POST-HARVEST EVALUATION OF MAIZE GENOTYPES FOR RESISTANCE TO MAIZE WEEVIL (*SITOPHILUS ZEAMAIS*) AND LARGER GRAIN BORER (*PROSTEPHANUS TRUNCATUS*) INFESTATION

BY

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CHAPTER ONE: INTRODUCTION

1.1 Background Information

Agriculture in Kenya is the mainstay of the economy. The sector directly contributes 24% of the Gross Domestic Product (GDP) and 27% of GDP indirectly through links with manufacturing, distribution and other related sectors (IFPRI, 2012). It is estimated that, of the 58 million hectares of land in the country, 48.1% is suitable for agriculture and 9.8% capable for crop production (IFPRI, 2012). In 2012 top crops that contributed to food production included sugarcane, maize, potatoes, mangoes, bananas, cassava and sweet potatoes (IFPRI, 2012).

Maize is the third most important cereal crop in the world after rice and wheat. In Kenya, it is the most important food crop (Kimanya *et al.,* 2008), a staple food to 90% of the Kenyan population and an essential subsistence food to the majority of households and staple to about 90% of her population (Melinda *et al.,* 2003). Other important uses of maize include animal feed, source of starch and an ingredient for brewing alcohol (Ranum *et al.,* 2014). Kenya produces 24-33 million bags of maize each year (Biwott, 2014), amounts perceived to be the highest rates of maize production in Africa.

Maize production faces a number of constraints; Low yields due to reduced soil fertility, drought, fungal contamination, pests and diseases (Suleiman and Rosentrater 2015). Huge production and productivity of maize has been achieved through the development of high yielding stress tolerant varieties (Tester and Langridge, 2010). In spite of this intervention at production level, food insecurity remains the greatest challenge arising from food

storage losses (Kamanula *et al*., 2011). Livelihoods of farmers across Africa are threatened by storage losses due to pests (Kamanula *et al*., 2011).

The maize weevil (*Sitophilus zeamais* Motschulsky) is one of the most serious stored product pests that cause huge losses in the tropics (Mwololo *et al*., 2012). The maize weevil affects the crop before harvest and multiplies further after storage (Demissie et al., 2008a). Derera *et al*. (2001) reported maize loss of up to 20 - 90 % due to maize weevil infestation on stored untreated maize. Larger Grain Borer (LGB) is responsible for losses of maize grain worldwide. Kumar, (2002) reported losses of up to 45% due to LGB in stored grain in Kenya. It thrives effectively in dry, hot and humid environments where drought and crop failure are prevalent (Kumar, 2002).

1.2 Problem statement and justification

Post-harvest insect management technologies among small scale holders in rural communities include the application of pesticides that are expensive to buy and the use of botanicals which are unreliable in terms of availability and lack of knowledge in using them (Rugumamu, 2011). Use of pesticides to curb storage pests is popular with farmers because pesticides are readily available in the market, easy to administer and provide rapid action against the pests (Calvert *et al*., 2001). However, the widespread use of pesticides has caused enormous problems including environmental degradation, pesticide resistance development, chemical accumulation in food and feed and has also caused death to non-target organisms (Dhuyo and Ahmed, 2007).This problem is mostly serious to small scale farmers who produce and store their harvested maize grains often under conditions that favour insect multiplication (Dobie *et al*., 1984; Abebe *et al*., 2009). It is more sensible and economical to protect the harvested crop instead of trying to make up

for the losses through increased production. Most studies that have been conducted concentrate on growing of crops in the field with little or no attention paid to post-harvest grain protection. Measures to contain the devastating effects by maize weevils and LGB are still going on. Despite these efforts, their populations are still increasing causing enormous grain losses of up to 14-36% (Tefera *et al*., 2016). Integrated Pest management (IPM) initiatives being undertaken to curb weevil and LGB menace do not include the concept of crop breeding whose major advantage is incorporation of resistance in crops. This study was conducted to identify genotypes for post-harvest insect resistance from new tropical and sub-tropical maize germplasm to act as sources of resistance in maize breeding programs in Kenya. Potential hybrids will be deployed to farmers for use in their farms.

1.3 Study Objectives

1.3.1 General Objective

To assess levels of maize weevil and LGB resistance on selected maize genotypes so as to reduce grain losses in storage.

1.3.2 Specific Objectives

1 To identify selected maize inbred lines and hybrids for resistance to maize weevil (*Sitophillus zeamais)*

2 To identify selected maize inbred lines and hybrids for resistance to larger grain borers *(Prostephanus truncate)*

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany, Ecology and maize importance

Maize (*Zea mays* L.) is a member of the grass family Poaceae (Gramineae), a classification it shares with many other important agricultural crops, including wheat (*Triticum aevestium* L.*)* and rice (*Oryza sativa* L.) (Buckler and Holtsford, 1996). It is a diploid species with a chromosome number of 2n=2x=20. Maize performs best in well drained, aerated and loamy soils having a pH value of 5.5-7. It grows in a wide range of agro-ecological zones in Kenya ranging from 0-2200 meters above sea level and temperature of 30^0C (Njoroge *et al.,* 2008). Well distributed rainfall of between 600-900mm is most appropriate for maximum maize production (Njoroge *et al.,* 2008). As Kenya's staple food, it is estimated that an individual can consume 98 kg per annum, thus a lot of emphasis is laid on maize production (Nyoro *et al*., 2004). Small scale farmers produce about 75% of the total maize produced and the rest by large scale farmers (EPZ, 2005). Maximum maize production in Kenya stands at about 36 million bags annually (Biwott, 2014). Maize is the third most important cereal crop after rice and wheat in the world.

2.2 Constraints to maize production in Kenya

Production of maize can significantly drop depending on various factors including drought, climate change, soil nutrient deterioration, lack of adequate extension services, poor infrastructure, pests and diseases in the field and post-harvest losses due to stored product pests.

2.2.1 Abiotic constrains

Drought is one of the most important factor that limit maize production, where farmers particularly depend on rain-fed agriculture (Patrick, 2006). Yield loss of up to 60% caused by drought has been reported in maize (Zaidi *et al*., 2008). Farmers have felt the impact of climate change because of dependence on rain-fed agriculture. Climate change has caused unpredictable rainy seasons which have greatly affected farmers' ability to plan their farming activities (Kibet, 2011). Also, the continuous cropping without commensurate nutrient replenishment is reported to contribute to low nutrient content of many soils (Bunemann, 2003; Smaling *et al*., 1997), therefore reducing maize production. Maize yield can increase from an average of 1300 kg/ ha to as high as 6000–7000 kg/ha by improving soil fertility (Zambezi *et al.*, 1993).

2.2.2 Biotic

The most commonly reported biotic constraint in food crops are diseases (Gerpacio and Pingali, 2007). Endemic diseases include leaf blight (LB), common rust, maize steak virus (MSV), gray leaf spot (GLS), ear rots(ER), leaf spot and the recently reported maize lethal necrotic disease (MLND) (Wangai *et al*., 2012; Mwangi, 1998,), which is associated with reduction of maize yield in the country. Maize steak virus and leaf blight can cause grain yield losses of up to 70% (Ngwira and Khonje, 2005) while the recently reported MLND can cause yield loss of up to 90% (Ochieng *et al*., 2012).

Demand for maize for use as food, fuel production and feed grain in the livestock industry is increasing (Edgerton, 2009). To meet the demand, farmers have intensified maize production using the high yielding varieties (Edgerton, 2009). However, biotic

constraints at storage level have adversely affected maize yields (Reynolds *et al*., 2015). Some of the major post-harvest stored produce pests that cause enormous losses of grain among small holder farmers include maize weevils (*Sitophilus zeamais)* and larger grain borer *(Prostephanus truncates)* (Tefera *et al.,* 2016). Over one thousand species of insects are directly involved in damage of stored grains worldwide (Chomchalow, 2003) and about 10–40% of grain damage is caused by insect pests (Mathews, 1993) posing a huge challenge to stored maize. The maize weevil and the larger grain borer cause enormous qualitative and quantitative grain yield loss by feeding on the kernels and burrowing into them for oviposition (Mwololo, 2013; Parwada *et al*., 2012). The degree of damage to stored maize depends on various factors including the length of storage, variety and the species of insect (Suleiman *et al.,* 2013). Apart from maize weevil and LGB, other post-harvest insect pests include the red flour beetle (*Tribolium castaneum*) and the Indian-meal moth (*Plodia interpunctella)* (Maier *et al*., 2006). Primary storage pests attack grains that are intact and stable whose temperature and moisture content are below the levels needed for germination (Savidan, 2002). They reproduce and multiply very quickly and thus cause great damage within a short period of time.

The red flour beetle is a worldwide pest which attacks stored grain and food products including maize, beans, nuts and biscuits causing loss and damage (Weston and Rattlingourd, 2000). The red flour beetle causes grain damage, but more problems by contaminating grain (Calvin, 1990). Sometimes the red flour beetle may be mistaken for the new beetle (Walter, 1990) of African origin.

The Indian meal moth (IMM) *(Plodia interpunctella)* is a household pest that feeds on various stored products. The larva of the IMM feeds on a variety of grain products, dried

fruits and pet foods (Kammerling, 2014). The larval stage is responsible for grain damage (Kammerling, 2014). However, the biggest quality reduction is due to contamination of larva droppings and silken webs in the grain, which results in excessive moisture accumulation thus quickening deterioration of the stored product (Choe *et al*., 2013).

The lesser grain borer (*Rhyzopertha dominica*) feeds on a variety of foods. It prefers cereals such as maize, rice, wheat and millet, but can also feed on flour, beans, dried potato and wood (Linda, 2010). The adult are long lived and can fly to initiate new grain infestation.

Olivier, (*Sitotroga cerealella)* resides in sub-tropical and warm temperate regions of the world. It is a primary colonizer of stored grains (Bhargava *et al.,* 2007). The larval stage causes substantial damage by tunneling inside the kernel thus making the grain more susceptible to other insect pests (Weston and Rattlingourd, 2000). The adults cause weight loss of about 5% by hollowing them out at infestation (Omar and Kamel, 1980).

The rice weevil (*Sitophillus oryzae)* is a small reddish brown to black insect. It is very similar to the granary weevil (*Sitophilus granaries*) and it causes substantial qualitative and quantitative losses to maize and other grain products depending on the duration of storage (Hell *et al*., 2000).

2.2.3 Socio economic constraints

The agricultural sector extension service plays a key role in disseminating knowledge, technologies and agricultural information. However, there is limited access to extension services in most parts of Kenya with the ratio of national extension staff to farmer standing at 1:1,500 (Kibet, 2011). To curb this problem, it is important to recruit more staff and involve relevant stakeholders so as to accelerate extension services to farmers.

Poor infrastructure including poor rural roads, markets and transport systems that result in high transactions costs for farmers and inaccessibility to input and output markets are among the main concerns for the sector (Cheptoo, 2014). Inadequate and poor infrastructure has also led to the poor market integration in the country (Ter-Hemen, 2015). Stakeholders in the agricultural sector may need to ensure provision and maintenance of the necessary infrastructure to facilitate movement of agricultural produce to markets (Alila and Atieno, 2006).

2.3 Biology and lifecycle of maize weevil

Figure 2.1: Maize weevil (*Sitophilus zeamais* Motschulsky). Photo: ICRISAT-2014

Maize weevil (Fig 2.1) belongs to the order Coleoptera and family Curculionidae. It is about 2.5-4.5 mm in length with its head protruded into a proboscis (Tefera *et al*., 2010). It is generally reddish brown in color, sometimes dark brown or almost black (Tefera *et al*., 2010). It is further identified by the presence of four light reddish brown or yellowish pale spots on the elytra (Khare, 1994). The pre-oviposition period is about three days. It remains highly productive throughout its lifetime although effective egg

laying period is 50% of the first 5 weeks of its lifespan (Tefera *et al*., 2010). The female lays up to four eggs in a single maize kernel. The egg is white in color and oval in shape and the larvae is also white and about 4 mm in length (Hill, 1983). Eggs are deposited inside the kernels and covered with a jelly-like material which hardens thus protecting it from external destruction. The larvae moults several times before pupation. Both larval development and pupation takes place inside a single grain kernel (Tefera *et al*., 2010). After emergence from the pupae, the adult eats through the outer layer of the grain leaving a circular hole (Kranz *et al*., 1997). The weevils then use their elongated snouts to bore into the grain. Due to the high fecundity of females, if not controlled the rate of increase is extremely high (Tefera *et al*., 2010). However the weevils do not breed well at temperatures below 20^{o}C or above 32^{o}C, or in food with moisture content below 11% (Tefera *et al*., 2010). It is also vital to establish that mating does not take place before the adults are three days old (Walgenbach *et al.*, 1987). *Sitophilus zeamais* completes development from egg to adult in 31-64 days at 30^{o}C on maize with 13% moisture content. The actual development period of maize weevil depends on the type and quality of grain being infested (Tefera *et al*., 2010). At 25^{o}C temperature, the female weevil is capable of producing about 300 to 400 eggs within a period of 4-5 weeks, the eggs then hatch and feed inside the grain (Hill, 1983). Various factors may influence the number and life span of adults (Tefera *et al*., 2010). Adam (1976) and Gomez *et al*. (1983) have reported that both diet and varietal differences within cereals can affect developmental time and reproductive capacity of *S. zeamais.*

2.4 Biology and lifecycle of larger grain borer *(Prostephanus truncates)*

Figure 2.2: The larger grain borer (LGB) (*Prostephanus truncates*). Photo: ICRISAT-2014

A mature LGB has a cylindrical bostrichid shaped body and is dark brown in color (CABI, 2015). Its body resembles a flattened tube whose surface is pitted and has many small wart-like growths. Apart from maize, LGB can damage a range of products including dried cassava, bamboo, plastic, soup, timber and timber products (CABI, 2015). Adult females lay small yellow eggs in tunnels of maize or dried cassava. The egg then hatches to larvae which are white, fleshy and covered with minute hairs having short legs and a small head (Mailafiya *et al*., 2008). The lifecycle of LGB can be completed in 24-25 days under optimum conditions of 32^0C and 70-80% relative humidity (Hill *et al*., 2003). Losses due to LGB are particularly serious in stored maize (CABI, 2015). In West Africa LGB has been reported to cause losses of up to 45% on maize (Pantennius, 1988). Areas with high incidences in Kenya and Tanzania, have recorded losses of up to 34% after 3 months of storage (Hodges *et al*., 1983).

Prostephanus truncates is a long-lived species having an extremely rapid larval

development stage (Machingura, 2014). It is closely related to *Rhyzopertha dominica*, lesser grain borer which is from the same insect family. After boring into maize kernels, LGB produces large quantities of dust creating tunnels in the grains affected (Bell and Watters, 1982). The adult female then lays eggs within the tunneled grain and covers with maize dust. Oviposition begins 5-10 days after adult emergence, reaching the peak at 15-20 days (Bell and Watters, 1982). Under optimum conditions the development period of the LGB egg is 3 days, for larvae (3 instars) 13.2 days, pre-pupae 3.9 days and pupae 2.4 days (Demianyk and Sinha, 1988). The female LGB tends to outlive the male with 16 days; mean survival time for females and males is 61 and 45 days respectively (Shires, 1980; Bell and Watters, 1982).

2.5 Definitive host of maize weevils and LGB

Maize weevils prefer attacking maize, rice, sorghum and wheat while LGB is a pest of stored maize and dried cassava (Phiri and Otieno, 2008). Although the maize weevil cannot readily breed in finely processed grains it can easily breed in products such as macaroni and noodles and milled cereals that have been exposed to excessive moisture (Alter, 2013). Multiplication of storage pests in maize depends on the form in which maize is stored. Maize stored in shelled form show increased establishment and multiplication of insects than that stored as ears (Vowotor *et al*., 1994). Other factors which provide conducive conditions for insect pests in host materials include ambient temperature, moisture and adequate relative humidity (Phiri and Otieno, 2008).

2.6 Role of insect pests in aflatoxin contamination in maize and seed damage

Maize seeds will lose viability, weight and are bound not to germinate if there is direct

damage by storage pests. Insect pest infestation interferes with the storage of seed intended for the next season (Dhliwayo and Pixley, 2003). Grain damage by insects plays a major role in the spread of *Aspergillus flavus* thus aflatoxin contamination. Insect damage and aflatoxin contamination are positively correlated (Bowen and Mack, 1991; Lynch and Wilson, 1991; Lynch *et al*., 1991; Gorman and Kang, 1991). Maize free from insect damage may have no aflatoxin contamination but maize cobs damaged by insects have most of the kernels contaminated with aflatoxin (Hell *et al*., 2000). Maize weevil *(Sitophilus zeamais)* is considered a poor vector of *Aspergillus flavus* in the field (La prade and Manwiller, 1977). It, however, has a major role in *A. flavus* infection and subsequent aflatoxin contamination during storage (Beti *et al*., 1995). Storage insect pests can change conditions within bulk grain to encourage growth of storage fungi (Christensen and Kaufmann, 1969). In the USA, maize kernels infested with insect pests and contaminated with *A. flavus* had higher levels of aflatoxins than *A. flavus* inoculated maize without insect pests (Beti *et al*., 1995). The presence of these insects resulted in increased kernel moisture content which was positively correlated with aflatoxin contents (Beti *et al*., 1995). Insects employ different mechanisms which perpetuate contamination. They can break the pericarp while feeding, thus rendering grain more susceptible to invasion by storage fungi (Tuite *et al*., 1985; Wicklow, 1988). During metabolism, insects increase relative humidity thereby providing a conducive environment for *A. flavus* establishment (Sauer and Burroughs, 1980; Mills, 1983). Furthermore, Lynch and Wilson (1991) reported that insects could carry fungal spores on their bodies and drop them to other grains as they move about. Therefore, development of maize lines that are resistant to stored-product insect pests should also reduce aflatoxin contamination of

stored corn. Throne *et al*. (1995) identified maize lines that were relatively resistant to both maize weevils and *Aspergillus flavus*.

2.7 Management of stored product pests

Maize weevils and LGB are very destructive grain pests (Danjumma *et al*., 2009). They can cause 'almost complete destruction' of grain under storage. Various control measures against these pests are practiced by farmers (Yakubu *et al*., 2011). Some have been deployed to farmers for use while others are still under experimentation (Yakubu *et al*., 2011). Immediate control measures which target reduced insect infestation before it occurs include proper sanitation and use of hermetic storage system (Kasozi, 2013). Use of host plant resistance is a control measure which minimizes insect pest population in grain after infestation has occurred (Derera *et al*., 2001).

2.7.1 Sanitation

Sanitation is a key factor for preserving grain products in good condition for a long time (Rotundo et *al.*, 1995; Suss and Locatelli, 1993) which greatly saves stored products against losses due to infestation. Regular checking should be conducted on the maize produce in the store for the presence of insect pests, damaged cobs or rotten grains and measures applied immediately (Vinuela *et al*., 1993). This is a critical grain management program which can prevent many stored grain insect problems including maize weevils.

2.7.2 Hermetic storage system

Hermetic storage is a technique which controls moisture and allows depletion of Oxygen with increase in carbon dioxide through respiration of insects (Jonfia-Essien *et al.*, 2010). These conditions will suffocate the insects and cause them to die. It can achieve 100% 'kill' of insects, control molds and free fatty acids (FFA) (Villers *et al*., 2010). A

study by Yakubu *et al*. (2011) reiterates that hermetic storage system is effective, easy and safe due to its freedom from chemicals. A recent research on the hermetic storage system in insect prone areas in Kenya confirms its effectiveness in controlling maize weevils and other storage pests (De Groote *et al*., 2013). Examples of hermetic storage devices include Postcosecha galvanized steel silo (Bern *et al*., 2013) which was first developed in central America in 1980 and is now available in almost every part of the world, Purdue Improved Crop Storage (PICS) bag (Forbes, 2007) first developed in Purdue University in the United and the Grain Pro ultra-hermetic bags designed by Grain Pro Inc. of Concord, Massachusetts.

2.7.3 Chemicals

Synthetic chemicals are insecticides used to help protect grains from damage caused by insects (Rajashekar *et al.,* 2012). A wide range of synthetic insecticides are available for use in the world. Pyrethroids are believed to affect the central and peripheral nervous systems of insects (Cash, 2012) and kill them. Phosphides are also commonly used to prevent insects especially where large amounts of grain need protection (Kasozi, 2013). However insects can develop physiological and behavioral resistance to insecticides thereby rendering them ineffective (Rajashekar *et al.,* 2012). Kasozi, (2013) reitarated the use of insecticides to control maize weevil which causes human and animal health hazards. Use of chemicals as a control strategy against maize weevils and LGB comes with many disadvantages including improper chemical accumulation in the environment and causing adverse effects to the higher food chain.

2.7.4 Biological control agents

Biological control involves the use of live natural enemies or antagonists of the insect

pests. These methods involve biologically based tactics (Pal and Gardener, 2006). Alternative cheap and safe methods to chemicals, such as biological measures have therefore been tested. *Beauveria bassiana* is effective against storage pests if used as a preventive treatment (Hluchy and Samsinakova, 1989). In a study, *B. bassiana* was selected and evaluated against maize weevils because of its potential use as a biopesticide (De Muro *et al*., 2003). In another study, Nboyine *et al*., (2015) assessed the compatibility of *Teretrius nigrescens* and *Beauveria bassiana* isolates for the management of LGB and found positive results. Other prominent insect pests that have been found to be effectively controlled by *B. bassiana* include *Leptinotarsa decemlineata* (Anderson and Roberts, 1988), *Plutella xylostella* (Correa-Cuadros *et al*., 2014), *Anastrepha ludens* (Toledo *et al*., 2006). *Bacillus thuringiesis* (Bt) is a strain of bacteria that contains an insecticidal protein that kills insect pests directly by blood poisoning in the gut (McGaughey and Beemen 1985). Tsuchiya *et al*., (2002) reported significant suppression of cigarette beetle, *Lasioderma serricorne* by *Bacillus thuringiesis (Bt).* It has also emerged that single and multiple releases of *Anisopteromalus calandrae* can suppress maize weevil populations by 90% (Wen and Brower, 1994).

2.7.5 Botanicals

Plant and plant products have been found to be useful in managing pests because of their effectiveness and ability to act as natural enemies (Ascher, 1993; Schmutterer, 1990; Rattan and Sharma, 2011). Several plant and plant products have been reported to be potential pesticides. Neem, pyrethrum and tephrosia have been used to prevent a number of storage pests including maize weevils and LGB (Mbaiguinam *et al*., 2006; Greenberg *et al*., 2005; Akhtar and Isman, 2004). Muzemu *et al*. (2013) reiterated the importance of

botanicals in suppressing *S. zeamais* in maize grains in a study involving *Eucalyptus tereticornis, Tagetes minuta* and *Carica papaya*. In another study conducted by Yeshaneh, 2015, *Carissa schimperi* and *Tagetes minuta* were found to effectively control *S. zeamais* in sorghum. Effective treatment has also been observed in using leaves from *Datura stramonium, Phytolacca dodecandra* and *Schinese molle* causing high weevil mortality (Eticha and Tadesse, 1999). Several plants have been reported to control larger grain borers. Castor beans, neem, pyrethrum and velvet leaves can effectively curb the establishment and survival of larger grain borers in stored grains (Stoll, 2003). According to Ukeh *et al*. (2008), botanicals are cheap, readily available, and easily biodegradable and more importantly cannot contaminate food products.

2.7.6 Inert materials

Several inert substances are shown to be effective in insect pests in curbing stored products (Chomchalow, 2003). They include wood ash, diatomaceous earth, silicosec and mineral industrial filter cakes (Dimissie *et al*., 2008a). Results from studies conducted by Achiano *et al*. (1999) indicate that a mixture of wood ash with maize kernel can result to 100% death of *S. zeamais* after 20 days of treatment. Another study by Gemu *et al*. (2013) confirmed the efficacy of coffee husks and wood ash in controlling *S. zeamais* and *S. cerealella*. Spinosad is an inert chemical made from the bacterium species *Saccharopolyspora spinose* and has been reported to be effective against storage pests (Athanassiou *et al*., 2008). Therefore, use of inert materials is an alternative control strategy especially for the resource poor farmers due to low costs involved, effectiveness and availability.

2.7.7 Host plant resistance (HPR) to insects

Host plant resistance are heritable characteristics possessed by the plant which influence the ultimate degree of damage done by insects (Smith, 2018). It is effective, cheap to administer and environmentally safe, as an integral pest management strategy against insect attack. It has no special technology which the farmer needs to employ besides risks associated with insecticide applications are avoided (Throne and Eubanks, 2002). Host plant resistance has been described as a phenomenon that comprises three components which include antibiosis, antixenosis and tolerance (Parsa *et al.,* 2011). Antibiosis involves plants producing allelochemicals which adversely affect the survival and development of insects. Antixenosis is a group of plant characteristics which make it unpalatable thus protecting it from insect attack. Tolerance on the other hand is the ability of a plant to withstand injury imposed by insects. Insect resistance against maize weevils and LGB has been identified in a number of studies. Tefera *et al*. (2011) determined levels of resistance among 54 maize hybrids against *Sitophillus zeamais* and found 5 hybrids to confer resistance. Abede *et al*., (2009) identified resistance in BHQP-542 among the 13 screened maize varieties. In another study, Derera *et al*. (2001) evaluated F_2 hybrids and classified the genotypes as either resistant (2%) or moderately resistant (29%) in reference to resistance against maize weevils. A study conducted by Mwololo *et al*. (2012) on maize weevil resistance observed high heritability values and resistance in 7 genotypes among the 120 inbred lines evaluated. Host plant resistance in 19 maize genotypes was identified by Kumar, (2002) through a series of infestation, selection and inbreeding thus generating S3 maize ears which showed high levels of resistance against *P. trancatus.* In another study, Munyiri and Tabu (2013) evaluated 25

landraces and found appreciable levels of resistance against LGB. Mwololo *et al*. (2012) identified 8 hybrids and 4 landraces as potential sources of resistance against LGB from the 163 genotypes tested. Likewise Nhamuncho *et al*. (2014) evaluated 17 maize genotypes and selected 25% of the genotypes as being resistant and the remaining 75%, susceptible against LGB. These studies show the possibility of utilizing host plant resistance as a strategy to curb maize weevil and LGB infestation.

2.8 Mechanism of resistance to stored product pests

Many studies agree that, despite storage pests causing damage to products at storage, the genesis of infestation starts from the field (Hagstrum *et al.*, 2012; Upadhyay and Ahmad, 2011; Youdeowei, 1989). The magnitude of storage damage will depend upon the degree of infestation while the crop was in the field. A number of characteristics have been reported that contribute to maize resistance against stored product pests. Resistance of stored maize to storage pests attack emanates from physical and biochemical characteristics exhibited by a maize variety (Adedire *et al*., 2011). These protective mechanisms ensure safety of grain from both biotic and abiotic damage (Phytomedizin, 2009).

2.8.1 Physical mechanisms

In order to oviposit inside the maize kernel, the insect has to penetrate by chewing through it. Studies have reported the significance of kernel hardness to influence resistance against storage pests (Garcia-Lara *et al.*, 2004; Arnason *et al.*, 1997). Diferulic acid, structural proteins, peroxidases and phenolic acid of grain hull were found to enhance resistance against insect pests (Santiago and Malvar, 2010). Diferulic acid was found to concentrate on insect resistant maize genotypes (Bergvinson, 2002). Garcia-Lara

et al. (2004) demonstrated the existence of Diferulic acids and glycoproteins as being positively correlated to grain hardness. These chemicals contribute to cross linking of cell wall polysaccharides thus hardening the testa (Barros-Rios et al., 2015).

Garcia-Lara *et al.,* (2007) reported the significance of peroxidase enzymes as components present in the outer membrane of resistant grains. The study established differences in the composition of peroxidase enzyme activity between resistant and susceptible genotypes. Another study by Gafishi, (2012) reported positive correlations between soluble peroxidase and maize weevil resistance. These enzymes facilitate cross- linking of the cell wall resulting to its hardening thus making it act as a physical barrier against insect pests. A study by Derera *et al.* (2001) eluded the pivotal role played by phenolic acid towards resistance to grain weevils in maize through mechanical means. Protection against infestation in the field is partly enhanced by husk cover. Maize cobs consisting of numerous and tightly packed husks tend to be more resistant to insect infestation in the field. Therefore, strong correlations exist between insect resistance and husk protection (Suleiman *et al.*, 2014; Kossou *et al.*, 1993).

Osipitan and Odebiyi (2007) reported grain shape and size as factors of resistance. Dent maize is susceptible to grain infestation by insect pests (Paliwal *et al*., 2000). Suleiman (2014) also reported that dent maize was more susceptible to *S. zeamais* than flint maize. Flint maize is characterized by hard external layers on the endosperm (Haros *et al*., 2001) making it less prone to insect damage (Suleiman, 2014; Paliwal *et al.,* 2000). Therefore, promoting flint maize could be a viable approach to reduce problems associated with insect infestation. Taulu (2013) observed better resistance to stored product pests in large

sized maize grains than the small sized ones. Another study by Lale and Kartay (2006) concluded that maize varieties associated with large seeds and thick testa were more resistant to maize weevils than small seeded and thin testa varieties.

Tipping *et al*. (1988) reported more damage in rough grain surfaces than smooth ones as a result of firm grip by insects on rough grain surfaces compared to smooth grain surfaces. Therefore, grain type has significant influence in the resistance against insects. Shelled maize is less prone to insect infestation than the unshelled maize (Lebaka, 2000). This is because the softer parts of the unshelled maize is not easily accessible by insects hence oviposition and emergence of adults is impossible. The shelled maize has the softer parts exposed and insects can easily access them resulting to infestation (Lebaka, 2000). Knowledge on physical resistance mechanism enriches the basis for enhancing storage pests control measures, especially the breeding strategies which ought to capitalize on this mechanism when improving maize germplasm against maize weevil and LGB.

2.8.2 Biochemical mechanisms

The chemical constitution of the kernel is an important component that ultimately determines insect pest resistance in maize (Santiago et al., 2013) This chemical constitution is crucial in the sense that, the insect larvae dwells and obtains their nutrients from the grain (Santiago et al., 2013). The type of resistance at work in this stage is antibiosis. Antibiosis is a key factor that contributes to biochemical mechanism of insect pest resistance in maize. Derera *et al*. (2001) reiterated the importance of antibiosis as a factor that hinders reproduction, growth and feeding by storage pests. A number of authors have attempted to explain resistance in maize grains insect pests based on antibiosis (Santos *et al*., 1998; Santos and Foster 1983). A Study on effects of different

carbohydrates on the survival of weevils (Chippendale, 1972) has provided essential information of effectively managing the weevils and other storage insect pests through regulation of these carbohydrates. Increased amylopectin and glycogen in maize supports the survival of insects while their reduction enhances mortality (Lebaka, 2000). The study reveals the significance of amylopectin and glycogen in the survival of storage insect pests. Similarly, maltose and glucose results into high insect mortality (Lebaka, 2000) which could be the result of inability to digest polymers associated with these compounds. Amylose is also an important carbohydrate which enhances resistance of storage pests. Dobie (1974) established a positive correlation between kernel hardness and amylose. Many studies have shown the significance of amylose in contributing to insect resistance (Franco *et al*., 2002; Warchalewski *et al.,* 2002). These findings show the significance of carbohydrates in maize kernels as a component of resistance against storage pests.

Proteins have been associated with resistance to insects. Opaque-2 is a mutant gene that changes the protein composition and increases the content of lysine and tryptophan components (Demissie *et al*., 2015). These protein components have been reported to contribute to resistance against maize weevil (Demissie *et al*., 2015). Kaster and Gray (2005) reported antibiosis effects due to high protein concentration in maize which prevented establishment of some field pests. Another study by Santos *et al.* (1996) emphasized on the existence of variation among the Quality Protein Maize (QPM) genotypes in regard to their resistance against maize weevils. It was thus concluded that, apart from carrying genes that confer enhanced protein, QPM also carry genes for resistance to insect.

Consequently, resistance of insects to stored maize has also been attributed to high concentration of lipids (Tipping *et al*., 1988). In contrary, Ram and Singh (1995) reported that lipid content was not a factor involved in insect resistance. Secondary metabolites also have a crucial role to play in antibiosis. They are not directly involved in growth and development of plants but rather function by defending the plant against biotic attack (Mazid *et al.,* 2011). Phenolics are such compounds and they include ferulic acid, lignin, tannins and phenolic amides. Phenolics contents were found to concentrate in the pericarp, testa and aleurone layer of the seeds of resistant maize genotypes which play a very important role in suppressing insect development (Mihm, 1997). Arnason *et al.* (1997) reported correlation between maize and ferulic acid which contributed to resistance against *S. zeamais* and larger grain borer (LGB). Ferulic acid creates cross links along the cell wall thus resulting to kernel hardness which eventually contributes to insect resistance (Arnason *et al*., 1997). The peripheral endosperm can contain unbound ferulic acid which may adversely affect insect pests (Arnason *et al*., 1997). Lignin is a highly branched heterogeneous polymer in both primary and secondary walls whose indigestible structural frame provides a strong barrier against insect attack (Adeyemi and Mohammed, 2014). Tannins are polyphenolic biomolecules that function as antifeedants to herbivores. In insects, tannins bind to salivary proteins and digestive enzymes thus resulting to protein inactivation (Adeyemi and Mohammed, 2014) contributing to loss of weight, emaciation and eventual death of the insects. Phenolic amides such as diferoyl and dicoumaroyl putrescine have also been reported to contribute to antibiosis against

insect pest (Lebaka, 2000). Ether extracts, sugar content, free sterols, p-coumaric acid and dichloromethane are also secondary metabolites that influence grain resistance against storage pests (Serratos *et al.,* 1987). Therefore these compounds may be used by breeders as indicators of resistance during screening of materials for resistance to maize weevils and larger grain borers.

Proteinaceous α- amylase inhibitor production is triggered due to insect infestation. It has been reported to confer resistance against insects whose diets make them highly dependent on α-amylase activity (Mugnozza *et al.,* 1999; Franco *et al.,* 2002). Action of these compounds in insect guts adversely affects their development and survival. A study by Serratos *et al.* (1994) has reported negative correlation between protein inhibitor and maize susceptibility to *Sitophilus zeamais*.

2.9 Genetic diversity and useful genetic markers in plant breeding

Genetic diversity within a population refers to the number of different genes and the frequency with which they appear (Bajpai *et al.,* 2014). For better understanding of genetic diversity of insect pest resistance, it is critical to analyze genes that constitute host plant resistance. Effective exploration of variations among populations has been enhanced through the use of genetic markers (Bergvinson and Garcia-Lara, 2004). Genetic diversity within and between populations is assessed using these markers. They include morphological, isozyme and DNA (or molecular) markers (Govindaraj *et al.,* 2015; Collard *et al.,* 2005). Morphological markers constitute observable traits such as seed shape, pigmentation and flower color, isozymes are markers captured by electrophoresis and specific staining, molecular markers are those that scrutinize DNA sequence variations (Collard *et al.,* 2005; Govindaray *et al.,* 2015). Morphological and

isozyme markers were earlier used because they were cheap and easy to use. However, they are less preferred today due to a number of limitations including low polymorphism, low heritability and being easily affected by the environment. Development and successful use of DNA markers has extensively enhanced knowledge on plant genetics resulting to effective selection of preferred crop accessions (Jiang, 2013). Molecular markers are based on DNA polymorphism as a result of mutations (Pinto *et al.,* 2003) and have been utilized to achieve substantial milestones in maize research. Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) are other types of molecular markers that have been extensively used for application in plant breeding and in other disciplines including human genetics, animal genetics and breeding (Jiang, 2013). Insect resistance in maize can also be identified by analysis of Quantitative Trait Loci (QTL). Castro-Alvarez *et al.* (2015) identified 15 QTL locations for various maize weevil parameters using composite interval mapping. Another study by Garcia-Lara *et al.* (2009) analyzed QTL associated with maize weevil resistance and found dominant and additive gene actions as the main genetic effects. It was also noted that both parents contributed to the resistance.

2.10 Genetics, heritability and breeding methods for insect resistance

Combining ability in plants comes about during the process of fertilization (Kabir *et al.*, 2014). Two types of combining ability, general (GCA) and specific (SCA), have been recognized in quantitative genetics. A method proposed by Griffing (1956) explains how total genetic variation is portioned into GCA of the parents and SCA of the crosses. This is widely used today (Machikowa *et al.,* 2011). The GCA is the average performance of a

particular inbred in a series of hybrid combinations, whereas SCA refers to the performance of a combination of specific inbred in a particular cross (Sprague and Tatum, 1942). The GCA and SCA variances provide an estimation for addititve and non-additive gene actions respectively (Falconer, 1967).

Free and no-choice experiments have been suggested to influence how additive and non-additive gene actions operate in maize weevil resistance. Widstrom *et al*. (1975) reported additive effects as operative components under no-choice experiments, in contrast to Kang *et al.* (1996), who reiterated that additive effects were important in free-choice experiments. A study by Derera *et al.* (1998) emphasized the importance of additive, non- additive and maternal effects in no-choice experiments; however, maternal effects were not important in free choice.

Additive and non-additive gene actions are responsible in determining grain damage, grain weight loss, weevil emergence and susceptibility index (Matewele, 2014). A study by Mwololo *et al.* (2012) reported significant differences for grain damage, grain weight loss and susceptibility index. Resistant parameters such as grain damage, grain weight loss, progeny emergence, median development period and Dobie index of susceptibility were found to be heritable. Dari *et al.* (2010) reported significant differences in additive and non-additive gene actions in the studied inbred lines and hybrids for grain weight loss. It was thus concluded that, for a selection process to be effective, additive, non-additive, dominant and maternal gene effects should be deployed.

Heritability is defined as the proportion of the observed variation in the progeny that is inherited (Sleper and Poehlman, 2006). It can be expressed as a ratio of additive genetic

variation to phenotypic variation. A large genetic variation in a progeny in relation to the environment will result to a high value of heritability; likewise a small genetic variation in relation to the environment gives a low heritability value (Sleeper and Poehlman, 2006). Effective selection is obtained when genetic variation in relation to environment is high. Heritability is an important component to plant breeders as it can be utilized in choosing suitable segregating generations that exhibit best expression of genes of a particular trait (Wannows *et al*., 2010). Heritability estimates of >70% is considered very high; 50-70% high; 30-50% moderate and < 30% low (Hallauer and Miranda, 1988). Heritability can either be broad sense or narrow sense. Broad sense (H) refers to heritability estimate on the basis of total genetic variance while narrow sense (h^2) is the heritability estimate from the additive portion of the genetic variance (Sleeper and

Poehlman, 2006). Heritability in the broad sense is estimated as follows (Lush, 1940):

$$H = \sigma_g^2 / \sigma_p^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where:

$\sigma_g^2 = Genotyic\ variance$

$\sigma_p^2 = Phenoypic\ variation$

Several studies have reported the concept of heritability in the recent past. A study by Dari *et al.,* (2010) reported broad sense heritability for maize weevil resistance to be 0.6 and 0.5 for inbred lines and hybrids respectively. Additive and non-additive gene actions were responsible for weevil resistance. Another study by Gafishi (2012) reported existence of maize weevil resistance in maize inbred lines. Results on broad sense and narrow sense heritabilities showed resistance to be moderately heritable. Analysis of variance was performed using linear mixed model. Moderate to high broad sense

heritability for maize weevil resistance and grain yield were reported by Musundire *et al.* (2015). Genetic analysis was performed as described by Hallauer and Miranda (1988) using North Carolina Design II in SAS. The results perceived high genetic variance concluding that the traits could be upgraded by selection. Mwololo *et al.* (2012) studied dual resistance to maize weevil and larger grain borer and found high heritability values ranging from 0.7 to 0.9. Broad sense heritability was calculated using the Allard (1960) model. Apart from heritability Mwololo *et al.* (2012) also encompassed the concept of phenotypic and genetic variability which were estimated according to Manggoel et al., (2012). According to Mwololo *et al.* (2012), the possibility of selecting resistant materials for both LGB and maize weevil resistance is feasible because resistance for storage pests is controlled by the same genes.

Transfer of insect resistance can be effectively accomplished through appropriate breeding procedures. Recurrent selection is a breeding technique that can be used to effectively improve levels of insect resistance (Ortega *et al.,* 1980). A study by Kang *et al.* (1996) suggested recurrent selection as an appropriate breeding technique for developing hybrids resistant to maize weevils. In the back cross method of inbred line development, a trait is incorporated effectively by handling one or two genes (Mandal, 2014). Disease and pest resistance have successfully been imparted in lines using backcrossing (Ye *et al*., 2009). Three to five backcrosses are required to incorporate trait of interest into a plant. Modifications of backcrossing emphasize transfer of transgenes to elite inbreds (Wilcox and Cavins, 1995).

Therefore, this study was designed to select lines and hybrids which could be used to breed for insect resistance using appropriate breeding methods. Consequently, the

selected hybrids could be deployed to farmers for usage.

CHAPTER THREE

DETERMINATION OF SELECTED INBRED LINES AND HYBRIDS FOR RESISTANCE TO MAIZE WEEVIL *(SITOPHILUS ZEAMAIS)*

Abstract

Maize weevil (*Sitophilus zeamais* Motschulsky) is a major maize (*Zea mays* L.) storage insect pest in the tropics which reduces the quantity and quality of maize. The objective of this study was to determine maize weevil damage on selected inbred lines and hybrids. Twenty eight inbred lines and 22 hybrids, with two checks (MTPO701-reistant and Duma 41-susceptible) were used in this experiment. Thirty unsexed adult insects were introduced into 250 ml glass jars with grains of the lines and hybrids at room temperature. The experiment was arranged in a randomised complete block design. Assessment of grain damage was done at 10, 60 and 120 days after maize weevil infestation. Each category of storage period was replicated four times and experiment set at the same time. Data was collected on per cent weevil damage, grain weight loss and number of live and dead weevils on each inbred line. Percent weight loss among inbred lines and hybrids at 10, 60 and 120 days differed significantly ($P<0.05$). Weight loss at 10 days was less than 1%. The selection of the resistant genotypes was based on percent weight loss after 60 days. The lines, KEN2/TZL2.2.5# and LEPOOL-1/TZL2-2-1, showed resistance to maize weevil damage at 60 and 120 days of storage and could be stored up to 4 months. KEN2/TZL2-2-5# showed consistency in resistance to maize weevils at all storage periods. Weight loss on the susceptible check Duma 41 and resistant check MTPO701 was 10.98 and 5.05, respectively. KH631Q emerged as the most resistant among the 22 hybrids evaluated and was found to be consistent in all the

three storage periods. The hybrid PH4 was a moderately resistant hybrid. The resistant check MTPO701 emerged as truly resistant to weevil attack as was envisaged. Correlation coefficients among grain damage, grain weight loss, live and dead maize weevil were highly significant. Heritability values were moderate. The resistant lines can act as sources of resistance and be considered for inclusion in breeding programs whereas resistant hybrids can be recommended for deployment to farmers for planting.

3.1 Introduction

Maize (*Zea mays L.*) is the most important cereal crop in Kenya and is consumed in various ways by the entire population in the country (Kang'ethe, 2011). While farmers may achieve high yields in the farm, they experience grain losses during storage due to insect pests. Research has focused on increased field maize productivity while post-harvest handling has received little attention yet insect pests at maize storage cause devastating yield and quality losses (Tefera *et al.,* 2011). After harvest, farmers are sometimes faced with the problem of surplus maize. This, together with low market prices force farmers to store their maize to take advantage of higher prices when the demand is high. Moreover, small scale farmers may store maize for longer periods for home consumption.

In most tropical countries, harvested grains are mainly stored by farmers for considerable periods in various types of storage structures made of mud, bamboo strips and plastic sacks (Ranjan *et al*., 1992; Bilgami and Sinha, 1987). These unimproved traditional storage methods inevitably provide suitable conditions for the growth of insects and microorganisms responsible for the quality loss in stored grains (Bilgami and Sinha, 1987). Most post-harvest insect pests are reported to be associated with stored maize

and their infestation has resulted to loss of food for man and animals. (Demianyk and Sinha, 1987). The main storage insect pests causing yield losses in maize include maize weevil, large grain borer, red flour beetle, Indian meal moth and lesser grain borer (USDA, 2015). These stored product pests are reported to destroy about 10- 15% of grain and contaminate the grains with undesirable odours and flavours (Das et al,. 2013). Among the pests, maize weevil has been identified to cause major grain losses in stored maize and creates a higher risk of establishment of aflatoxin and other mycotoxins in the grains (Tefera *et al*., 2011). The weevils generally lay eggs outside the grain kernels or in cracks in kernels (Tefera *et al*., 2010). The larvae then feed on broken kernels, although some can feed on the germ of the intact kernels (Tefera *et al*., 2010). Post-harvest maize weevil infestation commences in the field but most damage occurs during storage (Demissie *et al*., 2008b). This, therefore, demands control measures that are effective both in the field level and under storage.

Synthetic insecticides have been widely used on stored grains to control storage pests (Rajashekar *et al*., 2012). However, there is a global concern with respect to environmental hazards, chemical residues on food, insecticides resistance development and associated costs (Cherry *et al*., 2005).

Host plant resistance offers a sustainable control measure to weevil infestation in the field level, under storage and minimizes the major concerns associated with use of insecticides (War *et al*., 2012). Studies have found resistance to weevil infestation to be heritable (Derera *et al*., 1999). Most studies on host plant resistance to maize weevil have focused on grain factors contributing to resistance and inheritance mechanism of resistance (Derera *et al*., 2001). Despite the increased understanding of the inheritance of weevil

resistance and of the resistance mechanisms in the maize grains, there has been very little application of this knowledge in maize breeding programmes (Dhliwayo and Pixley, 2003).

Maize inbred lines represent a fundamental resource for studies in genetics and plant breeding towards crop improvement (Mwololo *et al*., 2012). These lines are mainly used in the development of hybrids. Progress has been made in developing maize cultivars resistant to post-harvest insect pests. Understanding the level of responses of different maize inbred lines especially against *S. zeamais* infestation is important to decide the course of resistance breeding strategy. Therefore, this study was undertaken to screen and identify resistant inbred lines to maize weevil attack for use in developing resistant maize hybrids.

3.2 Materials and methods

3.2.1 Site description

The study was carried out at the Kenya Agricultural and Livestock Research Organization-Kiboko, situated in Makueni County. Kiboko is situated within longitudes of 37.7235^0E and latitudes of 2.2172^0S. It lies at an altitude of 975 meters above sea level. The station receives between 545 and 629 mm of annual rainfall coming in two very short seasons. Average temperature is 22.6^0C, with mean annual maximum of 28.6^0C and mean annual minimum of 16.5^0C. Sandy-clay type of soil occupies this location.

3.2.2 Source of maize germplasm

Maize grains used in this study were from 28 inbred lines (Table 3.1) and 24 hybrids

(Table 3.2) which had been planted at the Kenya Agricultural and Livestock Research Organization (KALRO)-Kiboko nursery in July 2016. The genotypes used were provided by KALRO-Katumani. The inbred lines originated from Kenya, Zimbabwe and France while the hybrids were sourced from the local commercial enterprises within Makueni county.

Table 3.1: Source of inbred lines

	Origin	Status
CML 222	CIMMYT	Inbred line
CML 366	CIMMYT	Inbred line
CML312	CIMMYT	Inbred line
CML4	CIMMYT	Inbred line
HIFIL-57	KALRO	Inbred line
HIFIL-6	KALRO	Inbred line
Katumani 11-2-1	KALRO	Inbred line
Katumani 3-7-3	KALRO	Inbred line
KEN2/TZL2-1-2#	KALRO	Inbred line
KEN2/TZL2-2-3#	KALRO	Inbred line
KEN2/TZL2-2-5#	KALRO	Inbred line
KEN3/TZL2-2-6#	KALRO	Inbred line
Kikamba 4-3-3	KALRO	Inbred line
LEPOOL-1/TZL2-2-1	KALRO	Inbred line
PIP2ENTRY 108	France	Inbred line
PIP2ENTRY 135	France	Inbred line
PIP2ENTRY 14	France	Inbred line
PIP2ENTRY 143	France	Inbred line
RF291 3-10-11-1	France	Inbred line
RF291-10-5-3-9	France	Inbred line
RF291-8-3-4-9	France	Inbred line
TZL-1/DIPLO-1-2-2#	KALRO	Inbred line
TZL-1/DIPLO-1-2-3#	KALRO	Inbred line
TZL2/MUG1-2-4#	KALRO	Inbred line
TZL-2/MUG-1-2-5#	KALRO	Inbred line
TZL-3/DIPLO-1-1-6#	KALRO	Inbred line
TZL3/MUG1-4-10#	KALRO	Inbred line
ZIMLINE/MORO/BC18-1-1	Zimbabwe	Inbred line

CIMMYT-International Maize and Wheat Improvement Centre, KALRO-Kenya Agricultural and Livestock Research Institute

Table 3. 2: Source of hybrids

	Origin	Status
KH 600-15A	KALRO	Hybrid
DH01	KALRO	Hybrid
KH 631Q	KALRO	Hybrid
SC DUMA 41	KALRO	Hybrid
PH1	KALRO	Hybrid
WH 505	KALRO	Hybrid
MPTEH200804	KALRO	Hybrid
H614D	KALRO	Hybrid
SC DUMA 43	KALRO	Hybrid
PH3253	KALRO	Hybrid
MTP0701	KALRO	Hybrid
KH 500-33A	KALRO	Hybrid
WH 504	KALRO	Hybrid
H513	KALRO	Hybrid
PAN 67	KALRO	Hybrid
DH04	KALRO	Hybrid
PAN 691	KALRO	Hybrid
WH403	KALRO	Hybrid
SC SIMBA 61	KALRO	Hybrid
KH 500-31A	KALRO	Hybrid
KH 600-16A	KALRO	Hybrid
PH4	KALRO	Hybrid
Checks		
MPT0701	KALRO	Hybrid
DUMA 41	KALRO	Hybrid

KALRO-Kenya Agricultural and Livestock Research Institute

3.2.3 Field trial design and management

The entries were planted in the Kiboko experimental site. Field sizes were 87.5m x 18m and 87.5m x 30m respectively. Each plot measured 5m x 0.75m. Fertilizer was applied at a standard rate of 30kg of Calcium Ammonium Nitrate (C.A.N.) per ha and 30kg of Di ammonium Phosphate (D.A.P) per ha as recommended for the Kiboko site. Supplementary irrigation was administered when needed. The fields were kept free from weeds by hoe weeding. Number of rows per plot was 2 and distance between stations, 0.25m. The entries were arranged in a randomized complete block design (RCBD) with 4 replications. The experiment was done in a single season.

3.2.4 Grain preparation, insect culture and infestation

At harvest, sieving was done to remove any dirt, dust or broken grains. The mature maize weevil insects used for the evaluation were sourced from CIMMYT/KARI-Kiboko post-harvest testing laboratory. The insects were reared on commercial hybrid maize H614 under controlled conditions of 28°C and 70% relative humidity. Fifty grams of grains was put in 600 no-choice glass jars (experimental units) at room temperature which were infested with thirty unsexed adult maize weevils per glass jar (Tefera et al., 2011). Glass jars were then covered with a lid made of wire mesh to allow for adequate ventilation and prevent escape of the weevils. Before setting up the experiment the maize was divided into three groups based on the time of storage before assessing the grain for insect damage. One group had maize intended for storage for 10 days; the second had grain intended for storage for 60 days while the third group had maize for storage for 120 days. Each group consisted of 28 entries for inbred lines while 22 entries were for hybrids, replicated four times. The experimental set up for these genotypes was done at the same time.

3.2.5 Laboratory experimental design

The glass jars containing infested kernels were laid out in a randomized complete block design, an extension of the design obtained from the field. They were kept on wooden shelves at room temperature and 70-75% RH in the laboratory. The experiment consisted of 50 germplasm (lines and hybrids), replicated four times and put in three groups. A total of 600 samples were assessed in this experiment. MTP 0701(resistant check) and DUMA 4 (susceptible check) to weevil infestation were included in the experiment. Assessment of the trials was done at 10, 60 and 120 days of storage; these time periods for assessment were the treatments.

3.3 Data collection and assessment

Data was collected on % grain damage, % weight loss and number of live and dead weevils. On each assessment date (10, 60 and 120 days), the glass jars were opened, contents separated into grains, insects and dust using 4.7 and 1 mm sieves (Endecotts Ltd UK). Weevil mortality was assessed. All maize weevils were separated and removed (by hand) from the maize at the end of these three storage periods. Separation of the damaged and undamaged kernels was done using grain tunneling and holes as the criteria described by Tefera *et al*. (2011). The percent damage was determined using the converted percent damage method (Baba Tiertor, 1994):

$$\%GD = \frac{WDG\ X\ 100}{WDUD} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . \ldots\ldots (2)$$

Where:

$GD = Damaged\ grain$

$WDG = Weight\ of\ damaged\ grain$

$WDUD = Weight\ of\ damaged\ and\ undamaged\ grains$

Weight loss was determined by the count and weight method of Gwinner et al 1996.

$$Weight\ loss\ (\%) = (Wu\ \times Nd) - (Wd\ \times Nu) \times \frac{100}{Wu}\ (Nd + Nu) \ldots\ldots\ldots . . \ldots (3)$$

Where:

$Wu = Weight\ of\ undamaged\ grain$

$Nu = Number\ of\ undamaged\ grain$

$Wd = Weight\ of\ damaged\ grain$

$Nd = Number\ of\ damaged\ grain$

3.4 Data Analysis

3.4.1 Analysis of variance

The numbers of percentage weevil damage, grain weight loss, live and dead weevils were subjected to analysis of variance using GenStat 14th edition software to determine the difference in damage to the inbred lines and hybrids. The Tukey's test

was used to tell the significant difference of the genotypes. The data was also analyzed separately and combined for the three storage periods.

3.4.2 Heritability

Heritability was measured based on grain damage which is one of the measures of resistance to weevil damage. Heritability was estimated based on Johnson *et al*. (1955) procedure. Among the studied traits, percent damage as described by Golob (1981) was used to calculate heritability. The error mean square (EMS) was considered as error

variance (σ^2_e).

Genotypic variance (σ^2_g) was derived by subtracting error mean square (EMS) from the genotypic mean square (GMS) and divided by the number of replications (r) as given by the formula;

$$\sigma_g^2 = GMS - \frac{EMS}{r} \quad \ldots \ldots \ldots (4)$$

Where:

$\sigma_g^2 = Genotypic\ variance$

$GMS = Genotypic\ mean\ squares$

$EMS = Error\ mean\ squares$

$r = Replications$

Phenotypic variance (σ^2_P) was derived by adding genotypic variance with error variance as given by the formula:

$$\sigma_p^2 = \sigma_e^2 + \sigma_g^2 \quad \ldots \ldots \ldots (5)$$

Broad sense heritability was then calculated as:

$$H^2b = \sigma_G^2 / \sigma_P^2 \quad \ldots \ldots \ldots (6)$$

Where:

$\sigma_p^2 = Phenotypic\ variance$

$\sigma_g^2 = Genotypic\ variance$

3.4.3 Correlation Analysis

Pearson correlation coefficients were obtained using the GENSTAT 14th edition software. Correlations were computed between grain weight loss, grain damage, live and dead weevils. Pearson correlation formula (r) was computed by the formula:

$$r = \frac{COV_{xy}}{\sigma x . \sigma y} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

3.4 Results

3.4.1 Maize weevil damage of inbred lines

There were no significant differences among inbred lines in weevil damage after 10 days of storage (Table 3.3). Considering weevil damage after 60 days of maize storage, inbred lines differed significantly (Table 3.3). At 120 days of maize storage, the inbred lines showed significant drifts in the damage by weevils compared to storage at 60 days (Table 3.3). The genotype and time period interaction was significant for grain weight loss (Table 3.4). Maize weevil damage was at its peak after 120 days thus recording a mean damage of 66% (Table 3.4).

Table 3. 3: Mean squares of resistant parameters at three different storage periods among the inbred lines

Source	DF	10 days storage period				60 days storage period				120 days storage period			
		GD (g)	GWL (g)	LMW (count)	DMW (count)	GD (g)	GWL (g)	LMW (count)	DMW (count)	GD (g)	GWL (g)	LMW (count)	DMW (count)
Replication	3	9.22	0.0187	3.9639	3.2333	554.7	0.019	1854	43.422	73.31	33.26	2429.9	2053.9
Genotype	29	0.00138ns	0.0224ns	0.732ns5	0.654ns	647.9*	0.0224*	3809*	4.04ns	132.83*	96.37ns	1262.1*	3281*
Residual	87	0.00045	0.0173	0.8489	0.9345	106.5	0.017	1003	2.589	33	17.08	949.1	820.4
CV (%)		25	22	20	21	33	32	41	25	27	29	34	35

Table 3. 4: Combined mean squares at the three storage periods among inbred lines

Source	DF	GD (g)	GWL (g)	LMW (count)	DMW (count)
Replication	3	90.69	34.054	1463.4	793.6
Genotype	29	335.89*	26130.09*	176509.8*	579395.9ns
Days of storage	2	124820*	77.047*	2316.5*	1127.6ns
Genotype x days of storage	58	222.42*	28.413*	1377.7*	1079ns
Residual	267	51.51	8.033	668	283.1
CV (%)		30	31	37	34

Significance level * = $p<0.05$

DF-Degree of freedom, GD- Grain damage, GWL- Grain weight loss, LMW- Live maize weevils, DMW- Dead maize weevils, ns- not significant

The weevil damage on inbred lines was less than 1% and ranged from 0 to 1% at 10 days of storage (Table 3.5). PIP2ENTRY 108 was the only line that was slightly damaged at 0.1% by the maize weevil (Table 3.5). The remaining 27 lines were undamaged at this storage period. The resistant and susceptible checks MTP0700 and DUMA 41 were also not damaged after 10 days of storage. It was evident that weevil damage was very low after 10 days of storage.

The mean weevil damage after 60 days was 35% (Table 3.5). The damage range among the lines at 60 days of storage was 10.8% on KEN2/TZL2-2-5 to 62.3% on RF291-10-5-3-9. After these 60 days of storage, 26 inbred lines recorded less than 50% weevil damage while 4 were damaged beyond the 50% mark (Table 3.5). The highest weevil damage was recorded in RF291-10-5-3-9 as it was significantly more damaged than the susceptible check (Table 3.5).

On the other hand, lines KEN2/TZL2-2-5#, LEPOOL-1/TZL2-2-1 and CML 312 were less damaged (less than 20%) and were not significantly different from the resistant check MTP0701. The susceptible check Duma suffered 52% damage. (Table 3.5).

Maize weevil damage increased after 60 days and the maximum weevil damage was experienced at 120 days. The weevil damage at 120 days ranged from 41 to 72% (Table 3.5). Twenty six inbred lines recorded high weevil damage of 62 to 72%. The lines were the least damaged even at 60 days of storage. The susceptible (Duma 41) and resistant (MTPO701) checks recorded a mean of 69% and 64% respectively. The highly damaged line was RF291-10-5-3-9 at 62.3% (60 days of storage) and 72% (120 days of storage). Maize weevil damage sharply increased after 60 days of infestation in most lines (Table 3.5).

Table 3.5: Maize weevil damage of inbred lines at 10, 60 and 120 days of storage

Inbred line	10 days	60 days	120 days
CML 222	0.0	24.1	65.3
CML 366	0.0	35.9	67.8
CML312	0.0	16.3	62.5
CML4	0.0	44.8	69.0
HIFIL-57	0.0	23.8	63.9
HIFIL-6	0.0	28.9	65.4
Katumani 11-2-1	0.0	40.1	68.0
Katumani 3-7-3	0.0	39.4	68.0
KEN2/TZL2-1-2#	0.0	47.9	69.0
KEN2/TZL2-2-3#	0.0	42.1	68.0
KEN2/TZL2-2-5#	0.0	10.8	41.1
KEN3/TZL2-2-6#	0.0	48.2	69.9
Kikamba 4-3-3	0.0	28.6	65.2
LEPOOL-1/TZL2-2-1	0.0	16.1	51.2
PIP2ENTRY 108	0.1	47.2	69.4
PIP2ENTRY 135	0.0	35.4	66.5
PIP2ENTRY 14	0.0	20.3	63.5
PIP2ENTRY 143	0.0	38.3	67.1
RF291 3-10-11-1	0.0	34.8	66.1
RF291-10-5-3-9	0.0	62.3	72.6
RF291-8-3-4-9	0.0	42.2	68.3
TZL-1/DIPLO-1-2-2#	0.0	34.5	65.8
TZL-1/DIPLO-1-2-3#	0.0	37.1	67.6
TZL2/MUG1-2-4#	0.0	52.0	71.1
TZL-2/MUG-1-2-5#	0.0	32.4	65.3
TZL-3/DIPLO-1-1-6#	0.0	22.6	63.1
TZL3/MUG4-1-10#	0.0	23.7	63.9
ZIMLINE/MORO/BC18-1-1	0.0	52.1	71.5
Checks			
MTP0701(Resistant)	0.0	19.8	63.6
DUMA 41(Susceptible)	0.0	51.5	69.3
P-value	**0.2013**	**0.0132**	**0.0221**
Mean	**0.0**	**35.1**	**65.6**
LSD (genotype)		**5.8**	**7.4**
LSD (days)		**1.8**	
LSD (Gen x Days)		**8.7**	
CV (%)		**33.1**	**27.2**

Gen= genotypes, days= number of storage days

3.4.2 Heritability of maize weevil resistance

It was found that heritability varied with the storage period.

Table 3. 6: Heritability of weevil resistance on % damage in inbred lines after days of storage

Heritability was 34% at 10 days, 56% at 60 days and 43% at 120 days (Table 3.6).

	10 days	60 days	120 days
Environmental variance (V_E)	0.00045	106.5	33
Genotypic variance (V_G)	0.0002325	135.4	132.8
Phenotypic variance (V_P)	0.0006825	241.85	57.95
Broad sense heritability (H^2)	34	56.0	43.1

3.4.3 Maize weevil grain weight loss of inbred lines

Inbred lines did not differ significantly in grain weight loss after 10 days of weevil infestation in the inbred lines. There were significant differences in grain weight loss among inbred lines after 60 days of storage compared to storage after 10 days (Table 3.7). Weevil damage after 60 days resulted in weight loss of grains ranging from 4% to 16%. Genotypes were categorized according to the criteria described by Mwololo *et al*. (2012) and Tadele *et al*. (2011) where resistant genotypes are between 1 to 5%, moderately resistant (5.1 to 8%), moderately susceptible (8.1 to 10%), susceptible (10.1 to 13%) and highly susceptible (> 13%) after 60 days based on percent weight loss. The lowest grain weight loss was recorded in lines KEN2/TZL2-2-5# and LEPOOL- 1/TZL2-2-1(Table 3.7).

The inbred lines showed significant differences in grain weight loss after 120 days of storage. This stage had the maximum weight loss as compared to 10 days and 60 days of storage. The weight loss varied from 16 to 39%. KEN2/TZL2-2-5# lost up to 15% of grain weight (Table 3.7). The most grain weight loss was recorded in KEN2/TZL2-1-2# at 120 days. However, the grain weight loss among inbred lines increased

from 10 days to 120 days after weevil infestation. This increase in grain weight loss from 10^{th} day to 120^{th} day was observed in all lines. The most grain weight loss was after 120 days of storage (Table 3.7).

Table 3.7 Grain weight loss among inbred lines at the three storage periods

Inbred line	10 days	60 days	120 days	Remarks
KEN2/TZL2-2-5#	0.1	4.9	15.9	Resistant
LEPOOL-1/TZL2-2-1	0.1	4.9	22.3	Resistant
MTP0701(R)	0.1	**5.1**	**18.5**	**Resistant**
CML 222	0.1	7.2	25.2	Mod*
CML312	0.1	7.5	25.5	Mod*
TZL3/MUG4-1-10#	0.1	7.8	25.2	Mod*
TZL-3/DIPLO-1-1-6#	0.0	8.0	25.3	Mod*
HIFIL-57	0.3	8.1	26.4	Mod*
PIP2ENTRY 14	0.2	8.3	27.5	Mod*
CML 366	0.0	8.6	26.6	Mod*
RF291 3-10-11-1	0.1	9.5	28.4	Mod*
Kikamba 4-3-3	0.0	9.9	30.1	Mod*
Katumani 3-7-3	0.1	10.8	29.7	Mod*
TZL-1/DIPLO-1-2-3#	0.1	10.8	30.9	Mod*
DUMA 41(S)	**0.1**	**11**	**31.2**	**Susceptible**
TZL-2/MUG-1-2-5#	0.0	11.2	31.2	Susceptible
KEN3/TZL2-2-6#	0.0	11.7	33.8	Susceptible
PIP2ENTRY 108	0.0	11.7	35	Susceptible
PIP2ENTRY 135	0.1	12.3	35.8	Susceptible
RF291-8-3-4-9	0.1	12.5	35.0	Susceptible
CML4	0.3	12.5	30.2	Susceptible
KEN2/TZL2-2-3#	0.1	12.6	34.5	Susceptible
HIFIL-6	0.1	12.6	36.8	Susceptible
RF291-10-5-3-9	0.1	13.0	36.6	High*
TZL-1/DIPLO-1-2-2#	0.1	13.4	37.6	High*
PIP2ENTRY 143	0.1	13.8	37.5	High*
Katumani 11-2-1	0.1	15.5	37.9	High*
TZL2/MUG1-2-4#	0.1	15.6	36.6	High*
KEN2/TZL2-1-2#	0.1	15.9	38.7	High*
ZIMLINE/MORO/BC18-1-1	0.1	16.3	38.3	High*
P- value	**0.1720**	**0.0211**	**0.1422**	
Mean	**0.1**	**10.77**	**30.81**	
%CV		**32.3**	**29.0**	
LSD (genotype)		**2.4**	**3.5**	
LSD (days)		**1.3**		
LSD (Gen x days)		**10.3**		

Gen = genotypes, S = Susceptible, R = Resistant, Mod* = Moderately susceptible, High* = Highly susceptible

3.4.4 Number of live weevils in inbred lines

Among the inbred lines, 10 lines had retained the number of live weevils at 30. The rest of the inbred lines had 29 live weevils at this storage period (Table 3.8). Therefore, about 95% of introduced weevils were still alive after 10 days of maize storage in the jars. Inbred lines showed significant differences in the number of live weevils at 60 days of storage (Table 3.8).

Ninety-eight percent of inbred lines recorded increased number of live weevils. KEN3/TZL2-2-5 had the least number of weevils than the introduced number. The rest of the lines had more weevils than the initial number introduced at the start of the experiment. The increase in number of weevils varied from 19 to 128 weevils (Table 3.8). About 11 lines had at least 100 weevils at 60 days of storage. Katumani 11-2-1, PIP2ENTRY 143 and ZIMLINE/MORO/BC18 had more than 120 live weevils.

The number of live weevils varied from 76 to 134 weevils after 120 days of storage. The least number of live weevils was found in MTP0701. Nonetheless, this line had actually doubled the number of introduced weevils to 76 (Table 3.8). The highest number of weevils was found in KEN2/TZL2-1-2# at 120 days. The mean number of live weevils in this line was 134 (Table 3.8).

Analysis of variance indicated significant difference in inbred lines and storage periods. Their interaction was significant in response to damage, weight loss and number of live weevils. The number of live weevils increased with the number of storage periods. However, in PIP2ENTRY135, RF291-8-3-4-9, RF291-10-5-3-9, Katumani11-2-1, PIP2ENTRY

143 and ZIMLINE/MORO/BC18 the live weevils increased up-to the 60^{th} day and decreased by the 120^{th} day. The mean number of live weevils was 29 at 10 days, 82 at

60 days and 104 after 120 days of storage (Table 3.8). The number of live weevils in the susceptible check DUMA 41 was moderate, averaging 29, 56.25 and 106 weevils at 10, 60 and 120 days respectively (Table 3.8).

Table 3.8 Number of live weevils in inbred lines at the 3 storage periods

Inbred line	10 days	60 days	120 days
CML 222	29.3	49.0	122.3
CML 366	30.0	53.0	99.0
CML312	29.5	43.8	82.0
CML4	28.8	99.8	109.3
HIFIL-57	29.3	42.3	108.5
HIFIL-6	29.8	72.8	110.0
Katumani 11-2-1	30.0	127.3	99.5
Katumani 3-7-3	28.8	79.0	93.5
KEN2/TZL2-1-2#	29.3	103.0	134.0
KEN2/TZL2-2-3#	28.8	100.8	119.0
KEN2/TZL2-2-5#	29.3	18.5	84.5
KEN3/TZL2-2-6#	29.3	79.0	112.8
Kikamba 4-3-3	29.5	92.8	128.8
LEPOOL-1/TZL2-2-1	29.0	43.5	96.3
PIP2ENTRY 108	29.5	101.3	119.5
PIP2ENTRY 135	28.5	104.5	82.0
PIP2ENTRY 14	29.3	52.8	82.5
PIP2ENTRY 143	29.3	126.8	106.5
RF291 3-10-11-1	28.8	63.3	127.8
RF291-10-5-3-9	29.5	118.3	100.3
RF291-8-3-4-9	30.3	101.5	97.5
TZL-1/DIPLO-1-2-2#	29.3	121.5	126.5
TZL-1/DIPLO-1-2-3#	29.5	89.5	97.0
TZL2/MUG1-2-4#	29.0	114.3	132.0
TZL-2/MUG-1-2-5#	28.5	72.3	110.8
TZL-3/DIPLO-1-1-6#	29.3	60.5	85.8
TZL3/MUG4-1-10#	29.8	60.8	81.0
ZIMLINE/MORO/BC18-1-1	29.3	127.8	119.0
MTP0701	29.0	43.3	76.3
DUMA 41	29.0	56.3	106.0
P value	**0.1662**	**0.0134**	**0.0211**
Mean	**29.3**	**80.6**	**105.0**
CV%		**40.9**	**34.3**
LSD (Gen)		**9.1**	**6.2**
LSD (Days)		**3.6**	
LSD (Gen x Days)		**25.4**	

Gen= genotypes, days= number of storage days

3.4.5 Number of dead weevils in inbred lines

There were no significant differences in number of dead weevils among inbred lines after 10 days of storage. About 2 weevils were found to be dead after 10 days of storage in some inbred lines. Sixty-seven percent of inbred lines did not have dead weevils after 10 days of storage. Therefore, numbers of dead weevils at this storage period were few and ranged from 0 to 1 (Table 3.9). The susceptible check DUMA 41 had one dead weevil at this storage period. The inbred lines did not differ in the number of dead weevils after 60 days of storage. The numbers of dead weevils at this stage were still few and ranged from 0 to 4 (Table 3.9). Most dead weevils were found in RF291-10-5-3-9 and ZIMLINE/MORO/BC18 lines.

Analysis of variance results showed significant differences in number of dead weevils after 120 days of storage among inbred lines. The mean number of dead weevils was 122 at this storage period. This was the most recorded storage period with highest number of dead weevils (Table 3.9). At this storage period, the number of dead weevils in inbred lines varied from 55 to 175 weevils. Eighty-nine percent of inbred lines had at least 100 dead weevils (Table 3.9). The highest number of dead weevils was found in ZIMLINE/MORO/BC18. This corresponds to results after 60 days of storage in this line. DUMA 41 had about 80 dead weevils at this stage. This was among the lines with the least number of dead weevils at 120 days of storage (Table 3.9).

There were significant differences among inbred lines, storage periods and the interaction of inbred lines and storage periods in number of dead weevils (Table 3.9). The mean numbers of dead weevils were fewer (average of 2 weevils) at 10 and 60 days. However, most weevils died after 120 days of storage recording a mean of 122

dead weevils (Table 3.9). On averaging the 3 storage periods, dead weevils among inbred lines were found to range from 18 to 60 weevils. At 10 days of storage, the numbers of dead and live weevils were similarly few. However, there were remarkable differences in number of dead and live weevils at 60 and 120 days (Table 3.9). The lowest number of dead and live weevils was found in CML 312, DUMA41, KEN2/TZL2-2-5# and LEPOOL-1/TZL2-2-1-27-6 lines.

Table 3. 9: Number of dead weevils in inbred lines at the three storage periods

Inbred line	10 days	60 days	120 days
CML 222	0.8	1.5	103.3
CML 366	0.0	2.7	111.8
CML312	0.5	0.0	62.8
CML4	1.3	1.5	138.0
HIFIL-57	0.8	0.8	109.5
HIFIL-6	0.3	2.0	109.0
Katumani 11-2-1	0.0	1.3	148.3
Katumani 3-7-3	1.3	2.5	129.8
KEN2/TZL2-1-2#	0.8	3.0	114.5
KEN2/TZL2-2-3#	1.5	3.0	138.3
KEN2/TZL2-2-5#	1.0	0.3	55.0
KEN3/TZL2-2-6#	0.8	2.3	123.3
Kikamba 4-3-3	0.8	0.8	114.5
LEPOOL-1/TZL2-2-1	1.0	2.0	79.8
PIP2ENTRY 108	0.5	2.0	157.5
PIP2ENTRY 135	1.5	0.8	145.0
PIP2ENTRY 14	1.0	1.8	105.3
PIP2ENTRY 143	0.8	1.0	175.3
RF291 3-10-11-1	1.3	3.0	124.5
RF291-10-5-3-9	0.5	3.8	133.3
RF291-8-3-4-9	0.5	1.3	119.3
TZL-1/DIPLO-1-2-2#	0.8	2.8	135.5
TZL-1/DIPLO-1-2-3#	0.8	1.5	127.0
TZL2/MUG1-2-4#	1.0	1.0	160.8
TZL-2/MUG-1-2-5#	1.5	2.5	139.5
TZL-3/DIPLO-1-1-6#	0.8	2.5	111.0
TZL3/MUG4-1-10#	0.3	2.3	103.5
ZIMLINE/MORO/BC18-1-1	0.8	3.8	175.0
MTP0701(Resistant check)	1.3	1.5	117.0
DUMA 41(Susceptible check)	1.0	0.3	83.5
P-value	**0.2217**	**0.2911**	**0.0104**
Mean	**0.82**	**1.83**	**121.68**
%CV		**25**	**35.4**
LSD (Days)		**1.65**	
LSD (Gen x Days)		**12.3**	

Gen= genotypes, days= number of storage days

3.4.6 Correlations

The results show a linear association between weevil damage, weight loss, live and dead weevils measured after maize weevil infestation on inbred lines (Table 3.10). All the variates showed significant (at $P \leq 0.05$) and positive association. Weevil damage was found to correlate strongly with weight loss (r=0.9), live (r=0.8) and dead weevils (r=0.7). However, in all variables, weight loss (%) was strongly correlated to weevil damage (%). The number of live weevils and dead weevils gave a correlation coefficient of 0.5 (Table 3.10).

Table 3. 10: Correlation coefficients of maize weevil infestation in inbred lines

Parameter	Weevil damage	Weight loss	Dead	Live
Weevil damage	1			
(%) Weight loss	0.9192*	1		
Dead weevils	0.7482*	0.8964*	1	
Live weevils	0.807*	0.7602*	0.5219*	1

*=significant at 5% probability level

3.4.7 Maize weevil damage of hybrids

Genotypes did not differ significantly to weevil damage at 10 days of storage (Table 3.11). Maize weevil damage on genotypes after 10 days of infestation was negligible. The mean weevil damage was recorded as 0.0% (Table 3.13). It was evident that most genotypes had whole maize grains and were not damaged by the weevil.

Hybrids significantly differed in their response to weevil damage at 60 days of storage. Performance of hybrids to weevil damage was easily distinguished (Table 3.11). The mean weevil damage after 60 days of infestation was at 26.9%. Maize weevil attack on hybrids ranged from 4 to 48 % (Table 3.13). After 60 days of weevil infestation, MTPO701 (resistant check) was least damaged at 4% followed by KH631Q at 9.8 %. (Table 3.13). The susceptible check was damaged at

29% while the highly damaged hybrid was DK8031 at 48% (Table 3.13).

Hybrids were not significantly different in reaction to weevil damage at 120 days of storage (Table 3.11). Mean weevil damage in all hybrids after 120 days of storage was 51% (Table 3.13). However, the weevil damage among hybrids varied from 28% to 67%. H513 and WH403 were the highly damaged hybrids after 120 days of weevil attack (Table 3.13). The damage in these hybrids was 67%. Nevertheless, MTP0701 (Resistant check) and KH631Q were the least damaged hybrids. The damage in these hybrids was 28 to 30% respectively (Table 3.13). It was noted that after 120 days of weevil infestation, 5 hybrids namely KH 600-15A, KH 631Q, MTPEH200804, PH4 and SC DUMA 41 recorded damages of less than 50%.(Table 3.13). Interaction between hybrid damage and storage periods were significant (Table 3.12). Although no damage was recorded after 10 days, the highest damage was at 120 days of storage (Table 3.13). At 60 days there were significant damages on the hybrids grains but the damage peaked at 120 days. Weevil damage was consistently very low at all storage periods in the resistant check MTPO701 and hybrid KH631Q (Table 3.13). This hybrid was damaged by 9% and the check by 4%.

Table 3. 11: Mean squares of resistant parameters at three different storage periods among the hybrids

Source	DF	10 days storage period				60 days storage period				120 days storage period			
		GD (g)	GWL (g)	LMW (count)	DMW (count)	GD (g)	GWL (g)	LMW	DMW (count)	GD (g)	GWL (g)	LMW	DMW (count)
Replication	3	9.44	0.00382	0.5104	0.5694	385.6	578.8	1503.4	12.514	1695.2	2765.8	14620	153.01
Genotype	23	0.00159ns	0.0409ns	0.6698ns	0.7808ns	204.1*	287*	719.4*	7.257ns	472.8ns	761.2ns	6228ns	79.74ns
Residual	69	0.00019	0.0183	0.438	0.4535	104.7	142.5	406.8	6.833	481.4	787.3	4131	75.33
CV						0.27	0.36	0.33	0.41	0.35	0.39	0.32	0.30

Table 3. 12: Combined mean squares at the three storage periods among hybrids

Source	DF	GD (g)	GWL (g)	LMW (count)	DMW (count)
Replication	3	208.1	350	3071	82.69
Genotype	23	315.7*****	86355.2*	149216*	529.5ns
Days of storage	3	56294.9*	498.8*****	3084*	42.32**ns**
Genotype x Days of Storage	46	180.6*****	274.7*****	1932*****	22.73**ns**
Residual	213	216.3	343.4	1654	27.94
CV		0.51	0.27	0.35	0.28

Significance level * = p<0.05; DF-Degree of freedom, GD- Grain damage, GWL- Grain weight loss, LMW- Live maize weevils, DMW- Dead maize weevils, ns- not significant

Table 3. 13: Maize weevil damage on hybrids for the three storage periods

Hybrid	10 days	60 days	120
DH01	0.0	21.9	61.8
DH04	0.0	36.7	54.2
DK8031	0.1	48.4	62.1
H513	0.0	21.9	67.3
H614D	0.0	29.3	60.3
KH 500-31A	0.0	38.3	58.4
KH 500-33A	0.1	19.5	46.8
KH 600-15A	0.0	42.4	35.6
KH 600-16A	0.0	31.9	59.6
KH 631Q	0.0	9.8	37.7
MTPEH200804	0.0	18.7	30.6
PAN 67	0.1	20.5	51.7
PAN 691	0.0	44.5	54.3
PH 4	0.0	18.5	45.1
PH1	0.0	36.6	59.0
PH3253	0.0	28.1	61.6
SC DUMA 41	0.0	31.2	51.6
SC DUMA 43	0.0	29.5	58.3
SC Simba 61	0.0	28.7	51.9
WH 403	0.1	28.3	66.7
WH 504	0.0	36.5	58.9
WH 505	0.0	18.4	54.7
Checks			
MTP0701Resistant)	0.0	4.1	28.4
DUMA 41(Susceptible)	0.0	29.4	51.6
P-value	**0.211**	**0.011**	**0.129**
Mean	**0**	**26.92**	**50.73**
LSD (Gen)		**7.3**	**14.2**
LSD (Days)		**5.5**	
LSD (Gen x Days)		**8.3**	
CV (%)		**27**	**35**

Gen= genotypes, days= number of storage days

3.3.1 Heritability and variances of weevil resistance on hybrids

Negative heritability was recorded at 10 and 120 days of storage (Table 3.14).

Table 3. 14: Heritability and estimated variances in maize hybrids

	10 days	60 days	120 days
Environmental variance (V_E)	0.00019	104.7	481.4
Genotypic variance (V_G)	-0.0000075	24.85	-2.15
Phenotypic variance (V_P)	0.0001825	129.55	470.7
Heritability in broad sense (H^2)	-4.10958904	19.18	-0.46

3.4.8 Maize weevil grain weight loss of hybrids

There was no significant difference in weight loss among hybrids after 10 days of storage (Table 3.15).

Grain weight loss in all hybrids was less than 1% after 10 days (Table 3.15). At this storage period, the hybrids grain weight loss varied from 0 to 0.01. The susceptible check DUMA 41 was not damaged after 10 days of storage (Table 3.15). The hybrids showed differences in percentage grain weight loss after 60 days of storage. The mean grain weight loss after 60 days of storage was at 17. %. The grain weight loss of hybrids varied from 4% to 27%. According to the criteria of categorizing genotypes where resistant genotypes are between 1 to 5%, moderately resistant (5.1 to 8%), moderately susceptible (8.1 to 10%), susceptible (10.1 to 13%) and highly susceptible (> 13%) after 60 days based on percent weight loss (Mwololo *et al*.,2012; Tadele *et al*., 2011), KH631Q was resistant, PH 4 was resistant, DH01, MTPEH200804 and WH505 were susceptible and the rest were highly susceptible (Table 3.15). As expected, the resistant check, MTP0701 had the least damage and hence recorded the least grain weight (Table 3.15). The susceptible check DUMA 41 recorded a weight loss of 23.3 and hence

was grouped among the highly susceptible genotypes. Among the hybrids, PAN 691 and KH 500-31A had the most grain weight loss. The weight loss in these hybrids was 27 and 26%, respectively. At this stage about 50% of hybrids had lost more than 20% of the grain weight.

The hybrids did not differ significantly in grain weight loss after 120 days of storage (Table 3.15). At 120 days of storage, the hybrids had lost more grain weight than after 60 days, and the mean loss was at 39.5%. This storage period recorded the highest grain weight loss which varied from 8.7% to 57.6% (Table 3.15) .In all the hybrids, the resistant check; MTP0701 had the least weight loss at this storage period. On the contrary, PH3253, DK8031, WH 505 and WH 403 lost the most grain weight at this period. The grain weigh loss in these hybrids was above 50% (Table 3.15).

The hybrids differed significantly in grain weight loss and in the interaction of hybrids and storage days. However significant differences in storage days were recorded (Table 3.12). Grain weight loss increased gradually after the 60 days of storage and was most observed at 120 days in all hybrids. The least and most grain weight loss was recorded after 10 days and 120 days respectively (Table 3.15).

Table 3.15: Maize weevil grain weight loss on hybrids at the three storage periods

Hybrid	10 days	60 days	120 days	Remarks
MTP0701(R)	**0.0**	**4.5**	**8.7**	**Resistant**
KH 631Q	0.0	5.0	18.3	Resistant
PH 4	0.0	7.2	29.2	Moderately resistant
DH01	0.0	9.5	49.8	Susceptible
MTPEH200804	0.0	9.7	13.8	Susceptible
WH 505	0.0	9.9	55.9	Susceptible
PAN 67	0.0	12.4	35.3	Highly susceptible
H513	0.0	14.4	48.5	Highly susceptible
SC Simba 61	0.0	15.2	37.8	Highly susceptible
KH 500-33A	0.0	15.8	34.2	Highly susceptible
H614D	0.0	16.2	47.5	Highly susceptible
WH 403	0.0	17.0	57.6	Highly susceptible
KH 600-16A	0.0	18.7	47.2	Highly susceptible
PH3253	0.0	18.8	55.1	Highly susceptible
DK8031	0.0	20.1	55.9	Highly susceptible
DH04	0.0	20.2	41.5	Highly susceptible
SC DUMA 43	0.0	20.9	41.6	Highly susceptible
PH1	0.0	22.2	44.4	Highly susceptible
SC DUMA 41	0.0	22.3	32.7	Highly susceptible
KH 600-15A	0.0	22.7	17.1	Highly susceptible
DUMA 41(S)	**0.0**	**23.3**	**36.9**	**Highly susceptible**
WH 504	0.0	25.3	47.5	Highly susceptible
KH 500-31A	0.0	26.4	47.6	Highly susceptible
PAN 691	0.0	27.3	44.7	Highly susceptible
P value	**0.2171**	**0.0182**	**0.1317**	
Mean	**0.00**	**16.9**	**39.5**	
%CV		**36**	**39**	
LSD (Gen)		**8.2**	**5.6**	
LSD (Days)		**5.2**		
LSD (Gen x Days)		**2.5**		

Gen = genotypes, days = number of days

3.4.9 Number of live weevils in hybrids

After 10 days of storage, hybrids did not show differences in number of live weevils present in the grains. At the start of the experiment, 30 live insects had been introduced in each glass jar containing grains of hybrids. After 10 days of storage the live insects were ranging from 29 to 30 (Table 3.16). This showed that only one weevil had died in most

hybrids. However, 12 hybrids retained the number of live insects introduced at the start of the experiment.

There were significant differences in number of live weevils among hybrids when hybrids were stored for 60 days. At this storage period (60 days), a mean of 33 live weevils was recorded. Nonetheless, the number of live weevils varied from 5 to 64 at this storage period. The least number of weevils was recorded in the resistant check MTPO701. This check had a mean number of five weevils after 60 days of storage. In this check, number of live weevils had reduced by 25. Similarly, in nine hybrids, live weevils had reduced by 10 (Table 3.16). However, in 14 hybrids number of weevils had increased. DK8031 had the highest number of live weevils. This hybrid had doubled the number of live weevils to 64 at this storage period (Table 3.16).

Hybrids did not differ significantly in number of live weevils after 120 days of storage (Table 3.11). At this stage, live weevils ranged from 35 to 181.The mean number of live weevils had tripled after 60 days and was at 99 (Table 3.16). At this stage, 15 hybrids had at least tripled number of weevils than the introduced. The least number of live weevils were in hybrids; KH 600-15A and SC DUMA 41. The number of live weevils in the two hybrids was at 38 and 52 respectively. The resistant check MTP0701 had the least number of weevils (35 weevils on average). The susceptible check DUMA 41 had 54 weevils at this stage. However, 3 hybrids had lower number of live weevils than the susceptible check (Table 3.16).

Significant differences in number of weevils were recorded between storage periods, hybrids. The interaction of live weevils and time of storage was significant. The live weevils were retained at 10 days but increased after 60 days of storage and at 120 days

of storage, the live weevils had tripled the introduced number. In all storage periods, the highest number of live weevils were at 120 days of storage and lowest at 10 days (Table 3.16).

Table 3.16: Number of live weevils in hybrids at the three storage periods

Hybrid	**10 days**	**60 days**	**120 days**
DH01	29.5	29.0	124.8
DH04	29.5	35.3	106.5
DK8031	29.5	63.5	181.3
H513	29.3	24.8	101.3
H614D	29.0	35.5	91.0
KH 500-31A	29.0	49.5	128.5
KH 500-33A	28.8	24.3	84.5
KH 600-15A	29.0	43.3	38.3
KH 600-16A	29.5	43.3	107.8
KH 631Q	29.5	19.8	55.0
MTPEH200804	30.0	18.8	83.0
PAN 67	29.8	22.3	94.3
PAN 691	29.8	51.8	164.0
PH 4	28.8	19.3	79.5
PH1	29.3	51.3	103.5
PH3253	29.8	32.0	176.3
SC DUMA 41	29.3	43.0	52.8
SC DUMA 43	28.5	40.5	76.5
SC Simba 61	29.3	32.3	92.0
WH 403	30.0	36.0	139.0
WH 504	29.8	34.5	111.3
WH 505	29.8	23.3	109.0
Checks			
MTP0701	29.75	5.0	35.3
DUMA 41	29.75	21.3	54.3
P value	**0.3911**	**0.0182**	**0.2219**
Mean	**29.5**	**33.3**	**99.6**
%CV		**33**	**32**
LSD (Gen)		**21.4**	**10.1**
LSD (Days)		**15.3**	
LSD (Gen x days)		**8.2**	

Gen = genotypes, days = number of days

3.4.10 Number of dead weevils in hybrids

At 10 days of storage, there was no significant difference for number of dead weevils. However, only one weevil had died in 11 hybrids. The rest of the hybrids had live weevils (Table 3.17). Hybrids also did not show significant differences in number of dead weevils after 60 days. At this storage period, dead weevils had increased and were ranging from 0 to 7 (Table 3.17). Resistant check MTPO701 and WH504 lacked dead weevils at this stage.

The remaining hybrids had at least one or two dead weevils after 60 days of storage (Table 3.17). The number of dead weevils was also found not to be significant among hybrids after 120 days of storage. At this stage, dead weevils were at an average of 5 but varied from 1 to 22 among hybrids. At 120 days of storage, the highest numbers of dead weevils were in hybrid PH4. The check MTP0701 and DUMA 41 had dead weevils averaging 5 and 6 respectively (Table3.17). The highest number of dead weevils was at 5 after 120 days of storage. The number of dead weevils was considerably lower than live weevils at the three storage periods (Tables 3.16 and 3.17).

Table 3.17: Number of dead weevils in hybrids at the three storage periods

Hybrid	10	60	120
DH01	0.5	0.8	1.8
DH04	0.3	1.8	2.3
DK8031	0.5	1.3	3.8
H513	0.8	0.8	2.3
H614D	1.0	0.5	7.3
KH 500-31A	1.0	2.5	5.8
KH 500-33A	1.5	1.3	0.8
KH 600-15A	1.0	3.3	3.8
KH 600-16A	0.5	1.0	4.0
KH 631Q	0.5	0.8	2.0
MTPEH200804	0.0	1.5	1.0
PAN 67	0.3	1.0	2.8
PAN 691	0.3	2.8	2.8
PH 4	1.3	6.8	22.0
PH1	0.8	1.3	4.5
PH3253	0.3	2.3	5.5
SC DUMA 41	1.0	1.8	13.3
SC DUMA 43	1.5	0.8	3.5
SC Simba 61	0.8	1.0	5.0
WH 403	0.0	1.0	5.0
WH 504	0.3	0.3	3.8
WH 505	0.3	1.0	7.8
Checks			
MTP0701 (Resistant)	0.3	0.3	6.0
DUMA 41(Susceptible	0.3	1.3	5.0
P value	**0.1561**	**0.2160**	**0.2551**
Mean	**0.6**	**1.52**	**5.05**
%CV		**41**	**30**
LSD (Gen)		**5.5**	**4.2**
LSD (Days)		**6.1**	
LSD (Gen x Days)		**2.6**	

Gen = genotypes, days = number of days

3.4.11 Correlations

There was a significant correlation among all the variates. In all the variates, numbers of live weevils were strongly correlated to % weevil damage. The correlation coefficient in these variates was 0.99. Unlike the inbred lines where weight loss and % weevil damage had the most association, correlation coefficient was low in these factors (r=0.34) in hybrids.

Live weevils correlated well with dead weevils giving a coefficient of 0.81 while dead weevils and % weevil damage resulting in coefficient of 0.85 (Table 3.18).

Table 3.18: Correlation coefficients of maize weevil infestation in maize hybrids

Parameter	Weevil damage	Weight loss	Dead	Live
Weevil damage	1			
Weight loss	0.3358*	1		
(%) Dead	0.8491*	0.287	1	
Live weevils	0.9938*	0.3214*	0.8136*	1

***=significant at 5% probability level**

3.5 Discussions

3.5.1 Maize weevil damage of inbred lines and hybrids

Significant differences in response of the genotypes to weevil damage is attributed to genotypic effects because the genotypes were exposed to identical capacity of weevil infestation and environment. These differences in the resistance of the maize varieties indicated the inherent ability of the studied lines to resist *S. zeamais* attack. The resistance could either be due to antibiosis as a result of biochemical compounds which are toxic to insects or physical factors such as grain hardness (Garcia-Lara *et al*., 2004; Siwale *et al*., 2009; War *et al*., 2017). Resistance can also be attributed to pericarp

surface texture, nutritional factors such as amylose, lipid and protein content (Dobie, 1974; Tipping *et al*., 1988; Tefera *et al*., 2013) or non-nutritional factors, especially phenolic compounds (Serratos *et al*., 1987; Tefera *et al*., 2013). Gerema *et al*., (2017) reported that high level of grain damage depends on the number of emerging insects and grains permitting high level of adult emergence. Weevil damage increased progressively from 10 to 60 to 120 days in inbred lines and hybrids. These results are similar with those of Tefera *et al*. (2011) and Togola *et al*., (2013) who reported the same trend. According to Tefera *et al*. (2016), despite the shape, size and hardness of the grain, its chemical and nutritional composition are important primarily in resisting insect attack and damage, the length of exposure of the grain to the pest may affect the level of infestation of maize varieties by *S. zeamais* thus compromising extent of maize damage. The grain is then left exposed to micro- organisms leading to the production of mycotoxins thus lowering the quality and also rendering it undesirable for consumption (Mejia, 2007). Maximum weevil damage was recorded at 120 days. This showed that, resistance alone was not enough to suppress *S. zeamais* population build up but it can reduce losses due to weevil infestation since no maize grain was immune to attack by the weevil (Ivbiljaro, 2009). From the study, genotypes were undamaged for the first 10 days of storage. This showed that maize weevil damage does not commence immediately and hence maize grains can be stored for up to two weeks with minimal damage.

3.5.2 Maize weevil grain weight loss of inbred lines and hybrids

The maize weevil *Sitophilus zeamais* exhibit holometabolous type of post-embryonic development of 36 days period. This explains why after 10 days there was no grain

weight loss, and this was aggravated by the fact that no damage had occurred. Later, the larvae develop and start eating the grains from the inside (Abebe *et al*., 2009; Wangui, 2016). The adults too immediately start aggressive feeding, resulting in increased destruction of the grains as indicated by more weight loss after 60 and 120 days of storage (Dobie 1974; Dobie *et al*. 1984; Wangui, 2016). Given that both larvae and adults feed on grains, they create much dust and consequently, great maize weight losses as the storage period prolongs.

The degree of weight loss has been found to be an important measure of maize grain resistance or susceptibility to the maize weevil (Derera *et al*., 2014), therefore its use as a key trait in discriminating genotypes in resistant categories in this study. In this study, resistant varieties had the least grain weight as it was reported before by Siwale *et al*., (2009). Also, from the study, grain weight loss was relatively low and was less than 40% in all storage periods. According to Dobie, (1977), higher grain weight losses are expected when young weevils of particularly 0 to 3 weeks are used because they have a higher fecundity rate and increased feeding.

It has been noted that a number of factors contribute to genetic resistance of varieties to stored grain insect pests attack (Adentuji, 1998; Muzemu *et al*., 2013). However further evaluation of the identified resistant lines KEN2/TZL2-2-5 and LEPOOL-1/TZ2 2- 2-1 should be done to determine the specific factors causing resistance to weevil attack. Such factors will then be selected for when developing resistant inbred lines. The selected resistant lines should be regarded as potential sources of weevil resistance and thus be utilized in breeding resistant maize varieties. For hybrids, KH631Q was identified as resistant. This variety can be used as a source of resistance in breeding

programmes and subsequently be adapted by smallholder farmers to diversify the basis of resistance to this pest. The selected resistant hybrid exhibited genetic factors which confer resistance to maize weevil attack. Within the first storage period (10 days), none of the varieties suffered any significant damage or weight loss. For longer storage, there were increases in weevil numbers, leading to increased weight losses. According to Wangui (2016), despite the shape, size and hardness of the grain, its chemical and nutritional composition are important primarily in resisting insect attack and damage; the length of exposure of the grain to the pest may also affect the level of infestation of maize varieties by *S. zeamais.* This therefore, results in increased grain weight losses. Grain weight losses were generally lower at 10 days of storage with higher losses being at 60% at 120 days. Hossain *et al*. (2007) reported that grain loss of 12% to 20% is common, but up to 80% has been reported for untreated kernels.

3.5.3 Number of live weevils in inbred lines and hybrids

Number of live weevils remained constant even after 10 days of storage. This was attributed to the fact that the development stage of most weevils is 36 days therefore no new insects had emerged within 10 days. It was expected that the number of live weevils will be more in lines which had most damage. For instance, in the case of inbred lines, Katumani 11-2-1, PIP2ENTRY 143 and ZIMLINE/MORO/BC18 had the highest number of live weevils at 60 days and TZL2/MUG1-2# and KEN2/TZL2-1-2# at 120. Their percent damage was also high. This increased insect multiplication resulted into enormous damages in the grains of inbred lines. The resistant lines had lesser weevils indicating antibiosis kind of resistance among the inbred lines.

For hybrids, the number of live insects remained unchanged after 10 days since new insects had not emerged within this short period. According to Gafishi (2012), complete development for the life cycle of the maize weevil averages 36 days. This could also mean that short storages of studied hybrids up to two weeks are also possible. However, at 60 days number of maize weevils decreased and increased in some hybrids. The hybrids with highest number of weevils were mostly damaged as it was the case for DK8031. This variety has been found to be susceptible to maize weevil damage and hence a favourable host for maize weevil (Kalunde, 2011). In this study, numbers of live *S. zeamais* varied with the maize hybrid varieties used. Therefore, the shortest developmental times occurred on the varieties which had the largest number of weevils emerging. On the other hand, the longest developmental times occurred in varieties with the least number of live weevils. The development of an insect is influenced by nature of food the insect is reared on. Generally, more eggs are laid on and development is faster on a more favourable than a non-favourable hosts. Increased maize weevil emergence is a result of high susceptibility of a genotype on which weevils can feed easily and therefore produce many eggs and progeny.

3.5.4 Number of dead weevils in inbred lines and hybrids

For inbred lines, the numbers of dead weevils were fewer at 10 and 60 days of storage while more weevils died after 120 days. This indicates that the host lines were unfavourable for feeding and hence reproduction was not possible. Another reason would be competition for limited food resource amongst the weevils which could have resulted to death of insects. According to Sori and Keba (2013) and Abebe *et al*.

(2009), numbers of dead weevils or weevil mortality rates are generally low in most maize varieties. They also reveal that adult weevils can survive without food for more than 10 days indicating that the number of dead weevils is not a good indicator of weevil resistance in maize varieties. The number of dead weevils was relatively low in hybrids confirming the reports of Abebe *et al*., (2009) who also found a low mortality rate of maize weevils. However, the rate of mortality of weevils has been revealed not to be a good indicator for weevil resistance among maize varieties.

3.5.5 Heritability of maize weevil resistance

For the inbred lines, heritability was found to be below 50% at 10 and 120 days of storage. At 60 days, heritability was moderately high at 56%. High heritability at 60 days reveals that selection for weevil resistance in these inbred lines is effective at this stage.

Resistance in hybrids was low. Dhliwayo *et al*. (2005) reported that inheritance of weevil resistance is complex and heritability is likely to be small to moderate. Low heritability indicates slow progress in selection for this character. Low heritability levels could be because most of the evaluated hybrids were being developed for other agronomic traits and hence weevil resistance was not considered as a primary factor during selection.

3.5.6 Correlations

There was significant positive correlation among studied traits for both inbred lines and hybrids. The results revealed a strong association between weevil damage and grain weight loss. Also a strong association of live weevils and percent grain weight loss was recorded. These results were in conformity with reports of Dari *et al*.(2010) and Zunjare *et al*.(2016) who also found strong correlation in these factors indicating that they are key indicators of weevil resistance in maize.

3.6 Conclusions

This study exhibits variation that exists among the selected inbred lines and hybrids for maize weevil resistance. It has shown that percent damage, grain weight loss and number of live insects are important parameters to look for while investigating for weevil resistance. Also this study showed that resistance against weevil attack is heritable since resistant inbred lines and hybrids were selected. The selected resistant lines should be regarded as potential sources of weevil resistance and thus be utilized in breeding resistant maize varieties. Likewise, the selected resistant hybrids should be used in areas considered to be maize weevil hotspots. This offers a great opportunity to exploit the variability with the aim of reducing post-harvest insect-pest losses through genetic improvement

CHAPTER FOUR

DETERMINATION OF SELECTED INBRED LINES AND HYBRIDS FOR RESISTANCE TO LARGER GRAIN BORER (*PROSTEPHANUS TRUNCATUS)*

Abstract

The larger grain borer (LGB) is a major storage pest which has continued to devastate maize grains especially in the dry and hot ecologies of Kenya. Studies have shown that, without adequate moisture content control and insecticide treatment on stored maize incidences of LGB infestation could be rampant. Host resistance is a low cost and environmentally friendly form of pest management. The objective of this study was to identify selected inbred lines and hybrids with resistance against LGB. Twenty-eight inbred lines and 22 hybrids, MTP0701 (resistant check) and DUMA 41(susceptible check) were used in this study. Thirty unsexed adult insects were introduced into 250ml glass jars with grains of the genotypes at room temperature and experiment arranged in Randomised Complete Block Design (RCBD). Assessment of LGB damage was done at 10 days, 60 days and 120 days of maize storage. Data was collected on percent LGB damage, grain weight loss and number of live and dead LGBs on each genotype. Heritability and correlation of factors were also estimated. Results showed significant differences ($P \leq 0.05$) in all studied traits. The lines KEN2/TZL2-2-5# and LEPOOL-1/TZL2-2-1 showed resistance as evidenced by the low percent damage and weight losses at assessment dates of 60 and 120 days after infestation. Insect infestation was also adversely affected on KEN2/TZL2-2-5# as indicated by presence of only 23.8 live adult LGBs after 60 days of infestation. KH631Q was selected as the most resistant hybrid among the 22 hybrids evaluated. Heritability levels were moderate for the 3 storage

periods. Likewise, strong correlations of above 0.7 for various variates in the inbred lines indicated that increased number of LGBs results to high damages which cause grain weight losses. Level of resistance to LGB among studied hybrids was high compared to previous reports. The selected inbred lines should be utilized in developing resistant hybrids in breeding programmes. The resistant hybrids should be made available for farmers use.

4.1 Introduction

Most of the maize improvement programmes focus on agronomic traits and field insect pests' however little attention is on storage pests. While storage pests remain ignored, they can destroy up to 100% of the harvest considering the poor existing storage methods among most farmers in tropical areas (Pingali and Pandey, 2001). The larger grain borer (*Prostephanus truncatus* Horn) is a storage pest which is among the major pests responsible for serious losses of maize worldwide. Its damage results directly in wastage of food, and quality of stored maize (Pingali and Pandey, 2001). Developing high-yielding maize varieties with resistance to LGB has been regarded as a potential option to minimize the overall cost of production and storage of maize. Resistant varieties are environmentally friendly and can reduce the potential risk associated with consumption of treated maize with insecticides (Mugo *et al*., 2008).

Breeding for resistance LGB is crucial, especially as this influences the adoption of improved varieties. There are three resistance components of plants to insect pests namely, antibiosis, non-preference and tolerance which have been studied and found to be important for grain resistance to storage pests (Derera *et al*., 2001). Non-preference i s the heritable feature of the grain, which discourages insects from feeding, colonising and

oviposition or a combination of the three. Grain texture has been suggested as the basis of non-preference resistance. Antibiosis denotes plant characters that result in adverse effects on the insect's life history when the insect uses a resistant plant for food, while tolerance denotes a resistance whereby the plant shows an ability to grow and reproduce itself or to repair injury to a marked degree of pest damage. Evaluation of resistance to stored-grain pests should focus on measuring antibiosis and or non-preference because tolerance does not function in stored grain (Derera *et al*., 2001).

There are increasing efforts in developing resistant maize varieties to LGB in international research organisations such as CIMMYT and IITA. Most breeders are now focusing on accumulating antibiosis among maize cultivars as well as enhancing biochemical and physical factors that enhance antibiosis. It's therefore important to select resistant lines as a way of providing a wide genetic diversity for resistant genes. Likewise, it is necessary to evaluate existing hybrids for LGB resistance as a way of identifying the existing genetic variability among tropical maize varieties for breeding plans and resistant varieties for farmer use.

4.2 Materials and methods

4.2.1 Source of maize germplasm

Maize grains used in this study were from twenty eight inbred lines and twenty four hybrids which had been planted at the Kiboko nursery in July 2016. The genotypes used were provided by the Kenya Agricultural and Livestock Research Organization (KALRO) – Katumani. The inbred lines originated from Kenya, Zimbabwe and France (Table 3.1). The grains were selected on the basis of their high resistance to aflatoxin contamination.

4.2.2 Field trial design and management

The experimental materials were evaluated at Kenya Agricultural and Livestock Research Organization (KALRO) – Kiboko. This is a Research Centre situated in Makueni County, Eastern region. The mean annual rainfall is 530 mm and is spread over two very short rainy seasons. It lies at an altitude of 975 meters above sea level and between latitude 02^0 15' S and longitude 37^0 75' E. Sand-clay type of soil occupies this location. Temperatures are uniformly high with mean maximum value of 28.6^0C and the minimum of 16.5^0C (CIMMYT, 2013).

Field sizes were 87.5m*18m and 87.5m*30m respectively. Each plot measured 5m*0.75m. Fertilizer was applied at a standard rate of 30 kg/ha Calcium Ammonium Nitrate (C.A.N.) and 30kg/ha Di ammonium Phosphate (D.A.P) as recommended for the Kiboko site. Supplementary irrigation was administered when needed. The fields were kept free from weeds by hoe weeding. Number of rows per plot was 2 and distance between stations, 0.25m. Treatments were laid in a Randomized Complete Block Design (RCBD) with 4 replicates.

4.2.3 Grain preparation, insect culture and infestation

At harvest, sieving was done to remove any dirt, dust or broken grains. The mature maize weevil insects used for the evaluation were sourced from CIMMYT/KARI-Kiboko post-harvest testing laboratory. The insects were reared on commercial hybrid maize H614 under controlled conditions of 28°C and 70% relative humidity. Fifty grams of grains was put in 600, 250ml capacity no-choice glass jars at room temperature which were infested with thirty unsexed adult maize weevils per glass jars. Glass jars were then

covered with a lid made of wire mesh to allow for adequate ventilation and prevent escape of the weevils. Before setting up the experiment the maize was divided in to three groups based on the time of storage before assessing the grain for insect damage. One group had maize intended for storage for 10 days; the second had grain intended for storage for 60 days while the third group had maize for storage for 120 days. Each group consisted of 28 entries for inbred lines while 22 entries for hybrids, replicated four times. The experimental set up for these genotypes was done at the same time.

4.2.4 Laboratory experimental design

The glass jars containing infested kernels were laid out in a randomized complete block design and kept on wooden shelves at room temperature with 70-75% RH in the laboratory. The experiment consisted of 50 genotypes (lines and hybrids), replicated four times and put in three groups. A total of 600 samples were assessed in this experiment. Assessment of the trials was done at 10, 60 and 120 days of storage. These time periods for assessment were the treatments.

4.3 Data collection and assessment

Data was collected on % grain damage, %weight loss and number of live and dead weevils. On each assessment date, at 10, 60 and 120 days, the glass jars were opened, contents separated into grains, insects and dust using 4.7 and 1 mm sieves (Endecotts Ltd UK). Weevil mortality was also assessed. All maize weevils were separated and removed (by hand) from the maize at the end of these three storage periods. Separation of the damaged and undamaged kernels was done using grain tunneling and holes as the criteria described by Tefera *et al*. (2011). The percent damage was determined using the converted percent damage method (Baba Tiertor 1994):

$$\%GD = \frac{WDG\ X\ 100}{WDUD} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .\ldots\ldots (8)$$

Where:

$GD = Damaged\ grain$

$WDG = Weight\ of\ damaged\ grain$

$WDUD = Weight\ of\ damaged\ and\ undamaged\ grains$

Weight loss was determined by the count and weight method of Gwinner et al 1996.

$$Weight\ loss\ (\%) = (Wu\ \times Nd) - (Wd\ \times Nu) \times \frac{100}{Wu}\ (Nd + Nu) \ldots\ldots\ldots\ldots ..\ldots (9)$$

Where:

$Wu = Weight\ of\ undamaged\ grain$

$Nu = Number\ of\ undamaged\ grain$

$Wd = Weight\ of\ damaged\ grain$

$Nd = Number\ of\ damaged\ grain$

4.4 Data analysis

4.4.1 Analysis of variance

The numbers of percentage weevil damage, grain weight loss, live and dead weevils were analyzed using GenStat 14th edition software to determine the difference in damage to the inbred lines and hybrids. Where genotype means were significant, they were compared using LSD. The data was also analyzed separately and combined for the three storage periods.

4.4.2 Heritability

Heritability was measured based on grain damage which is one of the measures of resistance to weevil damage. The error mean square (EMS) was considered as error variance (σ^2_e).

Genotypic variance (σ^2_g) was derived by subtracting error mean square (EMS) from the

genotypic mean square (GMS) and divided by the number of replications as given by the formula;

$$\sigma_g^2 = GMS - \frac{EMS}{r} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

Where:

$\sigma_g^2 = Genotypic\ variance$

$GMS = Genotypic\ mean\ squares$

$EMS = Error\ mean\ squares$

$r = Replications$

Phenotypic variance (σ^2_P) was derived by adding genotypic variance with error variance as given by the formula:

$$\sigma_p^2 = \sigma_e^2 + \sigma_g^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

Broad sense heritability was then calculated as:

$$H^2 b = \sigma_G^2 / \sigma_P^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

Where:

$\sigma_p^2 = Phenotypic\ variance$

$\sigma_g^2 = Genotypic\ variance$

4.4.3 Correlation Analysis

Pearson correlation coefficients were obtained using the GENSTAT 14th edition software. Correlations were computed to establish the interaction between grain weight loss, grain damage, live and dead weevils. Pearson correlation formula (r) was computed by the formula:

$$r = \frac{COV_{xy}}{\sigma x . \sigma y} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

4.5 Results

4.5.1 LGB damage of inbred lines

There were no significant differences in percentage damage caused by large grain borer (LGB) among inbred lines at 10 days (Table 4.1). After 10 days all inbred lines were undamaged and % damage was at 0.01% (Table 4.3). Significant differences in percentage damage were recorded among inbred lines at 60 days of storage (Table 4.1). At 60 days, mean damage was 42% while the damage ranged from 20 to 73% among the lines. Inbred lines, LEPOOL- 1/TZL2-2-1, CML312, and KEN2/TZL2-2-5# were less damaged than the resistant check at 60 days of storage. LEPOOL-1-TZL2-2-1 was the least damaged at 20.4 (Table 4.3). The resistant check, MTP0701 was damaged by 25.5% and the susceptible check DUMA 41 by 56.7%. RF291-10-5-3-9 was the most damaged line at 60 days of storage. The damage in this line amounted to 73% (Table 4.3).

The damage caused to inbred lines by LGB at 120 days of storage differed significantly (Table 4.1). LGB damage in inbred lines ranged from 48 to 75% at this storage period. KEN2/TZL2-2-5#, LEPOOL-1/TZL2-2-1 and CML312 were less damaged than the resistant check at this storage period (Table 4.3). Among inbred lines, the most damaged line was RF291-10-5-3-9 with damages of 75.2%. The susceptible check DUMA 41 was damaged by 72% (Table 4.3). There was significant difference in storage periods, inbred lines and the interaction of the two factors (Table 4.2).The highest damage was at 120 days of storage with a mean damage of 68%. It was also evident that the damage by LGB was more than Maize weevil in inbred lines at all storage periods. Mean maize weevil damage was at 33.6% and for LGB at 36.7% for all the 3 storage periods (Table 3.5 and 4.3).

Table 4.1: Mean square of resistant parameters at three different storage period among the inbred lines

Source	DF	10 days storage period				60 days storage period				120 days storage period			
				LLGB	DLGB	GD (g)	GWL	LLGB	DLGB	GD (g)	GWL	LLGB	DLGB
Replicatio	3	0.011738	0.000626	0.1889	0.275	591.02	432.295	2185.56	108.63	369.43	544.62	3484.1	283.1
Genotype	29	0.00543**n**	0.00022**n**	0.4954**n**	0.4716**n**	633.16*	549.833*	3004.42*	100.59	324.8*	602.28*	2874.1*	232.1**n**
Residual	87	0.005471	0.000272	0.4992	0.4991	140.04	123.2	1151.21	36.23	78.5	126.05	786	230.5
CV						0.37	0.24	0.26	0.25	0.25	0.32	0.37	0.34

Table 4. 2: Combined mean square at the 3 storage periods among inbred lines

Source	DF	GD (g)	GWL (g)	LLGB (count)	DLGB (count)
Replication	3	195.35	50.03	435.31	143.34
Genotype	2	5178.77*	4735.44*	12292.63*	30439.57*
Days of storage	29	247.53*	235.69*	380.41*	88.52**ns**
Genotype x Days of storage	267	98.21*	41.45*	147.35*	122.32**ns**
Residual	359	37.11	32.02	70.68	89.85

Significance level *= p<0.05; DF-Degree of freedom, GD-Grain damage, GWL-Grain weight loss, LLGB-Live Larger Grain Borer, DLGB-Dead Lager Grain Borer, ns- not significant

Table 4.3: LGB damage on inbred lines at the three storage periods

Inbred line	10 days	60 days	120 days
CML 222	0.0	34.6	67.7
CML 366	0.0	37.8	69.4
CML312	0.0	20.6	64.2
CML4	0.0	49.2	71.9
HIFIL-57	0.0	27.0	65.1
HIFIL-6	0.2	31.8	68.4
Katumani 11-2-1	0.0	48.9	70.4
Katumani 3-7-3	0.0	47.7	71.5
KEN2/TZL2-1-2#	0.0	53.5	72.5
KEN2/TZL2-2-3#	0.0	43.8	70.9
KEN2/TZL2-2-5#	0.0	21.2	48.0
KEN3/TZL2-2-6#	0.0	55.8	71.7
Kikamba 4-3-3	0.0	32.3	67.3
LEPOOL-1/TZL2-2-1	0.0	20.4	55.4
PIP2ENTRY 108	0.0	57.8	71.7
PIP2ENTRY 135	0.0	48.7	68.1
PIP2ENTRY 14	0.0	32.3	65.2
PIP2ENTRY 143	0.0	42.9	69.1
RF291 3-10-11-1	0.0	37.3	69.9
RF291-10-5-3-9	0.0	73.3	75.2
RF291-8-3-4-9	0.1	49.3	71.1
TZL-1/DIPLO-1-2-2#	0.0	45.7	70.5
TZL-1/DIPLO-1-2-3#	0.0	46.6	68.0
TZL2/MUG1-2-4#	0.0	55.6	70.7
TZL-2/MUG-1-2-5#	0.0	35.5	74.1
TZL-3/DIPLO-1-1-6#	0.0	32.8	68.6
TZL3/MUG4-1-10#	0.0	31.2	66.3
ZIMLINE/MORO/BC18-1-1	0.0	61.3	65.8
Checks			
MTP0701(Resistant)	0.0	25.5	64.9
DUMA 41(Susceptible)	0.0	56.7	72.3
Mean	**0.01**	**41.9**	**68.2**
%CV		37	25
LSD (Gen)		2.1	5.1
LSD (Days)		4.1	4.5
LSD(Days x Gen)		3.9	12.3

Gen= genotypes, days= number of storage days

4.5.2 Heritability of LGB resistance of inbred lines

Four variates; LGB damage (%), grain weight loss (%) and number of live and dead LGBs were measured in this study. Among the variates, broad sense heritability was estimated based on % LGB damage. Heritability in broad sense was 0.17 %, 47.2%

and 43.9% at 10, 60 and 120 days of storage. At all storage periods, genotypic variance was found to be less than environmental variance (Table 4.4).

Table 4.4: Heritability and phenotypic variance estimates of inbred lines for resistance to grain damages by LGB

	10 days	60 days	120 days
Environmental variance	0.00543	140.04	78.50
Genotypic variance (V_G)	0.0000093	123.28	61.58
Phenotypic variance (V_P)	0.00547	263.32	140.08
Heritability in Broad Sense (H^2)	0.17	46.81	43.96

4.5.3 LGB grain weight loss of inbred lines

There was no significant difference in grain weight loss among inbred lines after 10 days of storage. At this period of storage, weight loss among inbred lines was lower and averaged to 0.0% (Table 4.5). Significant differences in grain weight loss among inbred lines were recorded after 60 days of storage. Grain weight loss at this stage increased and ranged between 13.9 to 26.5%. Their mean weight loss was 20.3%. The highest grain weight loss was in the line, TZL2/MUG1-2-4# at 26.5% (Table 4.5). KEN2/TZL2-2-5# and LEPOOL-1/TZL2-2-1 registered lower weight losses than the resistant check, MTP0701 at 13.9 and 14.5% respectively. After 120 days of storage, inbred lines differed significantly in grain weight loss (Table 4.1). Mean grain weight loss at this storage period was at 41.61%. Duma 41 check lost 44.0% weight while MTPO701 lost up to 30% of weight. KEN2/TZL2-2-5# was consistently resistant in all the storage periods and lost more weight than the resistant check, MTP0701 (Table 4.5).

In this study weight loss by LGB was more than Maize weevil in inbred lines at all storage periods. This trend was similar in checks and hence they were more damaged by LGB than maize weevil. The susceptible check DUMA 41 fell into the moderately

susceptible category.

Table 4.5: Grain weight loss of inbred lines at the three storage periods

Inbred line	10 days	60 days	120 days	Remarks
KEN2/TZL2-2-5#	0.0	13.9	26.3	Resistant
LEPOOL-1/TZL2-2-1	0.0	14.5	31.5	Resistant
MTP0701(R)	**0.0**	**15.1**	**30.0**	**Resistant**
TZL3/MUG4-1-10#	0.0	16.2	46.4	Mod*
ZIMLINE/MORO/BC18-1-1	0.0	16.2	49.3	Mod*
CML 222	0.0	16.3	34.1	Mod*
CML312	0.0	17.4	33.7	Mod*
TZL-3/DIPLO-1-1-6#	0.0	17.4	47.9	Mod*
CML 366	0.0	17.9	36.1	Mod*
PIP2ENTRY 14	0.0	18.8	38.4	Mod*
HIFIL-57	0.0	19.0	35.6	Mod*
Katumani 3-7-3	0.0	19.2	38.7	Mod*
RF291 3-10-11-1	0.0	19.5	37.4	Mod*
Kikamba 4-3-3	0.0	19.5	40.0	Mod*
TZL-1/DIPLO-1-2-3#	0.0	20.3	40.8	Mod*
HIFIL-6	0.0	21.0	46.8	Mod*
PIP2ENTRY 108	0.0	21.3	43.0	Mod*
DUMA 41(S)	**0.0**	**22.0**	**44.0**	**Mod* S**
KEN2/TZL2-2-3#	0.0	22.0	44.2	Mod* S
CML4	0.0	22.3	41.2	Mod* S
TZL-2/MUG-1-2-5#	0.0	22.6	44.0	Mod* S
KEN3/TZL2-2-6#	0.0	22.7	43.6	Mod* S
PIP2ENTRY 143	0.0	22.9	48.3	Mod* S
RF291-10-5-3-9	0.0	23.3	45.4	Mod* S

PIP2ENTRY 135	0.0	23.8	45.1	Mod* S
KEN2/TZL2-1-2#	0.0	23.8	47.1	Mod* S
TZL-1/DIPLO-1-2-2#	0.0	24.0	48.0	Mod* S
RF291-8-3-4-9	0.0	24.2	45.2	Mod* S
Katumani 11-2-1	0.0	25.6	48.5	Highly S
TZL2/MUG1-2-4#	0.0	26.5	47.6	Highly S
Mean	**0.0**	**20.3**	**41.6**	
%CV		**24**	**32**	
LSD (Gen)		**3.2**	**7.1**	
LSD (Days)		**2.5**		
LSD (Days X Gen)		**2.2**		

Gen= genotypes, days= number of storage days, S = Susceptible, R = Resistant, Mod* = Moderately resistance, Mod* S = Moderately susceptible , Highly S = Highly susceptible

4.5.4 Number of live LGB in inbred lines

The numbers of live LGBs were analyzed at three storage periods. Inbred lines did not show significant differences in number of live LGBs after 10 days of storage. The numbers of live LGB were still similar to the number introduced at the start of the experiment. Therefore, the mean of live LGB was at 30 at this storage period (Table 4.6).

After 60 days of storage, inbred lines differed significantly in number of live LGBs. The live LGBs had increased in all inbred lines. KEN2/TZL2-2-5# registered the lowest number of live LGBs at 24, whereas ZIMLINE/MORO/BC18-1-1 had the highest number of live LGBs at 139 at this storage period (Table 5.4). DUMA 41 and MTPO701 had 61 and 43 live LGBs, respectively. Significant differences in number of live LGBs among inbred lines were also observed after 120 days of storage. At 120 days of storage, the highest number of LGBs was recorded and 23 lines had more than 100 LGBs (Table 4.6). CML312 and PIP2ENTRY 135 recorded lower numbers of LGBs than the resistant check at 85 and 87, respectively. In all inbred lines, number of live LGBs had increased except in RF291-10-5-3-9, Katumani 11-2-1 and PIP2ENTRY 143. In these lines LGBs had reduced to 102, 120 and 123 respectively (Table 4.6).

Table 4.6: Number of live LGBs at the three storage periods

Inbred lines	10 days	60 days	120 days
CML 222	29.5	58.0	126.0
CML 366	30.0	64.0	120.0
CML312	30.0	49.3	85.3
CML4	30.0	102.5	124.0
HIFIL-57	29.5	45.8	139.8
HIFIL-6	29.8	78.0	132.3
Katumani 11-2-1	28.8	138.5	120.8
Katumani 3-7-3	30.0	88.5	115.0
KEN2/TZL2-1-2#	29.5	110.5	147.5
KEN2/TZL2-2-3#	29.5	106.8	100.8
KEN2/TZL2-2-5#	30.0	23.8	82.3
KEN3/TZL2-2-6#	29.5	84.8	119.8
Kikamba 4-3-3	29.8	89.0	135.0
LEPOOL-1/TZL2-2-1	30.0	51.0	110.8
PIP2ENTRY 108	30.0	107.3	132.0
PIP2ENTRY 135	30.0	114.0	87.5
PIP2ENTRY 14	30.0	50.0	94.0
PIP2ENTRY 143	30.0	134.0	123.0
RF291 3-10-11-1	29.0	74.0	143.0
RF291-10-5-3-9	29.3	124.8	102.3
RF291-8-3-4-9	29.3	108.3	106.8
TZL-1/DIPLO-1-2-2#	29.3	131.5	132.5
TZL-1/DIPLO-1-2-3#	30.0	98.8	113.3
TZL2/MUG1-2-4#	29.8	124.0	179.8
TZL-2/MUG-1-2-5#	30.0	75.0	124.8
TZL-3/DIPLO-1-1-6#	30.0	65.5	130.3
TZL3/MUG4-1-10#	29.5	71.8	118.3
ZIMLINE/MORO/BC18-1-	30.0	139.0	184.5
Checks			
MTP0701	30.0	43.3	88.5
DUMA 41	29.8	61.8	133.8
Mean	**29.7**	**87.1**	**122.1**
CV (%)		**26**	**37**
LSD (Gen)		**9.8**	**22.1**
LSD (Days)		**12.9**	
LSD (Days x Gen)		**17.3**	

Gen= genotypes, days= number of storage days

4.5.5 Number of dead LGB in inbred lines

Dead LGBs were recorded at 10, 60 and 120 days of storage. After 10 days of storage, there was no significant difference in number of dead LGBs among inbred lines. LGBs were live in most inbred lines. Even so, these lines had lost only one LGB at this storage period.

After 60 days of storage, lines differed in number of dead LGBs. The number of dead LGBs varied from 8 to 29 at this storage period. The mean number of dead LGBs had increased to 17. The least numbers of dead LGBs were recorded in the susceptible check DUMA 41 while the highest number of dead LGBs was found in PIPENTRY143, KEN2/TZL2-2-1, and LEPOOL-1/TZL2-2-1 (Table 4.7).

After 120 days of storage, lines were not found to differ significantly in number of dead LGBs. However, mean number of dead LGBs had increased to 32. At this stage, the dead LGBs varied from 20 to 51 LGBs. Fifteen lines at 120 day storage period had 30 dead LGBs. The highest number of dead weevils was reported in PIPENTRY 108 and the check MTPO701 (Table 4.7).

Table 4.7: Number of dead LGBs at the three storage periods

Inbred lines	10 days	60 days	120 days
CML 222	0.5	21.0	26.8
CML 366	0.0	15.0	30.8
CML312	0.0	22.0	29.8
CML4	0.0	13.3	28.8
HIFIL-57	0.5	16.0	32.5
HIFIL-6	0.3	19.0	28.0
Katumani 11-2-1	1.3	15.8	40.8
Katumani 3-7-3	0.0	12.3	36.5
KEN2/TZL2-1-2#	0.5	16.8	23.0
KEN2/TZL2-2-3#	0.5	22.8	30.3
KEN2/TZL2-2-5#	0.0	27.8	28.8
KEN3/TZL2-2-6#	0.5	15.3	35.5
Kikamba 4-3-3	0.3	20.3	28.8
LEPOOL-1/TZL2-2-1	0.3	29.0	25.0
PIP2ENTRY 108	0.0	20.0	50.5
PIP2ENTRY 135	0.0	14.0	23.3
PIP2ENTRY 14	0.0	12.3	28.5
PIP2ENTRY 143	0.0	26.0	31.3
RF291 3-10-11-1	1.0	16.8	31.0
RF291-10-5-3-9	0.5	15.3	33.5
RF291-8-3-4-9	0.8	18.3	36.3
TZL-1/DIPLO-1-2-2#	0.8	14.5	27.8
TZL-1/DIPLO-1-2-3#	0.0	12.3	30.3
TZL2/MUG1-2-4#	0.3	14.0	48.8
TZL-2/MUG-1-2-5#	0.0	18.0	20.3
TZL-3/DIPLO-1-1-6#	0.0	21.8	29.0
TZL3/MUG4-1-10#	0.5	14.5	27.5
ZIMLINE/MORO/BC18-1-1	0.0	9.5	39.8
Checks			
MTP0701(Resistant)	0.0	14.0	51.3
DUMA 41(Susceptible)	0.0	8.3	29.5
Mean	**0.3**	**17.2**	**32.1**
CV (%)		**25**	**34**
LSD (Gen)		**4.1**	**9.2**
LSD (Days)		**6.2**	
LSD (Days x Gen)		**5.7**	

Gen= genotypes, days= number of storage days

4.5.6 Correlations

Correlation was done for four factors that were measured in this study. From the results represented in Table 4.8, correlations were significant at $P \leq 0.05$. All variates showed positive association. Also, correlation among these variates based on LGB infestation was relatively high compared with infestation of maize weevil. Correlation of weight loss and % LGB damage was 0.92, dead LGB and % damage was 0.97 and dead LGB with weight loss was 0.85 (Table 4.8). The association among other variates was relatively high($r<0.50$).

Table 4.8: Correlation coefficients of large grain borer (LGB) infestation on maize inbred lines

Parameter	LGB damage	Weight loss (%)	Dead LGB	Live
LGB damage	1			
Weight loss (%)	0.9155*	1		
Dead LGB	0.9668*	0.8529*	1	
Live LGB	0.8272*	0.7189*	0.5332*	1

*= significant at 5% probability level

4.5.7 LGB damage of hybrids

There was no significant difference among hybrids in terms of damage caused by LGB after 10 days (Table 4.9 and 4.10). Damage caused by LGB was negligible after 10 days of storage and therefore mean damage was zero. After 60 days of storage, significant differences were recorded among hybrids (Table 4.11). Average damage for hybrids was 30% at 60 days. Percent damage varied from 6 to 44%. The resistant check MTP0701 was least damaged at 6% while PAN 691 recorded the highest percent damage at 44. The susceptible check DUMA 41 was damaged by 33% (Table 4.11).

The damage among hybrids differed significantly after 120 days (Table 4.9). Mean damage of large grain borer in hybrids at this stage was 59%. At 120 days MTPEH200804 was less damaged than the resistant check and recorded mean damage of 39%. The resistant check had been damaged by 36% while the Susceptible Check Duma 41 was damaged by 56% (Table 4.11).

Table 4.9: Mean squares of resistant parameters at three different storage period among the Hybrids

Source	DF	10 days storage period				60 days storage period				120 days storage period			
		GD (g)	GWL	LLGB (count)	DLGB (count)	GD (g)	GWL (g)	LLGB	DLGB (count)	GD (g)	GWL (g)	LLGB (count)	DLGB (count)
Replication	3	0.000944	0.00000382	0.5104	0.5694	385.6	578.8	1503.4	12.514	1695.2	2765.8	14620	153.01
Genotype	23	0.00159**ns**	0.0000409	0.6698*	0.7808*	204.1*	287*	719.4*	7.259**ns**	472.8**ns**	761.2**ns**	6228*	79.74**ns**
Residual	69	0.00019	0.0000183	0.438	0.4535	104.7	142.5	406.8	6.833	481.4	787.3	4131	75.33
CV						0.22	0.27	0.3	0.2	0.23	0.24	0.25	0.32

Table 4.10: Combined mean squares at the 3 storage periods among Hybrids

Source	DF	GD (g)	GWL (g)	LLGB (count)	DLGB (count)
Replication	3	208.1	350	3071	82.69
Genotype	23	315.7ns	86355.2*	149216*	529.5*
Days of storage	3	56294.9*	498.8**ns**	3084*	42.32**ns**
Genotype x Days of Storage	46	180.6**ns**	274.7**ns**	1932**ns**	22.73**ns**
Residual	213	216.3	343.4	1654	27.94
CV		0.25	0.26	0.29	0.26

Significance level *= $p<0.05$

DF-Degree of freedom, GD-Grain damage, GWL-Grain weight loss, LLGB-Live Larger Grain Borer, DLGB-Dead Larger Grain Borer, ns- not significant

Table 4.11: Mean LGB damage in hybrids

Hybrid	10	60	120
DH01	0.0	25.8	65.9
DH04	0.1	36.4	60.3
DK8031	0.0	43.5	67.5
H513	0.1	26.8	72.5
H614D	0.0	34.2	66.9
KH 500-31A	0.0	42.2	65.5
KH 500-33A	0.0	26.0	52.3
KH 600-15A	0.0	38.6	41.9
KH 600-16A	0.1	35.6	64.9
KH 631Q	0.1	12.0	43.1
MTPEH200804	0.0	23.8	33.8
PAN 67	0.1	24.2	57.2
PAN 691	0.0	43.8	60.0
PH 4	0.1	23.7	52.3
PH1	0.0	33.3	67.5
PH3253	0.0	31.6	66.6
SC DUMA 41	0.1	36.5	59.1
SC DUMA 43	0.0	32.2	65.0
SC Simba 61	0.0	30.6	55.1
WH 403	0.1	31.9	73.5
WH 504	0.0	35.2	63.7
WH 505	0.1	22.9	70.0
Checks			
MTP0701	0.0	6.3	35.5
DUMA 41	0.0	33.3	55.8
Mean	**0.0**	**30.4**	**59.0**
%CV		**22.3**	**23.0**
LSD (Gen)		**9.4**	**10.8**
LSD (days)		**5.5**	**9.5**
LSD (days x Gen)		**4.3**	**8.2**

Gen= genotypes, days= number of storage days

4.5.8 Heritability of LGB resistance

Heritability was calculated based on % damage for each storage period. From the results, heritability estimates were – 6.5 %, 38% and 39% after 10 days 60 days and 120 days respectively (Table 4.12). At all storage periods, genotypic variance was lower than phenotypic variance.

Table 4.12: Heritability estimates and components of phenotypic variance of hybrids resistance to large grain borer (LGB)

	10 days	60 days	120 days
Environmental variance (V_E)	0.00034	48.15	93.57
Genotypic variance (V_G)	-0.00002	30.00	59.19
Phenotypic variance (V_P)	0.00032	78.15	152.77
Heritability in Broad Sense (H^2)	-6.5	38.39	38.75

4.5.9 LGB grain weight loss of hybrids

At 10 days, all hybrids had not lost weight, therefore, no differences were observed among the hybrids. Significant differences in grain weight loss among hybrids were recorded at the second storage period (60 days). At this stage, mean weight loss was at 26 % (Table 4.13). Weight loss ranged from 5% in the resistant check MTP0701 to 43% in PAN 691. The susceptible check DUMA 41 registered 28% weight loss. One hybrid, KH631Q, recorded weight loss of less than 15% and hence was designated as resistant. Eight genotypes were moderately susceptible and another 13 were highly susceptible including the susceptible check DUMA 41(Table 4.13).

The weight loss increased at the third storage period (120days) causing significant differences among hybrids. Mean weight loss at 120 days of storage was 49%. The resistant check MTP0701 registered the least weight loss at 18% whereas WH505 had the highest weight loss at 66%. The susceptible check had weight loss of 40 % (Table 4.13). Large grain borer caused more weight loss compared to maize weevil. For instance, mean weight loss caused by LGB was 49% and 40% for maize weevil at 120 days (Table 3.15 and Table 4.13).

Table 4.13: Mean number of grain weight loss caused by LGB in hybrids

Hybrid	10 days	60 days	120 days	Remarks
MTP0701	**0.0**	**5.0**	**17.6**	**Resistant**
KH 631Q	0.0	9.1	27.4	Resistant
PH 4	0.0	21.3	40.9	Mod*
KH600-16A	0.0	23.0	65.8	Mod*
MTPEH200804	0.0	23.2	24.0	Mod*
KH 500-33A	0.0	22.7	45.9	Mod*
H513	0.0	23.0	59.8	Mod*
PAN 67	0.0	23.9	46.5	Mod*
H614D	0.0	24.2	57.2	Mod*
DH01	0.0	25.4	60.6	Mod*
DUMA 41	**0.0**	**27.8**	**40.3**	**Highly susceptible**
SC Simba 61	0.0	27.9	48.7	Highly susceptible
PH3253	0.0	28.4	62.2	Highly susceptible
SC DUMA 43	0.0	29.5	53.2	Highly susceptible
WH 403	0.0	30.5	65.6	Highly susceptible
SC DUMA 41	0.0	30.9	56.5	Highly susceptible
WH 504	0.0	32.5	59.0	Highly susceptible
DK8031	0.0	33.1	54.5	Highly susceptible
WH505	0.0	34.0	57.7	Highly susceptible
PH1	0.0	34.3	53.1	Highly susceptible
DH04	0.5	37.1	51.5	Highly susceptible
KH 600-15A	0.0	37.6	29.4	Highly susceptible
KH 500-31A	0.5	39.5	56.2	Highly susceptible
PAN 691	0.0	42.9	48.6	Highly susceptible
Mean	**0.1**	**27.8**	**49.3**	
%CV		**27.4**	**24.1**	
LSD (Gen)		**5.6**	**9.2**	
LSD (Days)		**2.1**		
LSD (Gen x days)		**3.2**		

Gen= genotypes, days= number of storage days, Mod* = Moderately susceptible

4.5.10 Number of live LGBs in hybrids

The numbers of live LGBs insects were unchanged after 10 days. However, at 60 days significant differences were recorded. Live LGBs increased at this storage period (4.14). Mean number of LGBs at this period was 51. The most live LGBs were found in DK8031 (4.14). This hybrid had 73 live LGBs. The least number of LGBs was found in the resistant check MTP0701. The susceptible check registered 54

LGBs.

Hybrids also showed significant differences in live LGBs at 120 days of storage. The numbers of live LGBs were most observed at this storage with a mean of 68 insects. The number of LGB insects ranged from 32 in the resistant check to 121 (Table 4.14). Among the hybrids, at least 17 had more than 50 insects. PH3253 and PAN691 had the most live insects totaling to 121 each. In MTP0701, PH 4, DH04, PH1 WH 505 and DK8031 the number of LGB reduced from day 60 to 120(Table 4.14).

Table 4.14: Number of live LGBs in hybrids

Hybrid	**10 days**	**60 days**	**120 days**
DH01	29.5	47.8	69.0
DH04	29.3	43.8	34.5
DK8031	30.0	73.3	57.8
H513	29.0	54.3	98.5
H614D	29.8	62.3	71.3
KH 500-31A	29.3	49.0	68.0
KH 500-33A	29.8	47.8	60.5
KH 600-15A	29.8	62.0	63.5
KH 600-16A	29.8	54.0	90.5
KH 631Q	29.8	46.8	48.8
MTPEH200804	30.0	41.0	61.0
PAN 67	30.0	57.0	106.3
PAN 691	29.5	49.0	121.5
PH 4	29.3	52.8	33.5
PH1	29.5	43.0	36.0
PH3253	29.5	52.8	120.8
SC DUMA 41	29.3	61.8	68.8
SC DUMA 43	29.5	49.3	85.0
SC Simba 61	30.0	49.8	79.0
WH 403	29.8	43.8	50.5
WH 504	30.0	52.0	89.0
WH 505	29.3	48.8	43.0
Checks			
MTP0701	29.8	36.5	32.8
DUMA 41	29.8	54.3	70.5
Mean	**29.6**	**51.3**	**68.3**
%CV		**30.2**	**24.8**
LSD (Gen)		**7.3**	**12.8**
LSD (Days)		**6.5**	
LSD(Days x Gen)		**5.7**	

Gen= genotypes, days= number of storage days

4.5.11 Number of dead LGB in hybrids

No LGB died at 10 days of storage Table 4.9. A number of LGBs had died by 60 days of storage and varied between 8 to 19 insects (Table 4.15). At 120 days, the mean number of dead LGBs increased to 27. MTP0701 and DUMA 41 had 20 and 28 dead LGBs at 120 days of storage respectively.

Table 4.15: Number of dead LGBs in hybrids

Hybrid	After 10 days	After 60 days	After 120 days
DH01	0.5	13.0	41.5
DH04	0.8	14.5	36.0
DK8031	0.0	14.5	30.0
H513	1.0	12.3	23.0
H614D	0.3	8.0	33.0
KH 500-31A	0.8	11.5	27.3
KH 500-33A	0.3	13.5	28.0
KH 600-15A	0.3	18.5	35.0
KH 600-16A	0.3	11.5	21.3
KH 631Q	0.3	13.5	25.3
MTPEH200804	0.0	15.0	24.0
PAN 67	0.0	15.0	33.5
PAN 691	0.5	11.3	19.3
PH 4	0.8	14.3	28.0
PH1	0.5	16.3	24.8
PH3253	0.5	14.3	28.5
SC DUMA 41	0.8	10.5	26.8
SC DUMA 43	0.5	10.3	23.8
SC Simba 61	0.0	14.0	33.5
WH 403	0.3	12.3	22.0
WH 504	0.0	13.0	28.3
WH 505	0.8	13.5	27.0
Checks			
MTP0701	0.3	10.8	20.3
DUMA 41	0.3	14.8	28.0
Mean	**0.4**	**13.2**	**27.4**
%CV		**20.0**	**32.2**
LSD (Gen)		**7.2**	**11.2**
LSD (Days)		**3.2**	
LSD (Days x Gen)		**3.7**	

Gen= genotypes, days= number of storage days

4.6 Discussions

4.6.1 LGB damage in inbred lines and hybrids

Grain % damage for both inbred lines and hybrids was found to be significant at 60 and 120 days. Percent damages were considerably high in most genotypes indicating that damage by LGB was propagated at these storage periods. It was noted that some inbred lines and hybrids showed resistance to LGB relative to the resistant check because they had lower % damages than the resistant check. Kumar (2002) and Tefera *et al.* (2010) reported that grains that showed a high level of resistance had lesser grain damage relative to a susceptible host. According to Suleiman and Kurt. (2015), large grain borer (*Prostephanus truncatus*) causes damages as high as 60% on untreated maize in East Africa after 2 to 3 months of storage. In this study, grain percent damage was significant at 60 and 120 days of storage. According to Nhamucho *et al*., 2014 much grain damage are observed in maize varieties which are susceptible and hence allow development of more LGBs. The low percentage of grain damage among the resistant genotypes indicates that they have genes that confer resistance to the LGB. According to Tefera *et al*. (2011), the level of damage and weight loss during storage is strongly correlated with the number of adult insects.

4.6.2 Heritability of LGB resistance in inbred lines and hybrids

Heritability estimates were relatively moderate in inbred lines and ranged from 0 to 47%. This moderate heritability reveals slow progress in selection for these traits. The low heritability is also because resistance to LGB is due to both non additive and additive effects (Matewele, 2014). However, inheritance to most insect pests focuses on antibiosis. Therefore it will be necessary to identify the biochemical and physical traits that confer the antibiosis in the lines. Biochemical components include phenolic

acids, hydroxyproline-rich glycoproteins, sugars, soluble peroxidase and protein inhibitors (García-Lara *et al*., 2004). Among physical factors, grain hardness has been found to confer resistance. Therefore, selection for resistance to LGB in early generation should focus on these biochemical and physical components to achieve more progress during breeding. Genotypic variance was also found to be low than the environmental variance indicating high influence of environment on these lines.

Heritability to LGB resistance in hybrids was found to be low which indicates low progress in selection for studied traits for LGB resistance. According to Mwololo *et al*. (2012) heritability values were high (70% to 90%) in the maize inbred lines they studied. This may be due to environmental influence which is also revealed by the high environmental variance than the genotypic variance (heritable component).

4.6.3 LGB grain weight loss in inbred lines and hybrids

Inbred lines varied significantly to weight loss caused by large grain borer which shows that there are genetic differences among the inbred lines. From the results, no weight loss was visible after 10 days showing that the feeding activity of LGB was still slow. Selection of genotypes was based on a standard criterion of selection (Mwololo *et al*., 2012; Tadele *et al*., 2011). In this study KEN2/TZL2-2-2# and LEPOOL-1/TZL2-2-1 were selected as the resistant inbred lines as they exhibited the least weight loss. KH631Q was selected as a resistant hybrid. Therefore parents of this hybrid could be utilized to breed for resistant varieties. Mwololo *et al*., (2012) and Tadele *et al*. (2011) also identified resistant genotypes against LGBs. Previous reports (Nhamucho *et al*., 2014; Mwololo *et al*., 2010) have shown LGB to cause serious weight losses on stored maize within a short period of 2 months. The extensive tunnelling in maize grain by *P. truncatus* adults characteristics allows it

to convert grain into flour within a very short time. These findings are in agreement with the current study, since after 2 months of storage, the genotypes under investigation exhibited extensive weight losses. Therefore, the chemical and physical factors of the maize substances which possibly confer antibiosis on these varieties may explain the resistance in these genotypes. Previous studies by Kumar, (2002); Garcia-Lara *et al*. (2004); Derera *et al*. (2011) show that chemical factors in resistant varieties tend to inhibit adult growth and development thereby supressing emergence of new weevils and reducing damages and consequently weight losses.

Considering that many traits are involved in insect resistance (Mwololo *et al*., 2012), a multi-trait breeding approach to LGB resistance breeding should be highly considered. Therefore, identifying the physical and biochemical factors that enhance antibiosis in the resistant lines can lead to a successful LGB resistance breeding programme.

4.6.4 Number of live and dead LGBs

Numbers of live LGB were found to be constant after 10 days. However, as the storage period extended live LGBs increased greatly. Most lines had more live LGBs at 120^{th} day. It was therefore evident that resistance in these lines could not be attributed to biochemical properties which retard growth and development of adult insects (Matewele, 2014). However KEN2/TZL2-2-5# had the least number of live LGBs than any other line after 60 and 120 days and hence experienced the least damage. This line was thus portrayed as an unfavorable host of LGB. The life cycle of LGB development from larvae to adult is 27 days. However in KEN2/TZL2-2-5#, this developmental period was delayed probably because the host was not favorable to the pest. This gives an assurance of existence of resistance in KEN2/TZL2-2-5#.

The lower number of living LGBs indicated resistance of this line to LGB damage and this is due to the fact that the insects could not feed and reproduce more adults. Abraham (1991) reported that damage severity on stored maize was dependant on the number of emerging adults and the duration of each generation.

As expected hybrids which harbored more live LGBs had the most damages than those with few insects. Therefore, adult insects were able to feed and reproduce more insects in susceptible hybrids. Report by Tefera *et al*. (2011) revealed that smallest insect densities (about 5 insects) can cause high grain damages and losses. High number of dead LGB's was observed in resistant hybrids indicating that the insects could not reproduce and feed therefore suggesting antibiosis type of resistance.

4.7 Conclusions

It is important to note that from the collection of genotypes studied, there exist both resistant and susceptible genotypes to *P. truncatus* attack. KEN2/TZL2-2-5#, LEPOOL-1/TZL2-2-1 and KH 631Q were among the resistant genotypes identified. The information on the resistant status of these varieties will help breeders in developing resistant varieties and also release to farmers for planting purposes.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

In this study, 10 days after infestation, both maize weevils and LGBs recorded no damages to the genotypes. This explains the possibility of storing maize for short term duration of up-to 2 weeks after harvest without serious damage experiences. However, beyond 10 days, damages may be severe as shown in genotypes stored for 120 days where maximum insect damage was recorded. This shows resistance alone was not enough to suppress insect pest population build up but it can reduce losses and contribute to integrated pest management since no maize grain was immune to attack by insect pests stored for longer periods. Reducing such losses could be enhanced through utilizing resistant hybrid varieties by farmers as demonstrated in this study. There were similarities in genotypic responses to grain damage by both maize weevils and larger grain borers. Inbred line KEN2/TZL 2-2-5 and hybrid KH 631Q emerged resistant when exposed to both insects. There inherent resistance describes their unique biochemical and physical properties which possibly confer antibiosis therefore explaining their resistant natures. Further analysis on these 2 genotypes should be conducted by breeders to identify and exploit their unique characteristics. Resistance in these 2 genotypes can also probably be attributed to pericap surface texture, nutritional factors such as amylose, lipid and protein content or non-nutritional factors like phenolic compounds. Moderately resistant varieties in this study for both insects should also not be ignored. There moderate ability to resist these insect pests indicates that they can be improved to attain resistant or near resistant status. This can be done through recurrent selection followed by inbreeding in order to come up with pure inbred lines. Test cross and S3 recurrent selection

methods under artificial infestation can also be adopted in order to improve these populations. Genetic engineering can also be exploited in an effort to impact favorable characteristics through gene transfer hence improve their performance. Breeders should also critically examine these genotypes for essential characteristics such as yield, protein content and disease resistance. This is to ensure wholesome inclusivity of essential properties in these genotypes

5.2 Conclusion

The present study reported the existence of sufficient variation for weevil and LGB resistance among the studied genotypes. In this study, 1 resistant inbred line, KEN2/TZL2-2-5 and 1 resistant hybrid namely KH631Q to maize weevils and larger grain borer were identified. This offers a great opportunity to exploit the variability with the aim of reducing post-harvest insect-pest losses through genetic improvement.

5.3 Recommendations

1. The selected resistant line should be regarded as potential sources of weevil and LGB resistance and thus be utilized in breeding resistant maize varieties.
2. The selected resistant hybrid against weevils and LGB should be released to farmers in Makueni County for planting purposes.

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