1.85	0.69	68.10	2.07	165.42	0.55	3.67	2.83	5.83
16	3×5	CKDHL120172 × CKSBL10082	2.92	0.76	67.87	2.45	184.92	0.57	2.67	2.83	4.83
17	5×7	CKSBL10082 × CKDHL120731	2.79	0.88	68.57	1.60	193.58	0.53	2.33	2.17	4.83
18	5×8	CKSBL10082 × CKDHL120517	2.78	0.83	68.27	2.90	183.50	0.58	2.67	2.50	6.00
19	1×6	CKSBL10027 × CKSBL10060	1.40	0.66	67.27	1.77	174.50	0.57	2.67	3.67	8.75
20	3×6	CKDHL120172 × CKSBL10060	3.49	0.76	67.14	2.49	205.17	0.56	2.00	2.33	4.25
21	6×7	CKSBL10060 × CKDHL120731	2.33	0.78	67.44	1.67	198.00	0.53	2.00	3.00	6.83
22	6×8	CKSBL10060 × CKDHL120517	2.24	0.72	68.50	2.17	189.58	0.56	3.00	2.67	6.67
23	1×3	CKSBL10027 × CKDHL120172	2.76	0.69	68.83	4.13	188.75	0.55	2.00	2.50	4.25
24	1×7	CKSBL10027 × CKDHL120731	2.62	0.82	69.33	1.67	208.67	0.53	2.00	2.17	5.33
25	1×8	CKSBL10027 × CKDHL120517	2.66	0.85	67.88	0.46	190.42	0.58	2.33	2.33	5.42
26	3×7	CKDHL120172 × CKDHL120731	3.03	0.70	71.50	3.57	208.17	0.52	1.67	2.17	3.75
27	3×8	CKDHL120172 × CKDHL120517	3.49	0.74	70.33	4.00	209.25	0.58	1.00	2.17	5.00
28	7×8	CKDHL120731 × CKDHL120517	2.66	0.75	69.17	2.83	201.75	0.52	3.00	2.50	5.92
29	CZH1258	CZH1258	3.06	0.76	69.33	2.33	183.42	0.51	2.00	2.67	4.67
30	CKPH12040	CKPH12040	2.93	0.78	88.17	1.00	194.83	0.56	1.67	2.83	5.50
31	WE1101	WE1101	1.89	0.59	71.83	2.60	187.00	0.56	2.67	3.00	5.08
32	PHB3253	PHB3253	2.50	0.72	71.00	4.00	197.25	0.55	1.67	3.17	4.67
	Min		**0.37**	**0.35**	**66.19**	**0.12**	**149.67**	**0.49**	**1.00**	**2.17**	**3.75**
	Max		**3.55**	**0.89**	**88.17**	**10.18**	**210.33**	**0.58**	**4.33**	**4.50**	**9.00**
	Mean		**2.64**	**0.75**	**70.61**	**2.74**	**189.27**	**0.54**	**2.24**	**2.75**	**5.53**
	StError		**0.12**	**0.02**	**0.72**	**0.40**	**2.76**	**0.00**	**0.14**	**0.10**	**0.23**

GY = grain yield; EPP = ears/plant, AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height

EPO = ear position; PA= Plant aspect, EA = ear aspect; PA = plant aspect; SEN= senascence

Appendix 5-2. Means of the Grain yield and other traits under across analysis for optimum environments

Entry	Cross	Pedigree	GY	EPP	AD	ASI	PH	EPO	PA	EA
1	2×4	CKSBL10011 × CKDHL121230	5.44	0.85	67.11	1.06	223.63	0.46	2.56	2.94
2	2×5	CKSBL10011 × CKSBL10082	4.90	0.97	67.17	0.39	217.80	0.51	2.83	2.89
3	2×6	CKSBL10011 × CKSBL10060	2.99	0.89	70.56	1.22	189.27	0.51	2.89	3.94
4	1×2	CKSBL10027 × CKSBL10011	5.27	0.94	69.00	1.33	225.50	0.52	2.78	2.78
5	2×3	CKSBL10011 × CKDHL120172	6.65	0.91	70.44	0.83	252.67	0.53	2.72	2.33
6	2×7	CKSBL10011 × CKDHL120731	6.01	0.91	70.00	-0.17	247.17	0.49	2.94	2.33
7	2×8	CKSBL10011 × CKDHL120517	6.39	0.93	69.78	0.22	242.30	0.52	2.56	2.56
8	4×5	CKDHL121230 × CKSBL10082	5.14	0.98	65.06	0.06	205.57	0.49	2.56	3.11
9	4×6	CKDHL121230 × CKSBL10060	5.59	0.94	65.83	1.11	219.40	0.47	2.44	2.78
10	1×4	CKSBL10027 × CKDHL121230	5.55	0.93	67.33	1.33	214.47	0.47	2.56	2.78
11	3×4	CKDHL120172 × CKDHL121230	6.22	0.93	67.56	1.39	227.37	0.48	2.61	2.61
12	4×7	CKDHL121230 × CKDHL120731	5.50	0.91	66.72	0.83	225.17	0.43	2.61	2.44
13	4×8	CKDHL121230 × CKDHL120517	5.46	1.02	65.94	0.89	205.90	0.47	2.22	2.72
14	5×6	CKSBL10082 × CKSBL10060	4.78	0.95	66.28	0.39	215.02	0.52	3.00	2.94
15	1×5	CKSBL10027 × CKSBL10082	3.76	0.96	69.06	0.72	203.53	0.49	2.89	3.33
16	3×5	CKDHL120172 × CKSBL10082	5.95	0.92	69.33	0.17	233.70	0.52	2.72	2.22
17	5×7	CKSBL10082 × CKDHL120731	5.35	0.95	68.00	0.56	229.93	0.47	2.72	2.72
18	5×8	CKSBL10082 × CKDHL120517	5.33	0.95	68.44	0.00	227.17	0.50	2.78	2.67
19	1×6	CKSBL10027 × CKSBL10060	4.32	0.89	66.89	1.67	213.77	0.52	2.72	3.00
20	3×6	CKDHL120172 × CKSBL10060	6.44	0.88	70.06	0.94	244.07	0.52	2.72	1.94
21	6×7	CKSBL10060 × CKDHL120731	6.09	0.89	67.94	0.78	246.10	0.50	2.61	2.44
22	6×8	CKSBL10060 × CKDHL120517	5.70	0.92	68.11	0.67	235.20	0.53	2.61	2.50
23	1×3	CKSBL10027 × CKDHL120172	5.80	0.93	70.00	2.00	240.40	0.50	2.56	2.33
24	1×7	CKSBL10027 × CKDHL120731	5.23	0.95	70.56	0.78	232.60	0.48	2.61	2.67
25	1×8	CKSBL10027 × CKDHL120517	5.01	0.96	69.39	1.33	230.50	0.51	3.00	2.89
26	3×7	CKDHL120172 × CKDHL120731	5.03	0.88	71.17	1.00	232.53	0.48	2.89	2.61
27	3×8	CKDHL120172 × CKDHL120517	5.65	0.91	71.44	0.61	242.83	0.54	2.94	2.50
28	7×8	CKDHL120731 × CKDHL120517	5.00	0.90	70.61	0.06	229.43	0.47	2.78	2.78
29	CZH1258	CZH1258	5.84	0.97	67.61	2.56	224.20	0.47	2.83	2.44
30	CKPH12040	CKPH12040	5.31	0.90	69.28	0.28	221.00	0.49	2.67	2.89
31	WE1101	WE1101	4.68	0.86	72.39	1.78	214.80	0.50	2.89	3.00
32	PHB3253	PHB3253	5.73	0.90	70.28	1.72	237.83	0.49	2.61	2.72
	Min		**2.99**	**0.85**	**65.06**	**-0.17**	**189.27**	**0.43**	**2.22**	**1.94**
	Max		**6.65**	**1.02**	**72.39**	**2.56**	**252.67**	**0.54**	**3.00**	**3.94**
	Mean		**5.38**	**0.92**	**68.73**	**0.89**	**226.59**	**0.50**	**2.71**	**2.71**
	StError		**0.13**	**0.01**	**0.32**	**0.09**	**2.66**	**0.00**	**0.03**	**0.07**

GY = grain yield; EPP = ears/plant; AD = days to anthesis; ASI = anthesis-silking interval; PH = plant height;
EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 5-3. Means of the Dobie's susceptibility index (dSI) and other traits under across analysis for maize weevil resistance

Entry	Cross	Pedigree	SI	MDP (#)	living MW (#)	WL (%)	SD (%)	PM	Oil	Protein	MOI	Starch	TEX
1	2×4	CKSBL10011 × CKDHL121230	9.21	46	74	4.21	29.18	20.37	5.23	9.49	12.73	69.68	3.60
2	2×5	CKSBL10011 × CKSBL10082	8.09	45	58	6.19	18.71	24.22	5.43	10.49	12.65	69.08	2.67
3	2×6	CKSBL10011 × CKSBL10060	9.42	46	90	4.03	25.37	25.08	5.26	9.99	12.49	69.99	2.07
4	1×2	CKSBL10027 × CKSBL10011	8.62	46	66	3.69	24.33	22.56	5.16	10.27	13.13	69.82	2.13
5	2×3	CKSBL10011 × CKDHL120172	9.21	44	78	3.85	28.31	19.78	5.34	9.09	13.88	69.83	4.33
6	2×7	CKSBL10011 × CKDHL120731	8.57	46	58	3.11	21.66	25.50	5.54	10.55	13.13	68.88	3.33
7	2×8	CKSBL10011 × CKDHL120517	6.61	46	36	2.09	15.69	29.68	5.38	11.21	12.94	68.78	4.20
8	4×5	CKDHL121230 × CKSBL10082	8.75	46	69	4.02	27.57	24.33	4.79	10.37	12.88	69.93	2.00
9	4×6	CKDHL121230 × CKSBL10060	8.02	46	49	2.27	20.04	25.02	5.18	10.17	12.78	69.33	4.04
10	1×4	CKSBL10027 × CKDHL121230	8.96	45	81	3.44	24.84	20.89	5.09	10.46	12.53	69.75	1.07
11	3×4	CKDHL120172 × CKDHL121230	9.47	45	91	5.09	32.45	21.36	4.81	9.62	12.86	70.24	3.67
12	4×7	CKDHL121230 × CKDHL120731	7.28	47	61	2.60	22.53	22.11	7.53	10.97	12.45	69.03	1.20
13	4×8	CKDHL121230 × CKDHL120517	8.23	45	62	3.42	21.32	26.56	5.03	11.09	12.56	70.04	2.53
14	5×6	CKSBL10082 × CKSBL10060	8.41	44	54	4.20	19.03	25.69	5.09	10.27	12.50	69.59	3.07
15	1×5	CKSBL10027 × CKSBL10082	8.66	45	80	3.25	21.26	25.11	5.06	10.81	12.93	69.67	1.53
16	3×5	CKDHL120172 × CKSBL10082	9.05	44	70	2.98	27.63	19.34	5.16	9.52	13.56	69.75	1.93
17	5×7	CKSBL10082 × CKDHL120731	8.67	46	74	3.78	24.73	24.22	5.44	11.02	12.56	68.64	1.67
18	5×8	CKSBL10082 × CKDHL120517	6.98	46	31	2.66	15.52	27.78	5.06	11.09	12.64	69.31	2.73
19	1×6	CKSBL10027 × CKSBL10060	8.29	47	77	2.86	21.27	25.00	5.02	10.25	12.76	69.68	1.87
20	3×6	CKDHL120172 × CKSBL10060	8.55	45	67	3.18	27.79	21.44	5.18	9.16	14.40	69.86	4.40
21	6×7	CKSBL10060 × CKDHL120731	7.37	44	50	2.19	18.75	23.56	5.70	10.71	12.91	68.45	3.27
22	6×8	CKSBL10060 × CKDHL120517	6.53	44	36	1.42	14.20	34.49	5.23	11.09	12.94	68.93	4.07
23	1×3	CKSBL10027 × CKDHL120172	10.01	44	99	7.15	31.41	17.44	5.06	9.90	13.16	70.26	2.27
24	1×7	CKSBL10027 × CKDHL120731	8.17	45	67	7.99	24.41	20.92	5.43	10.76	12.76	69.03	1.04
25	1×8	CKSBL10027 × CKDHL120517	7.68	46	48	7.21	16.66	27.09	4.95	11.06	12.85	69.59	2.13
26	3×7	CKDHL120172 × CKDHL120731	9.73	45	98	5.45	34.12	15.90	5.30	10.20	12.91	69.58	1.67
27	3×8	CKDHL120172 × CKDHL120517	8.85	45	74	2.96	25.20	22.32	4.80	9.73	13.04	70.15	3.67
28	7×8	CKDHL120731 × CKDHL120517	3.73	50	9	1.66	11.64	43.33	4.99	11.40	12.44	69.24	1.00
29	CZH1258	CZH1258	8.69	96	45	0.03	0.31	0.26	5.35	10.37	13.30	68.98	4.79
30	CKPH1204	CKPH12040	7.93	50	46	0.03	0.23	0.22	5.17	11.29	12.89	68.92	3.87
31	WE1101	WE1101	9.69	116	44	0.07	0.34	0.22	4.87	9.76	13.59	70.25	2.93
32	PHB3253	PHB3253	10.36	108	45	0.04	0.38	0.16	4.91	9.16	14.00	70.06	3.33
Min			**3.73**	**43.94**	**9.11**	**0.03**	**0.23**	**0.16**	**4.79**	**9.09**	**12.44**	**68.45**	**1.00**
Max			**10.36**	**116.44**	**99.11**	**7.99**	**34.12**	**43.33**	**7.53**	**11.40**	**14.40**	**70.26**	**4.79**
Mean			**8.37**	**51.42**	**62.07**	**3.35**	**20.21**	**21.31**	**5.24**	**10.35**	**12.97**	**69.51**	**2.75**
StError			**0.22**	**3.24**	**3.62**	**0.36**	**1.64**	**1.68**	**0.08**	**0.12**	**0.08**	**0.09**	**0.20**

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 5-4. Across and individual site general combining ability (GCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under drought conditions in Kenya 2017A and 2018A

	Genotype	GY	EPP	AD	ASI	PH	EPO	PA	EA	SEN
across	**CKSBL10027**	-0.48	-0.02	-1.74	0.20	-5.92	0.01*	0.07	0.21	0.58**
	CKSBL10011	-0.42	-0.06	0.70	-0.24	-0.17	0.00	0.18	0.40	0.70***
	CKDHL120172	0.62*	-0.03	1.11	1.54*	12.82***	0.01	-0.38*	-0.32	-1.34***
	CKDHL121230	0.62*	0.06	-0.21	1.23	-6.63*	-0.04***	-0.65***	-0.15	-0.78***
	CKSBL10082	-0.04	0.04	2.69	-1.52*	-13.32***	0.015**	0.74***	-0.04	0.05
	CKSBL10060	-0.61*	-0.07	-1.18	-0.94	-8.68**	0.01*	0.46**	0.54	1.22***
	CKDHL120731	0.06	0.02	-0.02	-0.26	14.88***	-0.03***	-0.32	-0.29	-0.40*
	CKDHL120517	0.26	0.04	-1.36	-0.01	7.01*	0.02***	-0.10	-0.35	-0.02
Site 4	**CKSBL10027**	-0.09	0.00	0.03	-0.80	-4.34	0.01	-	-0.07	0.58*
	CKSBL10011	-0.24	-0.04	0.14	0.58	-4.26	0.00	-	0.21	0.75**
	CKDHL120172	0.03	-0.08	1.64***	2.26***	11.97***	0.01	-	0.10	-1.11***
	CKDHL121230	0.20	0.08	-1.92***	-0.70	-4.78	-0.03***	-	-0.01	-0.22
	CKSBL10082	0.24	0.08	-0.92**	-1.92***	-7.95**	0.01	-	-0.29	-0.28
	CKSBL10060	-0.27	-0.07	-1.58***	-1.18*	-10.45***	0.01	-	0.26	1.03***
	CKDHL120731	-0.02	-0.02	1.53***	1.36**	11.58***	-0.02***	-	-0.01	-0.47
	CKDHL120517	0.16	0.05	1.08***	0.57	8.24**	0.025***	-	-0.18	0.24
Site 8	**CKSBL10027**	-0.88***	-0.03	-11.40***	3.09	-7.49***	0.02***	0.07	0.49**	0.57*
	CKSBL10011	-0.59**	-0.08***	0.93	0.04	3.92*	0.00	0.18	0.60***	0.65**
	CKDHL120172	1.21***	0.03*	-8.24**	3.26	13.67***	0.01	-0.38*	-0.74***	-1.57***
	CKDHL121230	1.04***	0.04**	7.18	0.97	-8.47***	-0.05***	-0.65***	-0.29	-1.35***
	CKSBL10082	-0.33	0.01	1.36	0.97	-18.69***	0.02***	0.74***	0.21	0.38
	CKSBL10060	-0.96***	-0.07***	14.36***	-2.50	-6.91***	0.01*	0.46**	0.82***	1.40***
	CKDHL120731	0.15	0.06***	-10.49***	-0.89	18.17***	-0.03***	-0.32	-0.57**	-0.32
	CKDHL120517	0.35***	0.04**	-4.38***	0.08	5.78**	0.02**	-0.10	-0.54**	-0.28

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant, AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position; PA= Plant aspect, EA = ear aspect; PA = plant aspect; SEN= senascence

Appendix 5-5. Across and individual site general combining ability (GCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

	Genotype	GY	EPP	AD	ASI	PH	EPO	PA	EA
Across	CKSBL10027	-0.44*	0.01	0.41	0.59***	-4.66	0.00	0.02	0.14*
	CKSBL10011	0.00	-0.02	0.69	-0.11	1.67	0.01*	0.05	0.14*
	CKDHL120172	0.68***	-0.02	1.68	0.24*	14.21***	0.02**	0.03	-0.40***
	CKDHL121230	0.21	0.01	-2.40*	0.19	-11.13***	-0.04***	-0.23***	0.07
	CKSBL10082	-0.42*	0.03**	-1.14	-0.54***	-9.16**	0.00	0.10	0.16*
	CKSBL10060	-0.29	-0.02	-0.72	0.20	-4.22	0.02**	0.01	0.10
	CKDHL120731	0.09	-0.02	0.84	-0.28*	9.12**	-0.02***	0.03	-0.18*
	CKDHL120517	0.15	0.02	0.63	-0.29*	4.17	0.01*	-0.01	-0.06
Site 1	CKSBL10027	-0.32	0.07*	0.68	0.50	-3.22	0.01**	0.13	-0.03
	CKSBL10011	-0.18	-0.05	1.72***	-0.27	4.87	0.02***	0.15	0.09
	CKDHL120172	-0.76**	-0.09**	2.50***	0.79*	13.42***	0.02***	0.21*	0.15
	CKDHL121230	1.41***	0.03	-3.50***	0.18	-10.58***	-0.06***	-0.74***	-0.13
	CKSBL10082	0.11	0.10**	-1.68***	-0.75*	-7.23*	0.00	0.01	-0.05
	CKSBL10060	0.40	-0.04	-1.22**	-0.20	-3.57	0.03***	-0.18	-0.01
	CKDHL120731	-0.43	-0.04	1.44***	-0.27	-1.22	-0.03***	0.32**	-0.02
	CKDHL120517	-0.24	0.01	0.06	0.01	7.51	0.00	0.10	-0.02
Site 2	CKSBL10027	-0.80***		0.01	0.76***	-9.29***	0.01**	-0.07	0.44***
	CKSBL10011	-0.46**		0.07	0.32*	4.04	0.00	0.26	0.44***
	CKDHL120172	0.86***		1.18***	0.21	15.43***	0.01	0.43**	-0.61***
	CKDHL121230	0.98***		-2.15***	0.15	-8.79**	-0.03***	-0.57***	-0.17
	CKSBL10082	-0.27		-0.76***	-0.68***	-11.43***	0.01	-0.07	-0.06
	CKSBL10060	-0.66***		-0.54**	0.21	-2.43	0.016**	0.10	0.17
	CKDHL120731	0.43*		1.24***	-0.57***	16.21***	-0.02**	0.10	-0.33***
	CKDHL120517	-0.07		0.96***	-0.40*	-3.74	0.00	-0.18	0.11
Site 3	CKSBL10027	0.03	-0.02	-0.35	0.82**	-2.18	-0.01	-0.03	-0.19
	CKSBL10011	0.03	0.02	0.54	-0.13	-5.07	0.01*	-0.19*	-0.03
	CKDHL120172	1.35***	-0.04*	2.93***	0.15	27.40***	0.04***	-0.58***	-0.31*
	CKDHL121230	-0.78***	0.06**	-2.51***	-0.07	-23.13***	-0.06***	0.53***	0.36**
	CKSBL10082	-0.69***	0.01	-2.01***	-0.51*	-19.78***	0.01	0.36***	0.42***
	CKSBL10060	0.00	0.02	-0.96***	-0.01	-6.28*	0.02**	-0.14	0.14
	CKDHL120731	-0.38*	-0.05*	1.10*	0.04	15.68***	-0.05***	0.03	-0.14
	CKDHL120517	0.44**	0.00	1.26**	-0.29	13.37	0.03**	0.03	-0.25

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; AD = days to anthesis; ASI = anthesis-silking interval; PH = plant height
EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 5-5. Continuation

	Genotype	GY	EPP	AD	ASI	PH	EPO	PA	EA
Site 5	CKSBL10027	-0.17	-0.04	0.39	-0.04	1.45	-0.01	0.10	0.17
	CKSBL10011	0.01	0.01	0.39	-0.15	-0.74	0.00	-0.35**	-0.11
	CKDHL120172	0.49**	0.05	-0.67	-0.43	2.17	-0.01	-0.24*	-0.22*
	CKDHL121230	-0.27	-0.02	0.50	0.29	-0.66	0.01	0.15	0.17
	CKSBL10082	0.19	0.00	0.33	0.18	4.09	0.00	-0.07	-0.28**
	CKSBL10060	-0.49**	-0.03	0.11	0.35	-1.63	0.00	0.54***	0.22*
	CKDHL120731	0.03	0.01	-1.11*	0.18	-5.66*	-0.01	-0.13	0.17
	CKDHL120517	0.22	0.03	0.06	-0.38	12.20	0.00	-0.01	-0.11
Site 6	CKSBL10027	-0.82***	0.02	0.15	1.01***	-9.22***	0.01	-0.14	0.5***
	CKSBL10011	0.37**	-0.05*	0.43**	-0.04	5.20***	0.01*	0.36*	0.22
	CKDHL120172	0.86***	0.00	1.32***	0.68***	12.56***	0.02***	0.31*	-0.78***
	CKDHL121230	0.08	-0.02	-2.13***	0.01	-12.58***	-0.04***	-0.53***	0.06
	CKSBL10082	-0.69***	0.05*	-1.13***	-0.82***	-12.13***	0.01*	0.03	0.22
	CKSBL10060	-0.86***	-0.02	-0.63***	0.46**	-6.94***	0.01**	-0.14	0.28*
	CKDHL120731	0.68***	0.00	1.32***	-0.93***	20.45***	-0.02***	-0.08	-0.44***
	CKDHL120517	0.38**	0.03	0.65***	-0.38*	-0.38*	0.01**	0.19	-0.06
Site 7	CKSBL10027	-0.48	0.03	1.38***	0.51**	-	-	0.14	-0.07
	CKSBL10011	0.24	-0.01	0.99***	-0.38*	-	-	0.08	0.21
	CKDHL120172	1.30***	-0.02	2.82***	0.01	-	-	0.08	-0.63***
	CKDHL121230	-0.15	0.01	-4.57***	0.57**	-	-	-0.25*	0.15
	CKSBL10082	-1.20***	0.00	-1.40***	-0.65***	-	-	0.31**	0.71***
	CKSBL10060	-0.10	-0.03*	-1.13**	0.40*	-	-	-0.14	-0.18
	CKDHL120731	0.23	0.00	1.10**	-0.15	-	-	-0.03	-0.18
	CKDHL120517	0.17	0.02	0.82*	-0.32	-	-	-0.19	-0.01

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; AD = days to anthesis; ASI = anthesis-silking interval; PH = plant height
EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 5-6. Across and individual site general combining ability (GCA) effects for Dobie's Susceptibility Index and other traits of 8 maize inbred lines evaluated under maize weevil infestation in Kenya 2017A and 2018A

	Genotype	SI	MDP (#)	living MW (#)	WL (%)	SD (%)	PM (%)	Oil	Protein	MOI	Starch	TEX
across	**CKSBL10027**	0.43*	0.06	10.89*	1.48*	0.46	-1.88	-0.17	0.14	-0.06	0.22	-1.04***
	CKSBL10011	0.33	0.00	1.37	0.07	0.31	-0.51	0.09	-0.26	0.10	-0.07	0.68***
	CKDHL120172	1.18***	-0.97	21.12***	0.65	7.58***	-5.45**	-0.18	-0.90	0.57**	0.50***	0.61***
	CKDHL121230	0.36	0.13	5.81	-0.28	2.75*	-1.61	0.13	-0.09	-0.25	0.25	-0.04
	CKSBL10082	0.14	-0.37	-2.70	0.06	-1.16	0.07	-0.12	0.14	-0.11	-0.09	-0.44***
	CKSBL10060	-0.20	-0.39	-4.68	-1.10	-2.49*	1.67	-0.02	-0.17	0.10	-0.10	0.74***
	CKDHL120731	-0.71***	0.80	-5.91	0.01	-0.60	0.88	0.53*	0.49	-0.19	-0.63***	-0.85***
	CKDHL120517	-1.53***	0.73	-25.90***	-0.89	-6.86***	6.83***	-0.24	0.65	-0.16	-0.08	0.34***
Site 1	**CKSBL10027**	0.65*	-0.17	20.36	3.30*	1.40	-1.83	0.02	0.38*	-0.16*	0.30	-0.99***
	CKSBL10011	-0.06	-0.17	-10.86	-1.54	-3.31	-0.83	0.15*	-0.45*	0.09	0.12	0.29
	CKDHL120172	1.22***	-1.17*	47.14***	3.18*	9.26**	-5.06***	-0.10	-0.93***	0.29***	0.94***	0.01
	CKDHL121230	0.24	-0.17	3.31	-0.61	3.28	0.17	-0.13*	-0.36	0.06	-0.06	0.46*
	CKSBL10082	0.11	-0.33	-2.92	-1.13	0.32	-3.28*	0.11	0.13	-0.16*	-0.52*	-0.32
	CKSBL10060	0.16	0.17	0.75	0.11	0.14	-0.39	0.09	-0.47*	0.16*	0.16	0.79***
	CKDHL120731	-0.48*	0.17	-7.69	-1.62	-2.31	3.83*	0.16*	0.39*	-0.15*	-0.54*	-0.88***
	CKDHL120517	-1.85***	1.67**	-50.08***	-1.70	-8.78**	7.39***	-0.30***	1.3***	-0.12	-0.39*	0.625**
Site 2	**CKSBL10027**	-0.25	0.17	0.71	4.73	-2.93	-0.81	-0.15***	0.22*	0.09	0.32**	-
	CKSBL10011	1.29***	-1.00	13.99*	1.18	3.63*	-0.58	0.23***	-0.27**	0.03	-0.04	-
	CKDHL120172	0.93*	-0.83	7.10	-2.08	5.49**	-1.69	-0.01	-0.92***	0.18	0.67***	-
	CKDHL121230	0.38	0.83	2.65	-2.93	1.99	0.86	-0.21***	-0.02	0.02	0.16	-
	CKSBL10082	0.34	-0.83	3.15	0.54	-1.67	4.19	-0.02	0.29**	0.15	-0.09	-
	CKSBL10060	-0.20	0.00	-2.57	-3.78	-2.19	0.19	0.01	-0.29**	-0.09	-0.08	-
	CKDHL120731	-1.36***	0.83	-10.57*	0.96	-0.86	-1.36	0.31***	0.52***	-0.25*	-0.88***	-
	CKDHL120517	-1.13**	0.83	-14.46**	1.38	-3.46*	-0.81	-0.16***	0.46***	-0.13	-0.05	-
Site 3	**CKSBL10027**	1.03*	-0.25	38.90**	1.07	5.12	-2.50	-0.04	0.05	0.07	0.35**	-1.26***
	CKSBL10011	0.01	-0.42	-8.82	-0.46	-2.20	-1.06	0.38***	-0.24*	0.01	-0.35**	1.01***
	CKDHL120172	1.14**	-1.42***	23.63*	1.33*	5.18	-3.94	-0.07	-1.29***	0.76***	0.30**	0.74***
	CKDHL121230	0.47	0.25	7.79	0.84	1.60	1.06	-0.29*	0.19	-0.51***	0.45***	-0.54***
	CKSBL10082	-0.11	-0.25	-6.82	-0.27	1.10	-2.39	-0.12	0.18	-0.24**	0.24*	-0.10
	CKSBL10060	-0.09	0.42	-12.21	-1.28	-2.51	-3.72	0.14	-0.04	0.25**	-0.27*	0.96***
	CKDHL120731	-0.71*	1.08*	-2.21	0.33	-0.46	3.50	0.13	0.58***	-0.40***	-0.52***	-0.10***
	CKDHL120517	-1.74***	0.58*	-40.26***	-1.57**	-7.85**	9.06***	-0.12	0.58***	0.06	-0.21*	0.18

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 5-6. Continuation

	Genotype	SI	MDP (#)	living MW (#)	WL (%)	SD (%)	PM (%)	Oil	Protein	MOI	Starch	TEX
Site 5	CKSBL10027	0.65	1.51	4.68	1.84***	3.94*	-5.71	-0.17	-0.23	-0.10	0.08	-1.19***
	CKSBL10011	0.53	1.85	7.57*	-0.16	1.34	-1.91	-0.09	-0.15	-0.09	0.02	0.70*
	CKDHL120172	1.73***	1.35	12.01***	0.49	12.08***	-16.97**	-0.17	-0.55*	0.04	0.20	0.75**
	CKDHL121230	-0.11	-0.38	-0.88	-0.25	-2.91*	-4.79	0.22	0.23	-0.07	0.17	0.03
	CKSBL10082	0.33	0.29	-5.15	-0.11	-3.06*	2.03	0.00	0.14	-0.04	-0.28	-0.47
	CKSBL10060	-0.66	-4.49*	-6.93*	-1.39**	-5.52**	10.00*	-0.09	0.08	0.10	-0.06	0.92**
	CKDHL120731	-0.81*	1.29	-3.10	0.27	0.22	0.90	0.17	0.04	0.08	-0.43*	-1.20***
	CKDHL120517	-1.67***	-1.43	-8.21**	-0.69*	-6.09***	16.45**	0.11	0.43*	0.07	0.31	0.47*
Site 6	CKSBL10027	0.49	-0.13	6.19	-0.52	-0.46	0.89	-0.72	0.21**	-0.11	0.16*	-1.13***
	CKSBL10011	0.13	-0.46	0.69	1.22	2.92*	1.00	-0.35	-0.25***	-0.04	0.07	0.93***
	CKDHL120172	1.15**	-1.79*	17.64***	0.54	6.70***	-1.11	-0.59	-0.85***	0.32*	0.63***	0.93***
	CKDHL121230	0.47	0.71	7.86*	0.18	4.34*	-1.44	1.44	-0.39***	-0.24	0.41***	0.15
	CKSBL10082	-0.25	-0.96	-7.75*	0.35	-3.92*	1.00	-0.66	0.23***	0.21	0.00	-0.90***
	CKSBL10060	-0.35	1.21*	-7.47*	-1.02	-3.27*	0.00	-0.43	-0.12	-0.13	-0.29**	0.54*
	CKDHL120731	-0.37	0.71	-3.25	-0.23	0.25	0.33	2.06*	0.72***	-0.02	-1.01***	-0.68***
	CKDHL120517	-1.29***	0.71	-13.92**	-0.51	-6.55***	-0.67	-0.75	0.45	0.01	0.03	0.14
Site 7	CKSBL10027	0.01	-0.79	-5.51	-1.56*	-4.29*	-1.30	0.02	0.20*	0.00	0.07	-0.65***
	CKSBL10011	0.04	0.21	5.65	0.19	-0.52	0.29	0.22***	-0.24**	0.61**	-0.30	0.46***
	CKDHL120172	0.93**	-1.96***	19.21*	0.47	6.80**	-3.91*	-0.22***	-0.93***	1.89***	0.41*	0.63***
	CKDHL121230	0.67*	-0.46	14.10*	1.09	8.20***	-5.48*	-0.17**	-0.16	-0.81***	0.33*	-0.27*
	CKSBL10082	0.42	-0.13	3.26	0.95	0.29	-1.14	-0.09	-0.07	-0.54*	0.10	-0.43***
	CKSBL10060	-0.06	0.38	0.38	0.75	-1.61	3.92*	0.11*	-0.22**	0.14	-0.15	0.51***
	CKDHL120731	-0.52*	0.71	-8.63	0.33	-0.42	-1.94	0.28***	0.66***	-0.47*	-0.27	-0.54***
	CKDHL120517	-1.49***	2.04***	-28.46***	-2.22**	-8.44***	9.55***	-0.15**	0.76	-0.82**	-0.19	0.29**

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 5-7. Across specific combining ability (SCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under drought conditions in Kenya 2017A and 2018A

Single Cross	Pedigree	GY	EPP	AD	ASI	PH	EPO	PA	EA	SEN
S12	CKSBL10027 × CKSBL10011	-0.45	0.05	-0.89	-0.45	1.35	0.02	-0.52	0.50	1.36***
S13	CKSBL10027 × CKDHL120172	-0.02	-0.02	1.04	-0.62	-7.22	-0.01	0.03	-0.12	-0.60
S14	CKSBL10027 × CKDHL121230	0.25	-0.06	-1.47	3.36*	-0.61	-0.01	-0.02	0.22	-0.49
S15	CKSBL10027 × CKSBL10082	-0.27	-0.09	-2.50	0.67	-4.42	-0.01	0.59	-0.06	-0.40
S16	CKSBL10027 × CKSBL10060	-0.15	-0.01	0.11	-0.35	0.03	0.01	-0.13	0.19	1.35**
S17	CKSBL10027 × CKDHL120731	0.40	0.07	2.67	-1.12	10.64	0.00	-0.02	-0.48	-0.46
S18	CKSBL10027 × CKDHL120517	0.24	0.07	1.06	-1.49	0.25	0.01	0.09	-0.25	-0.75
S23	CKSBL10011 × CKDHL120172	0.40	0.02	-1.35	0.62	5.86	0.01	0.59	-0.31	-0.56
S24	CKSBL10011 × CKDHL121230	0.70	0.14	-5.25	-2.29	8.47	-0.01	-0.47	-0.64*	-0.95*
S25	CKSBL10011 × CKSBL10082	0.40	0.05	7.35**	0.07	-4.34	-0.02	0.14	-0.25	0.22
S26	CKSBL10011 × CKSBL10060	-1.25*	-0.28**	-0.11	0.50	-30.56***	-0.01	1.42***	0.83*	1.47***
S27	CKSBL10011 × CKDHL120731	-0.19	-0.06	-0.82	1.65	6.55	0.00	-0.80*	0.16	-0.25
S28	CKSBL10011 × CKDHL120517	0.38	0.07	1.06	-0.10	12.66	0.01	-0.36	-0.28	-1.29**
S34	CKDHL120172 × CKDHL121230	-0.58	-0.03	2.56	3.79*	1.32	-0.01	0.42	0.41	1.26**
S35	CKDHL120172 × CKSBL10082	-0.31	-0.01	-4.72	-0.51	-3.65	0.00	0.03	0.47	0.51
S36	CKDHL120172 × CKSBL10060	0.83	0.11	-1.67	-1.04	11.96	0.00	-0.36	-0.62*	-1.24**
S37	CKDHL120172 × CKDHL120731	-0.30	-0.05	1.98	-1.87	-8.60	0.00	0.09	0.05	-0.13
S38	CKDHL120172 × CKDHL120517	-0.03	-0.03	2.15	-0.37	0.35	0.01	-0.80*	0.11	0.75
S45	CKDHL121230 × CKSBL10082	-0.26	-0.05	5.10	-2.22	-1.04	0.01	0.31	-0.03	0.62
S46	CKDHL121230 × CKSBL10060	-0.01	0.07	3.21	-1.13	3.90	0.00	-0.41	-0.12	-0.96*
S47	CKDHL121230 × CKDHL120731	0.05	-0.03	-1.29	-0.45	-5.07	0.01	0.37	0.05	0.15
S48	CKDHL121230 × CKDHL120517	-0.16	-0.03	-2.86	-1.06	-6.96	0.00	-0.19	0.11	0.36
S56	CKSBL10082 × CKSBL10060	0.39	0.04	0.70	-0.13	9.76	0.01	-0.47	-0.06	-0.88*
S57	CKSBL10082 × CKDHL120731	0.12	0.06	-3.29	0.49	2.96	0.00	-0.36	-0.23	-0.43
S58	CKSBL10082 × CKDHL120517	-0.08	-0.01	-2.64	1.64	0.73	0.00	-0.25	0.16	0.36
S67	CKSBL10060 × CKDHL120731	0.23	0.08	-1.36	1.03	2.73	0.01	-0.41	0.02	0.40
S68	CKSBL10060 × CKDHL120517	-0.05	-0.01	-0.89	1.11	2.18	-0.01	0.37	-0.25	-0.14
S78	CKDHL120731 × CKDHL120517	-0.31	-0.07	2.12	0.26	-9.21	-0.02	1.14**	0.41	0.72

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant, AD = days to anthesis; ASI = anthesis-silking interval, PH = plant height; EPO = ear position;
PA= Plant aspect, EA = ear aspect; PA = plant aspect; SEN= senascence

Appendix 5-8. Across specific combining ability (SCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

Single cross	Pedigree	GY	EPP	AD	ASI	PH	EPO	PA	EA
S12	CKSBL10027 × CKSBL10011	0.33	0.02	-0.66	0.06	1.59	0.01	0.00	-0.21
S13	CKSBL10027 × CKDHL120172	0.18	0.01	-0.65	0.38	3.95	-0.02	-0.21	-0.11
S14	CKSBL10027 × CKDHL121230	0.40	-0.02	0.75	-0.24	3.36	0.00	0.06	-0.14
S15	CKSBL10027 × CKSBL10082	-0.76	-0.01	1.22	-0.12	-9.54	-0.01	0.06	0.33*
S16	CKSBL10027 × CKSBL10060	-0.25	-0.02	-1.18	0.00	-4.71	0.01	-0.05	0.05
S17	CKSBL10027 × CKDHL120731	0.19	0.03	0.74	-0.32	1.26	0.00	-0.15	-0.02
S18	CKSBL10027 × CKDHL120517	-0.08	0.00	-0.22	0.24	4.09	0.00	0.29*	0.10
S23	CKSBL10011 × CKDHL120172	0.59	0.02	-0.48	-0.09	9.88	0.01	-0.07	-0.11
S24	CKSBL10011 × CKDHL121230	-0.15	-0.07*	0.26	0.18	6.19	-0.01	0.03	0.02
S25	CKSBL10011 × CKSBL10082	-0.06	0.02	-0.94	0.24	-1.62	0.00	-0.03	-0.11
S26	CKSBL10011 × CKSBL10060	-2.10***	0.00	2.03	0.34	-35.09***	-0.01	0.12	1.00***
S27	CKSBL10011 × CKDHL120731	0.53	0.01	-0.09	-0.57*	9.49	0.01	0.15	-0.35*
S28	CKSBL10011 × CKDHL120517	0.86	0.00	-0.10	-0.17	9.55	0.00	-0.19	-0.23
S34	CKDHL120172 × CKDHL121230	-0.05	0.01	-0.29	0.17	-2.61	0.00	0.10	0.23
S35	CKDHL120172 × CKSBL10082	0.30	-0.02	0.23	-0.32	1.75	0.01	-0.12	-0.24
S36	CKDHL120172 × CKSBL10060	0.66	0.00	0.54	-0.28	7.17	-0.01	-0.03	-0.47**
S37	CKDHL120172 × CKDHL120731	-1.13*	-0.01	0.08	0.26	-17.69**	-0.01	0.11	0.46**
S38	CKDHL120172 × CKDHL120517	-0.56	-0.01	0.57	-0.12	-2.45	0.01	0.21	0.25
S45	CKDHL121230 × CKSBL10082	-0.03	0.01	0.03	-0.39	-1.04	0.02*	-0.02	0.17
S46	CKDHL121230 × CKSBL10060	0.29	0.02	0.39	-0.07	7.85	0.00	-0.04	-0.10
S47	CKDHL121230 × CKDHL120731	-0.18	-0.01	-0.29	0.14	0.29	0.00	0.10	-0.18
S48	CKDHL121230 × CKDHL120517	-0.28	0.06*	-0.85	0.20	-14.04*	-0.01	-0.24	0.00
S56	CKSBL10082 × CKSBL10060	0.04	0.01	-0.67	-0.04	2.12	0.00	0.24	-0.02
S57	CKSBL10082 × CKDHL120731	0.29	0.01	-0.27	0.59*	3.08	0.00	-0.12	0.02
S58	CKSBL10082 × CKDHL120517	0.22	-0.02	0.39	0.04	5.25	-0.01	-0.02	-0.14
S67	CKSBL10060 × CKDHL120731	0.91*	0.00	-0.74	0.07	14.31*	0.01	-0.14	-0.21
S68	CKSBL10060 × CKDHL120517	0.45	0.00	-0.36	-0.03	8.35	0.01	-0.10	-0.25
S78	CKDHL120731 × CKDHL120517	-0.62	-0.03	0.57	-0.16	-10.75	-0.01	0.05	0.28

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; AD = days to anthesis; ASI = anthesis-silking interval; PH = plant height
EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 5-9. Across specific combining ability (SCA) effects for Dobie's Susceptibility Index and other traits of 8 maize inbred lines evaluated under maize weevil infestation in Kenya 2017A and 2018A

Cross	Pedigree	SI	MDP	Living MW	WL	SD	PM	Oil	Protein	MOI	starch	Tex
S12	CKSBL10027 × CKSBL10011	-0.40	0.75	-11.03	-1.67	0.50	0.62	-0.02	0.00	0.18	0.17	-0.12
S13	CKSBL10027 × CKDHL120172	0.14	-0.44	2.55	1.20	0.31	0.44	0.16	0.27	-0.45	0.03	0.11
S14	CKSBL10027 × CKDHL121230	-0.09	-0.38	-0.63	-1.58	-1.44	0.05	-0.12	0.08	0.01	-0.20	-0.46*
S15	CKSBL10027 × CKSBL10082	-0.17	-0.04	6.88	-2.10	-1.10	2.59	0.09	0.14	0.20	0.04	0.41*
S16	CKSBL10027 × CKSBL10060	-0.20	1.31	6.29	-1.34	0.24	0.89	-0.04	-0.11	-0.19	0.07	-0.45*
S17	CKSBL10027 × CKDHL120731	0.19	-1.21	-2.81	2.69*	1.48	-2.41	-0.18	-0.27	0.09	-0.06	0.28
S18	CKSBL10027 × CKDHL120517	0.52	0.02	-1.26	2.80*	0.00	-2.18	0.11	-0.12	0.16	-0.05	0.23
S23	CKSBL10011 × CKDHL120172	-0.55	-0.22	-8.76	-0.70	-2.64	1.41	0.18	-0.13	0.30	-0.10	0.44*
S24	CKSBL10011 × CKDHL121230	0.27	-0.16	2.55	0.60	3.06	-1.83	-0.24	-0.54**	-0.04	0.00	0.35
S25	CKSBL10011 × CKSBL10082	-0.63	-0.32	-5.66	2.24	-3.50	0.34	0.21	0.22	-0.25	-0.26	-0.18
S26	CKSBL10011 × CKSBL10060	1.04*	0.53	28.87*	1.24	4.50*	-0.40	-0.07	0.08	-0.59	0.67*	-1.95***
S27	CKSBL10011 × CKDHL120731	0.70	-0.49	-2.12	-0.79	-1.11	0.81	-0.33	-0.06	0.30	0.07	0.90***
S28	CKSBL10011 × CKDHL120517	-0.44	-0.09	-3.85	-0.92	-0.81	-0.96	0.27	0.43*	0.09	-0.57*	0.57**
S34	CKDHL120172 × CKDHL121230	-0.32	0.48	-0.20	0.90	-0.94	4.09	-0.38	0.22	-0.38	-0.01	0.49*
S35	CKDHL120172 × CKSBL10082	-0.52	0.15	-12.58	-1.55	-1.85	0.39	0.22	-0.11	0.18	-0.16	-0.84***
S36	CKDHL120172 × CKSBL10060	-0.69	1.00	-13.66	-0.20	-0.36	0.90	0.13	-0.14	0.94*	-0.04	0.40
S37	CKDHL120172 × CKDHL120731	1.00*	-0.52	18.18	0.97	4.07	-3.85	-0.27	0.29	-0.30	0.05	-0.70***
S38	CKDHL120172 × CKDHL120517	0.94*	-0.45	14.45	-0.63	1.42	-3.39	-0.03	-0.41*	-0.29	0.23	0.11
S45	CKDHL121230 × CKSBL10082	0.00	0.55	1.68	0.42	2.92	1.54	-0.46	-0.07	0.33	0.27	-0.13
S46	CKDHL121230 × CKSBL10060	-0.39	0.90	-16.68	-0.17	-3.28	0.63	-0.18	0.04	0.02	-0.32	0.69**
S47	CKDHL121230 × CKDHL120731	-0.62	0.38	-3.83	-0.95	-2.69	-1.49	1.62	0.18	-0.03	-0.10	-0.52*
S48	CKDHL121230 × CKDHL120517	1.15*	-1.78	17.10	0.77	2.37	-2.99	-0.24	0.08	0.08	0.36	-0.41*
S56	CKSBL10082 × CKSBL10060	0.22	-0.82	-3.28	1.42	-0.38	-0.37	-0.02	-0.09	-0.40	0.28	0.16
S57	CKSBL10082 × CKDHL120731	0.99*	0.05	18.17	-0.10	3.43	-1.05	-0.22	0.00	-0.06	-0.14	0.35
S58	CKSBL10082 × CKDHL120517	0.11	0.45	-5.22	-0.33	0.49	-3.45	0.18	-0.09	0.00	-0.03	0.23
S67	CKSBL10060 × CKDHL120731	0.02	-1.49	-3.96	-0.54	-1.22	-3.31	-0.08	-0.02	0.13	-0.27	0.79***
S68	CKSBL10060 × CKDHL120517	0.00	-1.42	2.42	-0.41	0.49	1.67	0.25	0.23	0.09	-0.39	0.38
S78	CKDHL120731 × CKDHL120517	-2.29***	3.28*	-23.63*	-1.28	-3.96	11.30**	-0.54	-0.13	-0.13	0.45	-1.10***

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 6-1. Means of the GY and other traits under across analysis for MLN virus infested environments

Entry	Cross	Pedigree	MLN1	MLN2	MLN3	MLN4	FW	GY	EA	NP
1	5×6	CKSBL10082 × CKSBL10060	2.83	4.17	5.00	5.17	0.55	0.43	3.67	14.00
2	4×5	CML494 × CKSBL10082	3.17	4.83	5.50	6.33	0.64	0.43	4.00	15.50
3	1×5	CML395 × CKSBL10082	3.17	4.17	5.67	6.50	0.21	0.12	4.33	16.00
4	2×5	CML442 × CKSBL10082	3.50	5.00	5.67	6.17	0.41	0.30	4.00	16.00
5	5×7	CKSBL10082 × CKDHL120731	3.17	4.33	5.17	6.00	0.66	0.47	4.00	15.67
6	5×8	CKSBL10082 × CKDHL120517	3.00	4.00	4.50	5.33	0.66	0.50	3.67	15.83
7	3×5	CKDHL120918 × CKSBL10082	2.50	3.00	3.67	4.17	1.12	0.72	3.33	14.83
8	4×6	CML494 × CKSBL10060	3.33	4.67	5.50	6.17	0.31	0.23	4.67	14.00
9	1×6	CML395 × CKSBL10060	3.00	4.17	5.83	7.00	0.20	0.12	4.67	13.02
10	2×6	CML442 × CKSBL10060	3.33	4.83	5.83	6.83	0.16	0.13	4.48	14.00
11	6×7	CKSBL10060 × CKDHL120731	2.83	4.00	4.83	5.17	0.69	0.49	3.67	15.83
12	6×8	CKSBL10060 × CKDHL120517	3.00	4.50	5.67	6.50	0.38	0.28	4.00	13.50
13	3×6	CKDHL120918 × CKSBL10060	2.00	2.33	2.83	3.00	1.44	1.07	3.33	16.33
14	1×4	CML395 × CML494	2.83	4.33	5.83	6.83	0.24	0.16	4.67	15.33
15	2×4	CML442 × CML494	4.33	5.50	6.33	6.83	0.19	0.12	4.33	14.00
16	4×7	CML494 × CKDHL120731	3.00	4.50	5.33	6.00	0.62	0.45	4.33	17.33
17	4×8	CML494 × CKDHL120517	3.50	5.00	5.83	6.33	0.25	0.18	4.00	12.67
18	3×4	CKDHL120918 × CML494	3.00	4.00	5.00	5.67	0.76	0.42	3.67	16.67
19	1×2	CML395 × CML442	3.50	5.00	5.83	7.33	0.00	0.00	-	13.00
20	1×7	CML395 × CKDHL120731	3.17	4.50	6.17	6.33	0.58	0.44	4.00	14.83
21	1×8	CML395 × CKDHL120517	3.33	5.00	5.33	6.33	0.11	0.07	5.00	11.83
22	1×3	CML395 × CKDHL120918	2.50	3.00	4.50	5.83	0.40	0.23	4.00	14.67
23	2×7	CML442 × CKDHL120731	4.00	5.17	5.50	6.17	0.47	0.30	4.33	13.33
24	2×8	CML442 × CKDHL120517	4.50	6.50	6.83	8.00	0.00	0.00	-	12.83
25	2×3	CML442 × CKDHL120918	3.00	4.17	5.17	5.83	0.38	0.31	3.67	13.83
26	7×8	CKDHL120731 × CKDHL120517	3.67	4.83	5.00	5.33	0.36	0.18	4.00	10.83
27	3×7	CKDHL120918 × CKDHL120731	2.67	3.83	4.50	5.00	0.68	0.38	4.00	17.33
28	3×8	CKDHL120918 × CKDHL120517	2.50	3.83	4.83	5.17	0.58	0.34	3.67	13.67
29	WE6109	WE6109	2.33	3.50	4.33	5.17	0.61	0.40	4.00	13.83
30	CKPH12040	CKPH12040	3.50	5.00	5.67	7.00	0.09	0.05	5.00	13.67
31	DK8031	DK8031	4.00	5.33	6.33	7.33	0.13	0.09	5.00	14.17
32	PHB3253	PHB3253	4.17	5.33	6.17	6.83	0.14	0.09	5.00	15.20
	Min		**2.00**	**2.33**	**2.83**	**3.00**	**0.00**	**0.00**	**3.33**	**10.83**
	Max		**4.50**	**6.50**	**6.83**	**8.00**	**1.44**	**1.07**	**5.00**	**17.33**
	Mean		**3.20**	**4.45**	**5.32**	**6.05**	**0.44**	**0.30**	**4.15**	**14.49**
	StError		**0.10**	**0.14**	**0.14**	**0.18**	**0.06**	**0.04**	**0.09**	**0.27**

MLN1- MLN4= MLN scores 1. early, 4- late, FW= Field weight, GY= Grain Yield, EA= Ear aspect, NP= number of plants

Appendix 6-2. Means of the Grain yield and other traits under across analysis for optimum environments

Entry	Cross	Pedigree	GY	EPP	PH	EPO	PA	EA
1	5×6	CKSBL10082 × CKSBL10060	4.96	0.97	206.64	0.51	2.72	3.06
2	4×5	CML494 × CKSBL10082	6.28	0.97	221.83	0.43	2.33	2.94
3	1×5	CML395 × CKSBL10082	5.90	0.93	226.97	0.50	3.00	2.39
4	2×5	CML442 × CKSBL10082	5.76	0.95	218.61	0.48	2.56	2.83
5	5×7	CKSBL10082 × CKDHL120731	5.76	0.92	223.53	0.44	2.39	2.83
6	5×8	CKSBL10082 × CKDHL120517	5.75	0.95	221.36	0.49	2.72	2.83
7	3×5	CKDHL120918 × CKSBL10082	5.40	0.93	199.67	0.48	2.72	2.61
8	4×6	CML494 × CKSBL10060	7.26	0.91	226.19	0.44	2.28	2.50
9	1×6	CML395 × CKSBL10060	7.23	0.89	251.00	0.53	2.56	1.89
10	2×6	CML442 × CKSBL10060	6.95	0.92	238.14	0.49	2.22	2.78
11	6×7	CKSBL10060 × CKDHL120731	6.23	0.94	240.39	0.47	2.67	2.28
12	6×8	CKSBL10060 × CKDHL120517	6.61	0.96	234.69	0.54	2.44	2.39
13	3×6	CKDHL120918 × CKSBL10060	6.14	0.96	222.89	0.48	2.67	2.61
14	1×4	CML395 × CML494	7.40	0.94	238.56	0.42	2.33	2.17
15	2×4	CML442 × CML494	6.84	1.53	242.42	0.45	2.50	2.61
16	4×7	CML494 × CKDHL120731	6.39	0.95	235.58	0.39	2.33	2.11
17	4×8	CML494 × CKDHL120517	6.79	0.91	240.14	0.47	2.67	2.50
18	3×4	CKDHL120918 × CML494	5.75	0.95	217.42	0.42	2.28	2.50
19	1×2	CML395 × CML442	6.25	0.88	231.69	0.51	3.17	2.39
20	1×7	CML395 × CKDHL120731	6.22	0.85	243.44	0.47	2.78	1.94
21	1×8	CML395 × CKDHL120517	6.13	0.89	242.81	0.52	2.94	2.28
22	1×3	CML395 × CKDHL120918	5.41	0.91	222.56	0.49	3.00	2.56
23	2×7	CML442 × CKDHL120731	6.50	0.94	233.17	0.46	2.28	2.44
24	2×8	CML442 × CKDHL120517	3.81	1.42	215.22	0.47	3.06	3.39
25	2×3	CML442 × CKDHL120918	5.85	0.93	233.00	0.48	2.78	2.64
26	7×8	CKDHL120731 × CKDHL120517	4.71	0.83	225.00	0.44	2.78	2.83
27	3×7	CKDHL120918 × CKDHL120731	5.16	0.92	228.33	0.45	3.00	2.72
28	3×8	CKDHL120918 × CKDHL120517	5.44	0.93	229.14	0.49	3.17	2.89
29	WE6109	WE6109	6.49	0.00	0.45	1.01	2.61	2.56
30	CKPH120	CKPH12040	5.78	0.06	0.48	0.88	2.89	2.78
31	DK8031	DK8031	5.84	0.94	0.53	0.89	3.22	2.78
32	PHB3253	PHB3253	6.66	2.06	0.47	0.91	2.78	2.56
	Min		**3.81**	**0.00**	**0.45**	**0.39**	**2.22**	**1.89**
	Max		**7.40**	**2.06**	**251.00**	**1.01**	**3.22**	**3.39**
	Mean		**6.05**	**0.94**	**200.38**	**0.53**	**2.68**	**2.58**
	StError		**0.14**	**0.06**	**13.71**	**0.03**	**0.05**	**0.06**

GY = grain yield; EPP = ears/plant; PH = plant height; EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 6-3. Means of the Dobie's susceptibility index (dSI) and other traits under across analysis for maize weevil resistance

Entry	Cross	Pedigree	SI	MDP (d)	living MW	WL (%)	SD (%)	PM (%)	OIL	PROTEIN	STARCH	MOI	TEX
1	5×6	CKSBL10082 × CKSBL10060	6.99	49.00	39	2.22	15.89	30.61	4.97	10.44	69.91	12.80	2.42
2	4×5	CML494 × CKSBL10082	7.64	48.33	54	2.39	15.67	23.89	4.97	11.36	69.16	12.97	1.83
3	1×5	CML395 × CKSBL10082	9.31	47.17	104	4.06	32.83	20.89	5.31	10.34	69.31	13.34	1.54
4	2×5	CML442 × CKSBL10082	7.95	48.83	73	3.67	20.94	22.67	5.19	10.38	69.71	12.83	4.33
5	5×7	CKSBL10082 × CKDHL120731	8.04	48.67	70	2.61	23.44	22.56	5.33	11.22	68.73	12.99	1.75
6	5×8	CKSBL10082 × CKDHL120517	6.87	50.11	42	2.94	19.06	26.22	4.99	11.32	69.34	13.12	2.67
7	3×5	CKDHL120918 × CKSBL10082	7.29	49.17	47	2.56	17.94	22.56	5.61	11.23	67.77	13.42	1.42
8	4×6	CML494 × CKSBL10060	7.48	49.50	60	3.00	21.39	29.78	5.06	10.18	69.50	13.29	3.83
9	1×6	CML395 × CKSBL10060	8.44	47.61	86	4.50	27.89	21.28	5.30	9.71	69.48	13.20	3.50
10	2×6	CML442 × CKSBL10060	8.21	49.00	68	3.17	23.00	22.78	5.94	9.84	67.39	13.92	4.56
11	6×7	CKSBL10060 × CKDHL120731	7.41	49.67	50	2.17	20.06	28.89	5.54	10.69	65.29	12.73	2.42
12	6×8	CKSBL10060 × CKDHL120517	6.95	49.83	44	2.61	14.94	35.83	5.16	11.24	69.00	12.86	3.17
13	3×6	CKDHL120918 × CKSBL10060	7.27	50.00	49	2.44	19.89	22.44	5.51	11.34	68.64	13.04	2.08
14	1×4	CML395 × CML494	9.27	47.83	106	4.06	34.94	16.67	5.20	10.48	69.26	13.69	2.00
15	2×4	CML442 × CML494	6.81	49.33	49	2.06	14.39	26.67	5.14	11.00	68.79	12.96	4.25
16	4×7	CML494 × CKDHL120731	7.03	49.00	43	3.56	18.06	36.44	5.17	11.71	68.63	12.78	1.67
17	4×8	CML494 × CKDHL120517	7.07	49.00	57	2.28	18.06	26.44	4.80	11.47	69.48	12.88	3.20
18	3×4	CKDHL120918 × CML494	7.23	49.50	51	2.67	18.22	23.56	5.57	12.11	68.07	13.07	1.67
19	1×2	CML395 × CML442	9.26	48.33	106	5.50	34.56	20.67	5.02	9.71	70.35	13.17	4.08
20	1×7	CML395 × CKDHL120731	8.99	49.17	104	5.00	32.56	19.33	5.21	10.66	69.39	13.00	1.24
21	1×8	CML395 × CKDHL120517	8.90	48.67	96	4.17	30.89	17.56	4.89	10.21	70.03	13.44	2.00
22	1×3	CML395 × CKDHL120918	9.27	47.83	99	6.50	30.78	14.72	5.42	11.12	68.79	13.11	1.33
23	2×7	CML442 × CKDHL120731	8.37	49.33	75	3.56	23.39	20.33	6.08	11.58	66.09	14.25	3.92
24	2×8	CML442 × CKDHL120517	7.77	48.83	53	2.83	15.56	22.61	4.62	11.19	69.90	13.18	3.86
25	2×3	CML442 × CKDHL120918	8.01	49.33	64	3.78	23.00	24.67	5.50	11.52	68.43	13.20	3.17
26	7×8	CKDHL120731 × CKDHL120517	3.63	52.00	7	2.28	12.06	57.06	5.01	11.63	69.06	13.04	1.67
27	3×7	CKDHL120918 × CKDHL120731	7.85	50.67	67	3.39	25.56	19.33	5.80	12.42	67.81	13.06	1.17
28	3×8	CKDHL120918 × CKDHL120517	7.35	50.67	46	1.89	18.06	24.06	5.40	11.77	68.56	12.82	1.25
29	WE6109	WE6109	7.34	48.17	58	0.03	0.21	0.25	5.43	10.88	68.96	12.69	3.42
30	CKPH12040	CKPH12040	7.38	49.33	42	0.02	0.19	0.20	5.11	11.17	69.19	12.99	3.73
31	DK8031	DK8031	8.93	48.00	93	0.04	0.32	0.20	4.76	10.48	69.58	14.37	4.08
32	PHB3253	PHB3253	8.67	48.33	85	0.03	0.29	0.27	5.12	9.78	69.86	13.55	2.83
Min	**Min**		**3.63**	**47.17**	**6.72**	**0.02**	**0.19**	**0.20**	**4.62**	**9.71**	**65.29**	**12.69**	**1.17**
Max	**Max**		**9.31**	**52.00**	**106.33**	**6.50**	**34.94**	**57.06**	**6.08**	**12.42**	**70.35**	**14.37**	**4.56**
Mean	**Mean**		**7.78**	**49.07**	**65.21**	**2.87**	**19.50**	**21.92**	**5.25**	**10.94**	**68.86**	**13.18**	**2.69**
StError	**StError**		**0.20**	**0.17**	**4.31**	**0.27**	**1.70**	**1.98**	**0.06**	**0.12**	**0.19**	**0.07**	**0.19**

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 6-4. Across and individual site general combining ability (GCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under MLN artificial infestation in Kenya 2017A and 2018A

	Genotype	FW	GY	MLN1	MLN2	MLN3	MLN4
Across	CML395	-	-	-0.10	-0.10	0.38**	0.72***
	CML442	-	-	0.68***	0.90***	0.71***	0.89***
	CKDHL120918	-	-	-0.65***	-1.10***	-1.07***	-1.19***
	CML494	-	-	0.18*	0.34**	0.40**	0.39***
	CKSBL10082	-	-	-0.13	-0.22*	-0.29*	-0.36**
	CKSBL10060	-	-	-0.29***	-0.35***	-0.24	-0.33**
	CKDHL120731	-	-	0.07	0.06	-0.07	-0.31**
	CKDHL120517	-	-	0.24**	0.48***	0.18	0.19
Site 5	CML395	-0.25***	-0.18***	-0.07	0.15	0.40**	0.68***
	CML442	-0.28***	-0.18***	0.82***	0.88***	0.68***	1.13***
	CKDHL120918	0.35***	0.21***	-0.74***	-1.13***	-0.93***	-1.10***
	CML494	-0.04	-0.04	0.26*	0.26	0.24	0.24
	CKSBL10082	0.17*	0.12*	-0.18	-0.18	0.01	-0.15
	CKSBL10060	0.08	0.09	-0.40**	-0.35*	-0.32*	-0.54***
	CKDHL120731	0.13*	0.08	0.10	0.15	-0.04	-0.26
	CKDHL120517	-0.15*	-0.11*	0.21*	0.21	-0.04	0.01
Site 10	CML395	-	-	-0.13	-0.36**	0.35*	0.76***
	CML442	-	-	0.54***	0.92***	0.74***	0.65***
	CKDHL120918	-	-	-0.57***	-1.08***	-1.21***	-1.29***
	CML494	-	-	0.10	0.42***	0.57**	0.54***
	CKSBL10082	-	-	-0.07	-0.25*	-0.60***	-0.57***
	CKSBL10060	-	-	-0.18*	-0.36**	-0.15	-0.13
	CKDHL120731	-	-	0.04	-0.03	-0.10	-0.35**
	CKDHL120517	-	-	0.26*	0.75***	0.40*	0.36**

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

FW= Field weight, GY= Grain Yield, MLN1- MLN4= MLN scores 1 at early stage and 4 at late stage

Appendix 6-5. Across and individual site general combining ability (GCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

	Genotype	GY	EPP	PH	EPO	PA	EA
across 1	CML395	0.39	-0.08	9.07**	0.02***	0.20**	-0.39***
	CML442	-0.04	0.14*	1.61	0.01	0.00	0.18*
	CKDHL120918	-0.51*	-0.04	-8.27**	0.00	0.17**	0.09
	CML494	0.75	0.07	3.26	-0.05***	-0.31***	-0.11
	CKSBL10082	-0.40	-0.02	-14.00***	0.00	-0.02	0.25***
	CKSBL10060	0.53*	-0.03	2.89	0.03***	-0.17**	-0.08
	CKDHL120731	-0.21	-0.07	4.47	-0.03***	-0.06	-0.14
	CKDHL120517	-0.50*	0.03	0.96	0.02***	0.20**	0.19*
Site 1	CML395	-0.31	-0.08**	8.10***	0.02***	0.38**	0.06
	CML442	-0.71**	0.00	-0.48	0.01*	0.15	0.39**
	CKDHL120918	0.34	0.02	-4.67*	0.00	0.15	-0.67***
	CML494	0.68**	0.01	5.97*	-0.04***	-0.24*	-0.06
	CKSBL10082	0.47*	0.10**	-8.28***	0.00	-0.24*	0.00
	CKSBL10060	0.92***	0.04	7.58**	0.02**	-0.46***	-0.17
	CKDHL120731	-0.15	-0.02	0.52	-0.03***	-0.24*	0.06
	CKDHL120517	-1.23***	-0.06*	-8.73***	0.02**	0.49***	0.39**
Site 2	CML395	0.58**	-	14.17***	0.03***	0.22	-0.41**
	CML442	0.26	-	-0.74	-0.01*	-0.06	0.18
	CKDHL120918	-0.71**	-	-1.99	0.01*	0.11	0.40**
	CML494	0.55**	-	-2.74	-0.05***	-0.11	0.03
	CKSBL10082	-0.62**	-	-13.88***	0.00	-0.17	-0.13
	CKSBL10060	0.23	-	1.31	0.02***	0.17	-0.02
	CKDHL120731	-0.18	-	9.20***	-0.01**	0.06	-0.25
	CKDHL120517	-0.12	-	-5.33*	0.01*	-0.22	0.20
Site 3	CML395	0.24	-0.03	7.17	0.03***	0.11	-0.10
	CML442	0.48	-0.06*	3.48	0.02*	-0.17	0.24
	CKDHL120918	-0.54	0.04	-14.22**	-0.02**	-0.06	-0.04
	CML494	0.90**	0.03	14.03**	-0.06***	-0.06	-0.21
	CKSBL10082	-0.74*	0.01	-22.00***	0.01	0.28	0.29
	CKSBL10060	0.84**	0.10***	6.67	0.04***	0.00	-0.26
	CKDHL120731	-0.58*	-0.04	-4.33	-0.05***	0.00	0.07
	CKDHL120517	-0.58*	-0.04*	9.17*	0.03***	-0.11	0.01

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; PH = plant height; EO = ear position; PA= plant aspect; EA = ear aspect

Appendix 6-5. Continuation

	Genotype	GY	EPP	PH	EPO	PA	EA
Site 5	CML395	1.01**	0.01	13.06***	0.02***	-0.08	-0.94***
	CML442	-0.98*	-0.02	1.42	0.00	0.14	0.89***
	CKDHL120918	-0.67	0.01	-14.36***	0.00	0.58***	0.22*
	CML494	1.29**	0.02	4.36**	-0.03***	-0.64***	0.00
	CKSBL10082	-0.31	0.03	-14.31***	0.01	0.03	0.78***
	CKSBL10060	0.57	-0.03	1.39	0.02***	-0.47***	-0.33***
	CKDHL120731	-0.22	0.00	6.58***	-0.02***	-0.14	-0.56***
	CKDHL120517	-0.70*	-0.03	1.86	0.01**	0.58***	-0.06
Site 6	CML395	0.16	-0.26	4.84**	0.01	0.21	-0.64***
	CML442	0.25	0.77*	1.23	-0.01	-0.07	-0.36***
	CKDHL120918	-0.76***	-0.24	-4.58**	0.00	-0.07	0.58***
	CML494	0.18	0.26	0.17	-0.05***	-0.46***	-0.03
	CKSBL10082	-0.61***	-0.25	-15.05***	-0.01	-0.07	0.25*
	CKSBL10060	0.44**	-0.27	-0.13	0.04**	0.04	0.19*
	CKDHL120731	0.34*	-0.25	13.09***	-0.02	0.10	-0.19*
	CKDHL120517	0.00	0.24	0.42	0.04**	0.32*	0.19*
Site 7	CML395	0.64**	-0.01	7.08	0.02*	0.36***	-0.33**
	CML442	0.45	0.00	4.75	0.02*	-0.03	-0.22
	CKDHL120918	-0.72**	-0.01	-9.78*	0.00	0.31**	0.06
	CML494	0.89***	0.02	-2.25	-0.06***	-0.36***	-0.39**
	CKSBL10082	-0.61*	0.00	-10.47*	0.02	0.03	0.33**
	CKSBL10060	0.17	-0.01	0.53	0.03**	-0.31**	0.11
	CKDHL120731	-0.47	-0.01	1.78	-0.05***	-0.14	0.06
	CKDHL120517	-0.35	0.02	8.36*	0.02**	0.14	0.39**

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; PH = plant height; EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 6-6. Across and individual site general combining ability (GCA) effects for Dobie's Susceptibility Index and other traits of 8 maize inbred lines evaluated under maize weevil infestation in Kenya 2017A and 2018A

	Genotype	SI	MDP (#)	living MW (#)	WL (%)	SD (%)	PM (%)	Oil	Protein	MOI	Starch	TEX
across	CML395	1.55***	-1.25**	0.27***	0.05***	0.14***	-0.10***	-0.09	-0.77***	0.17	0.79*	-0.38**
	CML442	0.37*	-0.18	0.08*	0.01	-0.01	-0.03	0.13	-0.28*	0.27	-0.19	1.70***
	CKDHL120918	0.02	0.51	0.01	0.00	0.00	-0.054*	0.30***	0.75***	-0.07	-0.53	-0.99***
	CML494	-0.27	-0.27	-0.05	-0.01	-0.04**	0.01	-0.17*	0.22*	-0.08	0.26	0.07
	CKSBL10082	-0.01	-0.47	-0.01	-0.01	-0.02	0.00	-0.10	-0.10	-0.09	0.41	-0.34**
	CKSBL10060	-0.24	0.09	-0.04	-0.01	-0.02*	0.04	0.08	-0.58***	-0.03	-0.38	0.66***
	CKDHL120731	-0.48**	0.73*	-0.08**	0.00	0.00	0.06*	0.19*	0.48***	-0.05	-1.06*	-0.69***
	CKDHL120517	-0.94***	0.84*	-0.17***	-0.02*	-0.06***	0.07**	-0.34***	0.29*	-0.12	0.69	-0.03
Site 1	CML395	1.36***	-0.25	0.28***	0.06***	0.16***	-0.04	-0.55	-0.97*	-0.36	2.93	-0.55**
	CML442	0.34	-0.75*	0.05	0.01	-0.04	0.03	0.70	0.09	1.77*	-2.24	1.94***
	CKDHL120918	-0.48*	-0.42	-0.11*	0.00	-0.01	0.00	0.53	0.57	-0.93	0.75	-1.23
	CML494	0.01	-0.25	0.00	0.00	0.00	-0.02	-0.47	0.23	-0.42	1.71	0.23
	CKSBL10082	0.00	0.75*	0.03	-0.01	0.02	-0.080*	-0.25	-0.42	-0.49	2.45	-0.22
	CKSBL10060	0.22	0.08	0.06	-0.02	-0.01	0.01	0.43	-1.47***	0.66	-2.96	0.69***
	CKDHL120731	-0.64*	0.25	-0.14**	0.00	-0.04	0.05	0.52	1.03**	0.26	-4.94*	-0.96***
	CKDHL120517	-0.81**	0.58*	-0.17**	-0.03*	-0.09**	0.04	-0.90	0.91**	-0.48	2.34	0.09
Site 2	CML395	0.97*	-1.32	0.17*	0.00	0.10***	-0.06***	-0.06	-0.98***	0.20**	0.65**	-
	CML442	1.17**	-1.26	0.22**	0.03	0.04*	-0.06***	0.05	-0.45***	-0.10	0.33*	-
	CKDHL120918	0.07	1.24	0.03	0.00	0.00	-0.01	0.42***	1.38***	-0.19	-1.66***	-
	CML494	-0.71	-0.60	-0.15*	0.00	-0.06**	0.02	-0.24***	0.21**	0.01	0.21	-
	CKSBL10082	0.27	-0.65	0.04	0.00	0.01	-0.01	-0.05	-0.01	0.02	-0.18	-
	CKSBL10060	-0.26	0.01	-0.06	0.01	-0.02	-0.02	0.02	-0.64***	-0.01	0.53**	-
	CKDHL120731	0.11	2.24**	0.06	-0.02	0.01	0.06***	0.12**	0.40***	0.04	-0.32	-
	CKDHL120517	-1.62***	0.35	-0.33***	-0.01	-0.09***	0.08***	-0.27	0.09	0.03	0.44**	-
Site 3	CML395	1.96***	-1.29***	0.35***	0.09***	0.16***	-0.07*	-0.01	-1.20***	0.37*	0.59***	-
	CML442	0.27	0.21	0.06	0.00	0.00	0.01	-0.08	-0.01	0.16	0.08	-
	CKDHL120918	0.19	0.04	0.04	0.02	-0.02	0.00	0.43***	0.91***	0.06	-0.74***	-
	CML494	0.05	-0.29	0.00	-0.03	0.00	0.03	-0.07	-0.11	0.19	-0.10	-
	CKSBL10082	0.13	0.04	0.02	-0.04	-0.05	-0.01	-0.12	-0.14	-0.38*	0.26*	-
	CKSBL10060	-0.53*	0.71*	-0.08	-0.03	-0.02	-0.01	0.13	-0.56***	0.11	-0.19	-
	CKDHL120731	-0.64*	-0.13	-0.13*	0.02	0.00	0.03	0.07	0.53**	-0.21	-0.13	-
	CKDHL120517	-1.43***	0.71*	-0.26***	-0.03	-0.06*	0.02	-0.36***	0.58***	-0.29*	0.23*	-

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage; PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 6-6. Continuation

	Genotype	SI	MDP (#)	living MW (#)	WL (%)	SD (%)	PM (%)	Oil	Protein	MOI	Starch	TEX
Site 4	CML395	2.28***	-2.08**	0.35***	0.08***	0.17***	-0.22***	0.11	-0.62*	0.02	0.28	-0.38
	CML442	-0.25	0.25	-0.03	-0.01	-0.03	-0.07	-0.18**	-0.66*	-0.02	0.57***	2.35***
	CKDHL120918	0.47	1.25	0.11*	0.01	0.03	-0.19***	0.24***	0.35	-0.05	-0.48***	-1.38***
	CML494	-1.02***	0.25	-0.17***	-0.02	-0.13***	0.19***	-0.09	0.80**	0.07	-0.45***	0.01
	CKSBL10082	-0.46	-1.08	-0.11*	-0.02*	-0.04	0.08	-0.06	-0.38	0.06	0.12	-0.60**
	CKSBL10060	-0.13	0.25	-0.02	0.00	-0.02	0.15**	0.13*	-0.15	-0.06	-0.18	0.96***
	CKDHL120731	-0.43	-0.42	-0.08	-0.02	0.00	0.04	0.29***	0.50	-0.03	-0.66***	-0.88***
	CKDHL120517	-0.46*	1.58*	-0.04	-0.01	0.02	0.02	-0.44***	0.15	0.01	0.79***	-0.10
Site 5	CML395	0.97***	0.33	0.21***	0.04*	0.08***	-0.07*	0.00	-0.85***	0.30	0.67***	-0.64***
	CML442	0.59*	0.17	0.13**	0.01	0.02	-0.04	0.02	-0.78***	-0.17	0.44***	2.47***
	CKDHL120918	-0.30	0.50	-0.05	-0.02	-0.01	-0.05	0.36***	1.67***	0.23	-1.38***	-1.25***
	CML494	-0.01	0.33	0.01	-0.03	-0.02	-0.07*	-0.32***	0.05	0.32	0.35***	0.03
	CKSBL10082	-0.27	-1.00	-0.08*	-0.01	-0.03	0.07*	-0.07*	0.06	0.08	-0.01	-0.58***
	CKSBL10060	-0.33	-1.00	-0.09*	-0.03	-0.03	0.05	0.09**	-0.59***	-0.06	0.14	0.75***
	CKDHL120731	-0.19	1.00	-0.01	0.04*	0.04*	0.06*	0.22***	0.49***	-0.30	-0.68***	-0.69***
	CKDHL120517	-0.46*	-0.33	-0.11**	0.00	-0.04*	0.04	-0.30***	-0.06	-0.39*	0.47***	-0.08
Site 6	CML395	1.73***	-2.88***	0.29***	0.03	0.16***	-0.14**	-0.09	-0.09	0.36	-0.02	0.03
	CML442	0.09	0.29	0.04	0.00	-0.03	-0.06	0.05	0.10	-0.19	-0.05	0.03
	CKDHL120918	0.17	0.46	0.05	0.00	0.00	-0.08	-0.12	-0.35	0.56	0.13	-0.08
	CML494	0.04	-1.04*	-0.02	0.00	-0.01	-0.06	0.17	0.13	-0.55	-0.27	0.03
	CKSBL10082	0.26	-0.88*	0.03	0.01	-0.01	-0.04	0.01	0.18	0.17	-0.13	0.03
	CKSBL10060	-0.38	0.46	-0.07	0.00	-0.03	0.05	-0.24*	-0.13	-0.82	0.44*	0.25
	CKDHL120731	-1.06***	1.46**	-0.19***	-0.01	-0.03	0.10*	0.00	-0.02	0.05	0.28	-0.25
	CKDHL120517	-0.85***	2.13***	-0.13**	-0.02	-0.07**	0.22***	0.21*	0.17	0.41	-0.37*	-0.03

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage; PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture

Appendix 6-7. Across specific combining ability (SCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under MLN virus infested conditions in Naivasha, Kenya 2017A and 2018A

Single cross	Pedigree	FW	GY	MLN1	MLN2	MLN3	MLN4
S12	CML395 × CML442	0.06	0.04	-0.24	-0.19	-0.52	-0.25
S13	CML395 × CKDHL120918	-0.16	-0.12	0.10	-0.19	-0.08	0.33
S14	CML395 × CML494	0.07	0.06	-0.40	-0.30	-0.22	-0.25
S15	CML395 × CKSBL10082	-0.17	-0.14	0.23	0.09	0.31	0.16
S16	CML395 × CKSBL10060	-0.09	-0.10	0.23	0.23	0.42	0.63*
S17	CML395 × CKDHL120731	0.23	0.22*	0.04	0.14	0.59*	-0.06
S18	CML395 × CKDHL120517	0.05	0.04	0.04	0.23	-0.50	-0.56*
S23	CML442 × CKDHL120918	-0.16	-0.04	-0.18	-0.02	0.25	0.16
S24	CML442 × CML494	0.04	0.01	0.32	-0.13	-0.05	-0.42
S25	CML442 × CKSBL10082	0.05	0.03	-0.21	-0.08	-0.02	-0.34
S26	CML442 × CKSBL10060	-0.11	-0.10	-0.21	-0.11	0.09	0.30
S27	CML442 × CKDHL120731	0.15	0.08	0.10	-0.19	-0.41	-0.39
S28	CML442 × CKDHL120517	-0.04	-0.03	0.43*	0.73	0.67*	0.94***
S34	CKDHL120918 × CML494	-0.01	-0.07	0.32	0.37	0.39	0.50*
S35	CKDHL120918 × CKSBL10082	0.14	0.07	0.12	-0.08	-0.25	-0.25
S36	CKDHL120918 × CKSBL10060	0.54**	0.46***	-0.21	-0.61**	-1.13***	-1.45***
S37	CKDHL120918 × CKDHL120731	-0.27	-0.23*	0.10	0.48*	0.37	0.52*
S38	CKDHL120918 × CKDHL120517	-0.08	-0.07	-0.24	0.06	0.45	0.19
S45	CML494 × CKSBL10082	0.05	0.03	-0.04	0.31	0.12	0.33
S46	CML494 × CKSBL10060	-0.19	-0.14	0.29	0.28	0.06	0.13
S47	CML494 × CKDHL120731	0.06	0.09	-0.40*	-0.30	-0.27	-0.06
S48	CML494 × CKDHL120517	-0.02	0.02	-0.07	-0.22	-0.02	-0.23
S56	CKSBL10082 × CKSBL10060	-0.16	-0.10	0.10	0.34	0.25	-0.12
S57	CKSBL10082 × CKDHL120731	-0.10	-0.06	0.07	0.09	0.25	0.69**
S58	CKSBL10082 × CKDHL120517	0.18	0.17	-0.27	-0.66	-0.66*	-0.48
S67	CKSBL10060 × CKDHL120731	0.01	0.00	-0.10	-0.11	-0.13	-0.17
S68	CKSBL10060 × CKDHL120517	-0.01	-0.02	-0.10	-0.02	0.45	0.66**
S78	CKDHL120731 × CKDHL120517	-0.09	-0.11	0.21	-0.11	-0.38	-0.53*

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

FW= Field weight, GY= Grain Yield, MLN1 - MLN4= MLN scores from early to late stage

Appendix 6-8. Across specific combining ability (SCA) effects for grain yield and other traits of 8 maize inbred lines evaluated under optimum conditions in Kenya 2017A and 2018A

Single Cross	Pedigree	GY	EPP	PH	EPO	PA	EA
S12	CML395 × CML442	-0.13	-0.15	-7.93	0.01	0.32*	0.03
S13	CML395 × CKDHL120918	-0.50	0.06	-7.19	0.00	-0.03	0.29
S14	CML395 × CML494	0.23	-0.02	-2.72	-0.03*	-0.21	0.10
S15	CML395 × CKSBL10082	-0.11	0.07	2.96	0.00	0.17	-0.04
S16	CML395 × CKSBL10060	0.29	0.03	10.09	0.01	-0.13	-0.20
S17	CML395 × CKDHL120731	0.01	0.03	0.96	0.00	-0.02	-0.09
S18	CML395 × CKDHL120517	0.21	-0.03	3.83	0.00	-0.11	-0.08
S23	CML442 × CKDHL120918	0.37	-0.14	10.71	0.01	-0.04	-0.20
S24	CML442 × CML494	0.10	0.36*	8.61	0.02	0.16	-0.03
S25	CML442 × CKSBL10082	0.17	-0.13	2.06	0.00	-0.07	-0.17
S26	CML442 × CKSBL10060	0.44	-0.15	4.70	-0.02	-0.26	0.10
S27	CML442 × CKDHL120731	0.72	-0.10	-1.86	0.01	-0.31*	-0.17
S28	CML442 × CKDHL120517	-1.68***	0.29*	-16.29**	-0.02*	0.21	0.45**
S34	CKDHL120918 × CML494	-0.52	-0.04	-6.52	0.00	-0.24	-0.05
S35	CKDHL120918 × CKSBL10082	0.28	0.02	-7.01	0.00	-0.08	-0.30
S36	CKDHL120918 × CKSBL10060	0.09	0.07	-0.68	-0.02	0.01	0.03
S37	CKDHL120918 × CKDHL120731	-0.15	0.06	3.18	0.01	0.23	0.20
S38	CKDHL120918 × CKDHL120517	0.42	-0.03	7.50	0.00	0.14	0.04
S45	CML494 × CKSBL10082	-0.10	-0.04	3.63	0.00	0.01	0.23
S46	CML494 × CKSBL10060	-0.05	-0.09	-8.90	-0.01	0.10	0.12
S47	CML494 × CKDHL120731	-0.18	-0.02	-1.09	-0.01	0.05	-0.21
S48	CML494 × CKDHL120517	0.51	-0.14	6.98	0.02*	0.12	-0.15
S56	CKSBL10082 × CKSBL10060	-1.19*	0.06	-11.20	0.01	0.26	0.31
S57	CKSBL10082 × CKDHL120731	0.34	0.04	4.11	0.00	-0.18	0.15
S58	CKSBL10082 × CKDHL120517	0.61	-0.01	5.46	0.00	-0.11	-0.18
S67	CKSBL10060 × CKDHL120731	-0.12	0.08	4.08	0.01	0.24	-0.08
S68	CKSBL10060 × CKDHL120517	0.54	0.01	1.90	0.02	-0.24	-0.29
S78	CKDHL120731 × CKDHL120517	-0.62	-0.09	-9.38	-0.02	-0.02	0.21

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

GY = grain yield; EPP = ears/plant; PH = plant height; EPO = ear position; PA= plant aspect; EA = ear aspect

Appendix 6-9. Across specific combining ability (SCA) effects for Dobie's Susceptibility Index and other traits of 8 maize inbred lines evaluated under maize weevil infestation in Kenya 2017A and 2018A

Cross	Pedigree	SI	MDP	Living MW	WL	SD	PM	Oil	Protein	MOI	starch	Tex
S12	CML395 × CML442	-0.39	0.61	-0.06	0.00	0.01	0.09	-0.16	-0.08	-0.26	0.37	0.19
S13	CML395 × CKDHL120918	-0.03	-0.59	-0.03	0.02	-0.03	0.00	-0.08	0.14	-0.15	-0.23	0.13
S14	CML395 × CML494	0.25	0.19	0.04	-0.01	0.05	-0.01	0.17	0.03	0.45	-0.54	-0.26
S15	CML395 × CKSBL10082	0.04	-0.27	-0.01	-0.01	0.01	0.06	0.21	0.22	0.10	-0.64	-0.31
S16	CML395 × CKSBL10060	-0.61	-0.38	-0.12	0.00	-0.05	0.00	0.01	0.06	-0.10	0.32	0.65*
S17	CML395 × CKDHL120731	0.18	0.52	0.06	0.00	-0.02	-0.05	-0.19	-0.06	-0.28	0.91	-0.23
S18	CML395 × CKDHL120517	0.56	-0.08	0.12	0.00	0.03	-0.08	0.03	-0.31	0.24	-0.20	-0.16
S23	CML442 × CKDHL120918	-0.11	-0.15	-0.02	0.01	0.01	0.05	-0.22	0.05	-0.16	0.40	-0.12
S24	CML442 × CML494	-1.02**	0.63	-0.20**	-0.02	-0.07**	0.03	-0.05	0.08	-0.37	0.02	-0.09
S25	CML442 × CKSBL10082	-0.15	0.33	-0.01	0.00	0.00	0.01	-0.13	-0.24	-0.50	0.75	0.40
S26	CML442 × CKSBL10060	0.34	-0.06	0.07	0.01	0.04	-0.05	0.44*	-0.30	0.52	-0.79	-0.38
S27	CML442 × CKDHL120731	0.74*	-0.37	0.14*	0.00	0.03	-0.06	0.47*	0.38	0.87	-1.40	0.34
S28	CML442 × CKDHL120517	0.60	-0.98	0.09	0.01	-0.02	-0.07	-0.36	0.12	-0.09	0.66	-0.34
S34	CKDHL120918 × CML494	-0.25	0.10	-0.05	-0.01	-0.02	0.02	0.15	0.14	0.06	-0.41	0.01
S35	CKDHL120918 × CKSBL10082	-0.45	-0.03	-0.09	0.00	-0.03	0.03	0.12	-0.41	0.42	-0.86	0.17
S36	CKDHL120918 × CKSBL10060	-0.25	0.25	-0.05	-0.01	0.00	-0.01	-0.16	0.17	-0.02	0.80	-0.16
S37	CKDHL120918 × CKDHL120731	0.57	0.26	0.13	0.01	0.05	-0.08	0.02	0.18	0.01	0.65	0.27
S38	CKDHL120918 × CKDHL120517	0.53	0.16	0.10	-0.02	0.02	-0.02	0.16	-0.27	-0.15	-0.35	-0.31
S45	CML494 × CKSBL10082	0.18	-0.09	0.02	-0.01	-0.03	-0.02	-0.05	0.24	-0.02	-0.25	-0.47
S46	CML494 × CKSBL10060	0.25	0.52	0.07	0.02	0.04	0.02	-0.14	-0.46	0.25	0.87	0.53*
S47	CML494 × CKDHL120731	0.04	-0.62	0.00	0.02	-0.02	0.05	-0.10	0.01	-0.29	0.55	-0.28
S48	CML494 × CKDHL120517	0.55	-0.73	0.11	0.00	0.04	-0.08	0.02	-0.04	-0.08	-0.22	0.56*
S56	CKSBL10082 × CKSBL10060	-0.50	0.23	-0.11	0.00	-0.03	0.05	-0.25	0.23	-0.16	0.87	-0.47
S57	CKSBL10082 × CKDHL120731	0.79*	-0.75	0.15*	-0.01	0.04	-0.08	-0.05	-0.16	-0.02	0.64	0.21
S58	CKSBL10082 × CKDHL120517	0.08	0.59	0.04	0.02	0.04	-0.04	0.15	0.13	0.18	-0.50	0.46
S67	CKSBL10060 × CKDHL120731	0.38	-0.31	0.07	-0.02	0.00	-0.03	-0.02	-0.21	-0.35	-2.02*	-0.12
S68	CKSBL10060 × CKDHL120517	0.38	-0.24	0.07	0.00	-0.01	0.03	0.13	0.52*	-0.14	-0.06	-0.04
S78	CKDHL120731 × CKDHL120517	-2.69***	1.27	-0.54***	-0.01	-0.08**	0.25***	-0.13	-0.15	0.05	0.68	-0.19

*** = significant at probability of 0.1%; ** = significant at 1%; * = significant at 5%

SI= Dobie's Susceptibility Index; MDP= Median development period; living MW= number of living maize weevil; WL= Weight loss; SD = Seed Damage;
PM = Parent mortality; Oil= grain Oil content; Protein= grain Protein content; MOI= grain moisture content; Starch=grain Starch content, TEX= grain texture