Diversity Analysis and Breeding for Maize Weevil (*Sitophilus zeamais* Motschulsky) and Larger Grain Borer (*Prostephanus truncatus* Horn) Resistance in Productive Maize Germplasm in Malawi

By

Macpherson Baxton Matewele

BSc (Crop Science), MSc Plant Protection (Pathology), Bunda College of Agriculture, University of Malawi

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African Centre for Crop Improvement

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

University of KwaZulu-Natal

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Thesis Abstract

Maize (*Zea mays* L.) is the main staple food crop in Malawi grown by 97% of small holder farmers. However, the potential maize yields are reduced by postharvest losses of grain in storage due to the larger grain borer (*Prostephanus truncatus* Horn) and maize weevil (*Sitophilus zeamais* Motschulsky). Limited research is conducted to improve larger grain borer and maize weevil resistance in productive varieties and to exploit their genetic potential for insect resistance breeding programmes. Little is also known about the existing genetic diversity among local maize varieties in Malawi, which is critical for selection of parents for such breeding programmes. In addition, the sustainability of insect resistant materials in farmers' agro-environments depends on their performance in the field and on farmers' perceptions on the varieties. Studies were conducted in Malawi between 2012 and 2014 focusing on genetic diversity analysis and breeding for maize weevil and larger grain borer resistance in productive maize germplasm. The objectives of the study were to: i) identify farmers' perceptions on yield, maize production constraints and storability of local maize varieties; ii) determine genetic diversity of the potential breeding sources for use in introgressing larger grain borer and maize weevil resistance genes in farmer-preferred local varieties; iii) determine levels of larger grain borer and maize weevil resistance in local maize varieties; iv) determine the value for cultivation of larger grain borer and maize weevil resistant hybrids, as reflected by combination of high productivity and stability, under farmer representative conditions in multi-location trials representing the target production environments in Malawi; v) estimate general combining ability (GCA) and specific combining ability (SCA) between maize lines and their hybrids for grain yield and resistance to larger grain borer and maize weevil.

Results of participatory rural appraisal showed that both hybrids and local varieties are grown by farmers. Maize hybrids are cultivated mainly because of their high yield potential and early maturity than local varieties, while local maize varieties are grown due to good tolerance to pests and diseases, large cobs, large grain size, good yields under low soil fertility, white color, superior poundability, drought tolerance and high storability than hybrids. Grain hardness, grain size, grain color, poundability and grain texture were the main characteristics used by farmers to select maize varieties tolerant to maize weevil and larger grain borer. The study indicated that farmer requirements should be incorporated in the conventional breeding programmes in Malawi. Storability and other traits should be bred in hybrids that are preferred by farmers.

Diversity analysis revealed that phenotypic variation exists among local maize varieties largely due to kernel weight, plant height and ear placement. Phenotypic data produced eight clusters. SSR markers revealed 97.56% polymorphism among the loci. A total of 165 alleles were detected, with a range of 2-9 alleles and an average of four (4) alleles per locus. The mean gene diversity (H_e) of 0.5115 and mean heterozygosity (H_o) of 0.5233 were observed. The furthest genetic distance of 0.9001 was between local varieties 206 and local 2 and the closest genetic distance of 0.2190 was between local varieties 203 and 811. SSR marker data revealed ten clusters. Both phenotypic and genotypic data support observation of large diversity and variation among open pollinated maize varieties and landraces, which could be exploited by the breeding programme in Malawi.

The analysis of resistance for maize weevil (MW) among local maize varieties showed that 14.5% of the varieties were resistant, 21.7% were moderately resistant, 24.6% moderately susceptible, 23.2% susceptible and 16% highly susceptible. Maize varieties, such as, 1772, 1983, 1992, 3243, 3244, 750 and 752 were resistant to maize weevil. For larger grain borer (LGB), all maize varieties were susceptible. However, varieties 1992, 2012, and 1983, representing Five (5) percent of the entire maize population had reasonable levels of resistance against large grain borer. Varieties 1992 and 1983 also showed high levels of resistance against maize weevil, qualifying them as suitable candidates for use in stacking MW and LGB resistance in new hybrids.

Designed crosses to combine for maize weevil and larger grain borer resistance in adapted maize lines resulted in the development of 4 to 67% maize weevil resistant hybrids and 4 to 9% larger grain borer resistant hybrids across sets. Stacking of maize weevil and larger grain borer resistance produced 67% maize weevil resistant hybrids, 14% larger grain borer resistant hybrids and 14% maize hybrids with resistance to both larger grain borer and maize weevil. Maize hybrids, MWA06A showed a yield potential of 10 tons/ha, MWMW15106 (9.07 tons/ha) and MWA10A (7.69 tons/ha) and useful resistance to maize weevil. Maize hybrids, lgMW087940 expressed a yield potential of 11.05 tons/ha and MWlg06264 (8.16 tons/ha) and good resistance to both maize weevil and larger grain borer. This demonstrated that the LGB and MW resistance genes can be incorporated into productive cultivars.

Analysis for gene action among maize weevil and larger grain borer resistant F_1 maize hybrids revealed that both additive and non-additive gene action were responsible for determining weevil resistance. Only additive gene action was responsible for grain yield in maize weevil resistant hybrids. For larger grain borer, additive gene action was responsible for both resistance and grain yield in the F_1 maize hybrids. This indicated that both selection and hybridisation strategies would be effective for breeding MW and LGB resistance in productive maize germplasm.

The study has demonstrated that maize breeding in Malawi should focus at developing both hybrids and local varieties that meet different needs and traits preferred by farmers. Storability is one of such important traits in local maize varieties. The expressed genetic variation in local maize varieties provides an opportunity to explore for new sources of resistance to maize weevil and larger grain borer. The variation observed in resistance against maize weevil and larger grain borer among local varieties can be exploited to develop new populations or improve resistance in productive maize populations. Breeding for high yielding insect resistant maize hybrids is achievable and would provide a sustainable way of reducing postharvest grain losses in storage and improve net gain in maize yields for smallholder farmers in Malawi. The stacking of maize weevil and larger grain borer resistance in single maize hybrids would offer an effective way of breeding for insect pest resistance to both maize weevil and larger grain borer .The preponderance of additive gene effects over dominance gene effects in the maize hybrids gives a practical option for selection to enhance resistance and grain yield among productive maize germplasm. The exceptional hybrids, which combined high grain yield potential with high levels of resistance to MW and LGB, will be advanced in the breeding programme in Malawi. Overall, findings from the completed research will be useful for devising effective strategies in breeding programmes that emphasize grain resistance to LGB and MW and to those that seek to incorporate selection for these principal postharvest pests in the conventional breeding programmes.

Declaration

I, Macpherson Baxton Matewele, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research work.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
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Signed

____

Macpherson Baxton Matewele (Candidate)

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

____

Prof. J. Derera (Supervisor)

____

Prof. H. Shimelis (Co-supervisor)

Dedication

I dedicate this work to the omnipotent God of my father and to my people for their support and understanding.

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List of Abbreviations

ACCI	African Centre for Crop Improvement
ADD	Agricultural Development Division
AFLP	Amplified Fragment Length Polymorphism
AGRA	Alliance for a Green Revolution in Africa
AMMI	Additive Main Effects and Multiplicative Interaction Effects
ANOVA	Analysis Of Variance
CIMMYT	International Maize and Wheat Improvement Centre
DMS	Department of Meteorological Services
DNA	Deoxyribonucleic Acid
DSI	Dobie Index of Susceptibility
FAO	Food and Agriculture Organisation
GCA	General Combining Ability
GDP	Gross Domestic Product
GEI	Genotype and Environment Interaction
GLS	Grey Leaf Spot
IFPRI	International Food Policy Research Institute
IPCC	Intergovernmental Panel on Climate Change
IPM	Intergraded Pest Management
LGB	Larger grain borer

MDP	Median Development Period
MDS	Multidimensional Scaling
MET	Multiple Environmental Trial
MPVA	Malawi Poverty Vulnerability Assessment
MSV	Maize Streak Virus
MW	Maize weevil
OPV	Open Pollinated Variety
PC	Principal Component
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PRA	Participatory Rural Appraisal
RAPDS	Random Amplified Polymorphic DNA
REML	Residual Maximum Likelihood
RFLP	Restriction Fragment Length Polymorphism
SCA	Specific Combining Ability
SPSS	Statistical Package for Social Scientists
SSR	Simple Sequence Repeat
UPGMA	Unweighted Pair –Group Method with Arithmetic Averages

Thesis Introduction

1.0 Importance of maize in Malawi

Maize (*Zea mays* L.) is one of the most important food crops grown in Malawi. It is grown by 97% of small holder farmers, covering approximately 70% of the arable land (Ngwira, 2001; Denning et al., 2009). In 2012, maize ranked among the top three food crops grown in the country, with an estimated yield production of 3618699 metric tonnes (FAO, 2014) (Table 1).

Table 1: Crop production in Malawi (2012)

Rank	Crop	Production (Int $1000)	Production (mt)
1	Cassava	490162	4692202
2	Potatoes	482771	4152204
3	Maize	427154	3618699
4	Sugar cane	91944	2800000
5	Bananas	107020	380000
6	Plantains	74325	360000
7	Groundnuts, with shell	160886	268081
8	Pigeon peas	111695	237210
9	Fruit, freshnes	80278	230000
10	Vegetables, freshnes	40609	215500

Source: (FAO, 2014)

Before 2006, maize production in Malawi had generally been low. As a response to perpetual decline in maize production, in 2005/2006 growing season, the Government of Malawi introduced the input subsidy programme through which smallholder farmers accessed improved seed and fertilizer. This intervention, coupled with good rains, resulted in the country realizing a surplus of 510,000 tonnes in the 2005/2006 growing season (Denning et al., 2009). Since then, Malawi has dramatically experienced unprecedented increase in food production, especially maize (Figure 1).

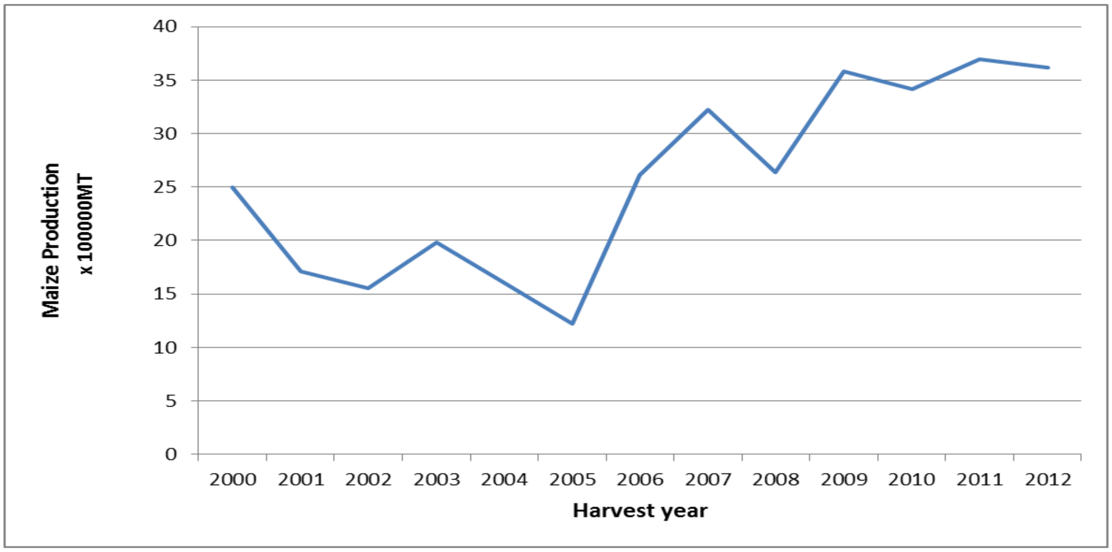

Figure 1: National maize yields in Malawi from 2001 to 2012. Data source: FAO (2014)

This increase in food production has enabled the country attain food sufficiency at the national level. However, there are still food shortages at the household level in many parts of the country due to different factors including plant diseases, insect pests, weeds, low soil fertility, soil acidity, soil erosion, climatic change, low rainfall, season length, high cost of farm inputs, insufficient maize materials that can withstand different production stresses, drought and post-harvest losses resulting from insect pest damage in storage (Zambezi, 1993; Sanchez et al., 1997; CIMMYT, 2000; Ngwira, 2001; Pingali and Pandey, 2001; Dorward et al., 2008; FAOSTAT, 2008; Makoka, 2008; Denning et al., 2009).

2.0 The problem of postharvest grain losses

Farmers in Malawi still use traditional methods and structures of keeping grain (Figure 2). Under such conditions, maize grain is more vulnerable to serious attacks from rodents, birds, micro-organisms and insects (Nukenine, 2010). For example, between 2004 and 2012, postharvest weight losses of maize grain in Malawi ranged from 19.3 to 22.5% (APHLIS, 2015). Damage caused by insects is a challenge for an African farmer and leads to loss after production (Mugo et al., 2002). The larger grain borer, *Prostephanus truncatus* Horn and the maize weevil, *Sitophilus zeamais* Motschulsky are the most important insect pests associated with grain storage (CIMMYT, 2000). *P. truncatus and S. zeamais* have most severe effects on grain damage and grain weight loss (Danjumma et al., 2009). Preservation of grain can only be possible if damage from pests is minimised (Bergvinson and Garcı´a-Lara, 2004). Sustainable strategies that are environmentally sound are required to protect farmers' harvest and the use of host resistance (resistant varieties) is the easiest, safest, most effective and economical way of controlling insect pests on stored grain (Ahmed and Yusuf, 2007).

Figure 2: Traditional storage structure for maize in Salima district (Central Malawi)

3.0 The larger grain borer (*Prostephanus truncatus* Horn)

The larger grain borer (LGB) is the single most important field and storage pest of dried cassava and maize in Africa (Farrell and Schulten, 2002) (Figure 3). LGB causes a wide range of grain losses in maize, which include: weight loss, nutritional loss, loss in grain quality, loss of seed viability, and loss of commercial value (McFarlane, 1989). Postharvest losses in susceptible varieties can range from 40 to 100% (Mushi, 1990; CIMMYT, 1999; Denning et al., 2009). However, according to APHLIS (2015), in Africa, between 2003 and 2014 postharvest weight loss of maize grain ranged from 16.8 to 19.9%. For Malawi, in 2012, postharvest losses due to larger grain borer was estimated at 1.2%, translating to a loss of 47000 tonnes of the total maize yields realised in the country (APHLIS, 2015).

Figure 3: Larger grain borer (*Prostephanus truncatus* Horn)

Source: Dr Werener–Freyberg Strasse (2012) (modified)

The larger grain borer in Malawi was first detected in the northern district of Karonga which borders with Southern Tanzania in 1991. Ever since, the pest has spread to many districts in the country (Binder, 1992). It has now become a major pest of maize in Malawi (Ching'oma, 2009), especially in the storage facilities (Makoka, 2008; Singano et al., 2009). The negative effect of LGB on maize grain in Malawi was so apparent, for instance, between 1995 and 2001, the weight loss of stored maize due to LGB increased from 5 to 16% (Singano et al., 2009). To reduce the spread of the pest in the country, an integrated pest management (IPM) strategy was put in place. The strategy comprised of bio-control agents, field and storage facility inspections, traps and chemicals (Paliani and Muwalo, 2001). However, the main focus of this strategy has been the use of chemical compounds, such as Actellic Super, a mixture of pirimophis methyl and permethrin, and biological agent (histerid beetle, *Teretrius nigrescens* Lewis) (Ching'oma, 2009). Despite the availability of Actellic Super and *Teretrius nigrescens*, larger grain borer is still on the increase in the country (Singano et al., 2009). In view of this, a more holistic IPM approach in LGB control is required, which among other aspects, necessitates integrating host resistance as part of the IPM strategy against LGB. Unfortunately, not much progress has been made in identifying genetic resistance of maize grain to storage insects (Derera et al., 2000). For instance, Kasambala (2009) evaluated the susceptibility of nine commercial maize hybrids, namely SC403, SC407, SC513, SC627, SC717, DK8033, DK8053, DK8073, MH18 and a local variety “Kanjerenjere” to LGB in Malawi. All the nine hybrids were found to be susceptible and only the local variety was resistant (Kasambala, 2009). The resistance of landraces to LGB has also been reported in other regions. Kumar (2002) reported the availability of 19 landraces from the Caribbean which showed resistance to LGB after undergoing selection. Nhamucho et al. (2014) reported the existence of LGB resistance among maize genotypes in Mozambique. The resistance observed was attributed to antibiosis (Kumar, 2002; Nhamucho et al., 2014).

Successes in breeding for maize materials with resistance to LGB have also been reported in Kenya through CIMMYT supported programmes. These materials would be available for use in other countries (Anonymous, 2008). Furthermore, Mwololo et al. (2010) reported existence of genetic diversity for resistance among maize materials against LGB in Kenya. According to Mwololo et al. (2010), genetic diversity is important in understanding different mechanisms responsible for host plant resistance and provides a platform for developing breeding materials with resistance to storage pests. Ndiso et al. (2007) also reported variation for resistance to LGB among varieties in Kenya. However, there are no available reports indicating the type of gene action responsible for the resistance. This was pursued in the study.

In Malawi, apart from the work reported by Kasambala (2009) on susceptibility of ten maize varieties to larger grain borer, no comprehensive work has been done to determine the extent of genetic diversity for LGB resistance among maize varieties. These initiatives and information offer an opportunity and thrust for Malawi to explore genetic diversity for LGB resistance among open pollinated maize varieties and further develop maize varieties with substantial resistance to larger grain borer. Development of materials with better pest and disease resistance is of paramount importance for Malawi (Denning et al., 2009), considering that maize is the staple food for millions of people in the country. For instance, in 2011, direct maize consumption in Malawi was around 2.03 million tones, representing 69% of the total maize produced. The average annual maize consumption per individual was around 131.2 kg, providing 1142 kcal/day (FAOSTAT, 2014).

4.0 Maize weevil (*Sitophilus zeamais* Motschulsky)

The Maize weevil (MW) is an important pest in developing countries with yield losses ranging from 5 to 15% (Tigar et al., 1994). Pest infestation starts in the field and is carried over to the storage facility (CIMMYT, 2000). In the tropics, yield losses of more than 80% have been reported, especially in untreated grain in storage facilities (Pingali and Pandey, 2001). Maize weevil is a major pest of maize in Malawi (Kamanula et al., 2011) (Figure 4).

Figure 4: Maize weevil (*Sitophilus zeamais* Motschulsky)

Source: Dr Werener–Freyberg Strasse II (2012) (modified)

The control of maize weevil has depended much on the use of chemical products (pesticides), which are mostly beyond the reach of smallholder farmers (Dhliwayo and Pixley, 2003). Plant resistance can be incorporated as part of integrated pest management strategy for controlling maize weevil. For example, apart from the responsible use of chemical pesticides as part of IPM strategy, the combination of weevil resistance with plant and grain characteristics, such as husk cover and grain flintiness can improve resistance to maize weevil (Kim and Kossou, 2003).

Improvement of resistance against maize weevil in maize materials is possible (Dhliwayo and Pixley, 2003) as variation for weevil resistance among maize genotypes exists. Abebe et al. (2009) found variation for resistance levels among Ethiopian maize varieties. Derera et al. (2000) reported variation for resistance against maize weevil among genotypes from Southern, Eastern and Western Africa. This resistance has been attributed to the presence of phenolic compounds in grains that confer both mechanical and antibiosis resistances (Arnason et al., 1992; Derera et al., 2000).

Unfortunately, no work has been done in Malawi to determine the extent of genetic diversity of maize weevil resistance in Malawian germplasm. Studies have also shown that additive gene actions, dominance gene action, and maternal effects play important role in maize weevil resistance (Derera et al., 2000; Kim and Kossou, 2003). However, not much has been done to exploit this genetic variation for breeding programmes for the development of maize weevil resistant materials (Dhliwayo and Pixley, 2003) and no varieties have been released on the basis of weevil resistance (Derera, personal communication). This is partly explained by not incorporating insect resistance in a conventional breeding programme. Pest resistance increases yield and differential reaction of genotypes to maize weevil can be exploited for breeding purposes (Kitaw et al., 2001) as such, breeding for maize weevil resistance is a practical option.

5.0 Performance of insect resistant maize varieties under farmers' conditions

The sustainability of insect resistant materials in farmers' agro-environments depends on their performance in the field and farmers' perceptions about the varieties. Previous studies had revealed differences in agronomic performance of insect resistant maize hybrids in the field (Tefera et al., 2012) and an increase in acceptability of varieties by the farmers through incorporation of farmers' views and knowledge in breeding programmes (Mukanga et al., 2011). It is therefore, imperative that LGB and MW resistant materials be productive (high yielding) and performs well under prevailing farmers' conditions. Differences in environmental and climatic conditions affect yields of maize mainly due to the differential reaction of genotypes to environmental factors, such as soil nutrients, light, pests, diseases, drought and physical injury (Yan and Kang, 2003; Mekonnen and Mohammed, 2009). Low N and drought are the most important stress factors affecting maize production in Eastern and Southern Africa (Bänziger and Diallo, 2001). Low soil fertility and drought conditions have a huge impact on maize productivity (Zambezi, 1993; FAOSTAT, 2008).

Due to continuous cultivation, without nutrient replenishment, soils under smallholder cultivation manifest serious nitrogen, phosphorous and potassium depletion (Sanchez et al., 1997). Low and declining soil fertility greatly contributes to low yields. Therefore, the use of nutrient utilization efficient cultivars becomes a prerequisite (CIMMYT, 2000). Furthermore, the majority of smallholder farmers in Malawi depend on rainfed agriculture, as such their farming system is prone to fluctuating maize production due to drought (Denning et al., 2009). The vulnerability of Malawians to drought cannot be overemphasized, for example, during the 2004/2005 growing season, the country experienced a drought that resulted in 36% reduction in maize production (FAOSTAT, 2008) and over five million Malawians survived on food aid (Makoka, 2008). In addition, some parts of Malawi, especially the low to mid altitude areas receive less than 50% of the national average rainfall (DMS, 2008). These areas are associated with relatively high temperatures, accompanied by dry spells (DMS, 2008). The case in point is Chikwawa (one of the districts in Shire Valley), where drought caused irreversible damages to maize crops in the 2009/10 growing season and most farmers had to re-plant. As a result, some farmers were planning to stop growing maize (Ngozo, 2010).

6.0 Phenotypic and molecular characterisation of maize

Through phenotypic and molecular characterisation, maize has been identified as one of the most diverse crops in the world. Interestingly, its potential in breeding programmes has been underutilized due to inability to identify variants largely within local varieties and lines (Tanksley and McCouch, 1997). For instance, studies have been conducted to identify genetic diversity in maize landraces using both phenotypic and molecular markers. The results have shown that landraces remain the main good source of genetic diversity and contain unique alleles not present in other maize varieties (Warburton et al., 2008). Unfortunately for Malawi, no comprehensive work has been done to determine the extent of genetic diversity among locally grown open pollinated varieties that can be exploited in breeding programmes, such as the development of insect resistant maize germplasm.

7.0 Summary of problem statement

Despite measures being put in place to control the spread of larger grain borer and damaging effects of the maize weevil, the pests are far from being contained as the pest populations are still on the increase. Interestingly, the IPM strategies employed to contain the spread of LGB and MW do not include the concept of breeding or improving grain resistance to the insect pest in maize varieties and worse still not much has been done to exploit genetic variation for maize weevil resistance in breeding programmes in Malawi. Nonetheless, plant resistance is an essential element of integrated pest management as it is cheap, environmentally friendly and acceptable by farmers. Furthermore, no comprehensive work has been done in Malawi to determine the extent of genetic diversity among locally grown open pollinated varieties that can be exploited in breeding programmes to improve larger grain borer and maize weevil resistance in productive varieties. In addition, the development of insect resistant maize materials without considering their yield potential under farmers' conditions, such as drought and low soil fertility is a risky strategy. This calls for development of insect resistant maize materials which should also perform well under specific farmer's agro-ecological environment. In view of this, the research therefore, focused on identification and characterisation of locally grown open pollinated maize varieties and exploration of maize weevil and larger grain borer resistant lines for the development of larger grain borer and maize weevil resistant hybrids that meet farmers' preferences and agronomic conditions.

8.0 Main objective

The main objective of this study was to develop maize varieties with resistance to larger grain borer and maize weevil, having desired agronomic performance, under smallholder conditions that will contribute towards improving food availability and self-sufficiency at the household level in Malawi.

9.0 Specific objectives

The specific objectives of the study were to:

1. Identify farmers' perceptions on yield, maize production constraints and storability of local open pollinated maize varieties (OPV).

2. Determine genetic marker diversity of the potential breeding sources for use in introgressing LGB and MW resistance genes in farmer-preferred local varieties.
3. Determine levels of LGB and MW resistance in local maize varieties.
4. Estimate general combining ability (GCA) and specific combining ability (SCA) between maize lines and their hybrids for grain yield and resistance to larger grain borer and maize weevil.
5. Determine the value for cultivation of LGB and MW resistant hybrids, as reflected by combination of high productivity and stability, under farmer representative conditions in multi-location trials, representing the target production environments in Malawi.

10.0 Hypotheses

The following hypotheses were tested:

1. Farmers have different perceptions of yield potential, production constraints and resistance to post-harvest grain pests in local varieties. Knowledge of this information is crucial in setting up the breeding priorities to improve grain storage ability in maize hybrids and local varieties.
2. Genetic diversity, productivity and grain resistance to LGB and MW are not mutually exclusive in the maize germplasm. Therefore breeding for LGB and MW resistance will not compromise grain yield of hybrids and populations.
3. Genetic variation exists among local varieties in Malawi for resistance against larger grain borer and maize weevil. This variation can be exploited in a breeding programme to improve the resistance in productive varieties.
4. Development of larger grain borer and maize weevil resistant maize varieties can substantially improve net maize yields in Malawi.
5. LGB and MW resistant maize hybrids developed have acceptable productivity in the field and stability in the target environments, which will be complemented by high levels of storage ability resulting in superior net yield on farm.

6. Larger grain borer and maize weevil resistant maize lines have good combining ability for resistance and grain yield which can be exploited in developing hybrids and synthetic populations.

7. There are maternal effects and additive gene effects which are responsible for controlling resistance to LGB and MW in maize germplasm, suggesting that selection can be used to enhance the resistance.

11.0 Thesis Structure

This thesis is divided into seven chapters as follows:

- Chapter 1: Literature review
- Chapter 2: Assessment of farmers' perceptions on maize production constraints, trait preference and storability of local maize varieties in central Malawi
- Chapter 3: Phenotypic and molecular genetic diversity of local varieties in Malawi
- Chapter 4: Variation in levels of resistance against maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (*Prostephanus truncatus* Horn) among local maize varieties in Malawi
- Chapter 5: Assessment of larger grain borer (*Sitophilus zeamais* Motschulsky) and maize weevil (*Sitophilus zeamais* Motschulsky) resistance and yield potential of F_1 maize hybrids in Malawi
- Chapter 6: Combining ability for grain yield and resistance among maize weevil and larger grain borer resistant maize lines
- Chapter 7: General overview

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Chapter 1

Literature Review

1.1 Introduction

The chapter on literature review outlines overarching information about the general status of agriculture in Malawi, the importance of maize in Malawi, constraints to maize production, underpinning on postharvest pests, control strategies and the role of host resistance in reducing the effects of storage pests. This chapter further describes the role of participatory rural appraisal (PRA), genetic diversity, and genotype x environment interaction in plant breeding. In the course of reviewing the literature, gaps were identified some of which were addressed in the current study.

1.2 Agriculture in Malawi

Agriculture is the single most important sector in Malawi, contributing 40% of GDP (Malawi Government and World Bank, 2006). It is estimated that of the 11.84 million hectares of land available in the country, 48.4% is suitable for agriculture and only 31.6% is suitable for crop production (FAOSTAT, 2014). Crop production in Malawi is largely dependent on rainfall, as a result, crop yields tend to fluctuate due to frequent dry spells experienced within the season (Denning et al., 2009). In 2012, the top crop productions mainly came from cassava, maize, potato, sugarcane, groundnuts, and bananas among other crops (Figure 1.1).

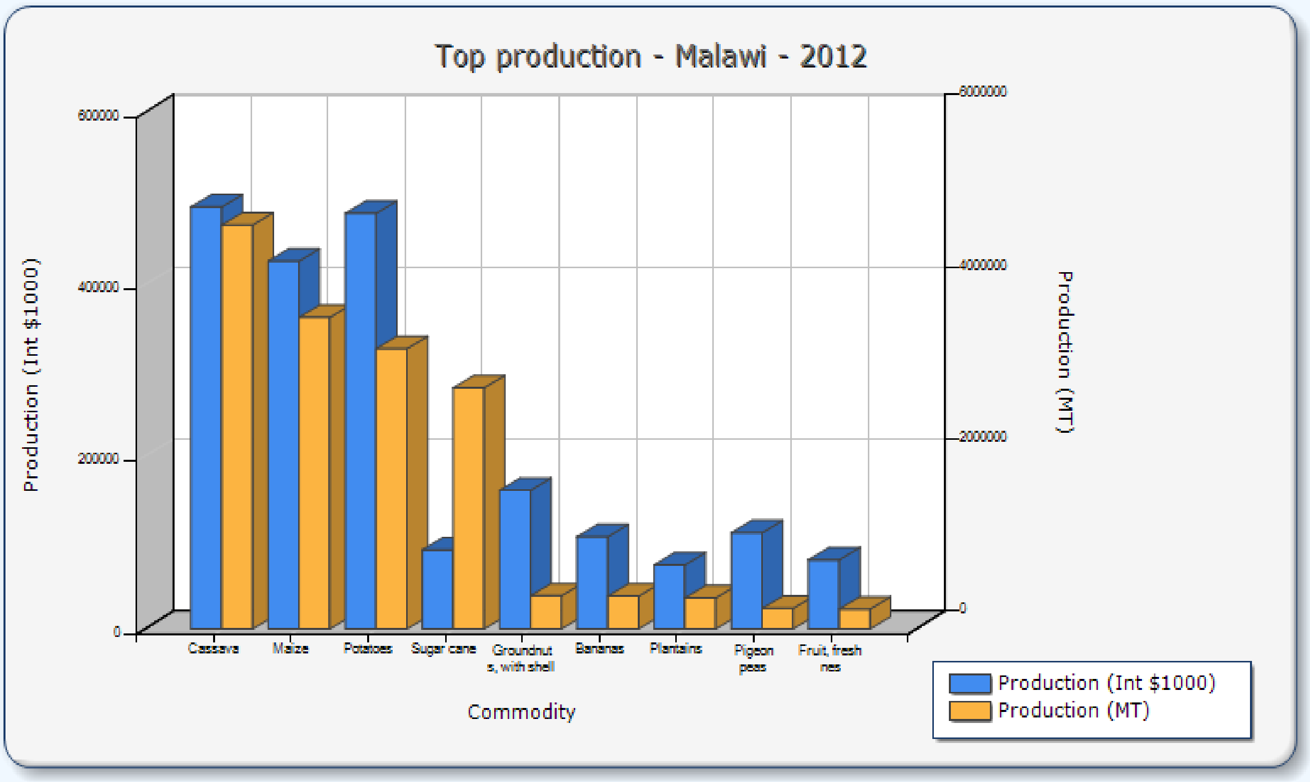

Figure 1.1: Crop production in Malawi. Source: FAOSTAT (2014)

1.3 Importance of maize in Malawi

Maize (*Zea mays* L) is the main food crop in Malawi. It is grown by 97% of farming households and accounts for 60% of total calorie consumption (Denning et al., 2009). The mean annual maize consumption per individual is around 131.2 kg (FAOSTAT, 2014). Before 2005, agricultural sector in Malawi experienced low maize productivity that resulted in only 20% of maize farmers realising surplus maize (FAOSTAT, 2008). In response to the low and declining maize productivity, the Government of Malawi introduced the national subsidy programme in 2005, through which smallholder farmers accessed cheap seed and fertilizer. This intervention greatly improved maize productivity in the country (Denning et al., 2009). However, sustaining the current levels of maize production is becoming a challenge due to high cost of agricultural inputs, low soil fertility, climate change, drought, diseases, and postharvest losses due to storage pests (Denning et al., 2009).

1.4 Constraints to maize production in Malawi

1.4.1 High cost of agricultural inputs

Agriculture development in general, and maize production in particular, is impeded by high cost of agricultural inputs that are most of the times beyond the reach of resource-poor smallholder farmers. The price of farm inputs has been going up in Malawi. According to Dorward et al. (2008), between 2007 and 2008, the cost of fertilizer in Malawi created a deficit on the national budget by US$80 million. Without government intervention, high costs of inputs result in smallholder farmers' inability to purchase and use inputs as per requirement to sustain maize production. For example, in 2008, the overall market value of farm inputs was at K5,500 (44 USD) but farmers were required to pay only K2,050 (16.40 USD) (Dorward et al., 2008).

1.4.2 Climate change

Climate change is undoubtedly one of the modern threats to agricultural production (IFPRI, 2007). Climate change has a direct effect on people's life especially in tropical climates (IPCC, 2001). In Sub-Saharan Africa, 95% of the cropping area is rainfed (Voortman et al., 2003) as such, the cropping system is prone to effects of climate change. For example, unprecedented high temperatures, short growing seasons and unpredictable rainfall pattern have of late been experienced in Malawi, there by affecting maize production (Denning et al., 2009). It is projected that by 2055, the world will experience a 10% reduction in maize production due to climate change and Africa will largely be affected due to its dependence on rainfed agriculture (Jones and Thornton, 2003).

1.4.3 Drought

Drought is one of the important factors limiting maize production, especially where farmers sorely depend on rainfed agriculture (CIMMYT, 1999). Yield losses due to drought are by far greater than any other causes (Farooq et al., 2008). Yield loss of up to 60% caused by drought has been reported in maize (Edmeades et al., 1999). Mild and severe water stress can reduce maize yield up to 63% and 85%, respectively (Earl and Davis, 2003). Economic estimates showed that by 2016, 13 countries in Eastern and Southern Africa could come out of the poverty trap by adopting drought tolerant maize varieties (La Rovere et al., 2010). Drought and heat tolerant maize varieties could yield 34% more than the current varieties in Malawi, Zambia and Zimbabwe (IFPRI, 2007).

1.4.4 Low soil fertility

Due to continuous cultivation, without nutrient replenishment, soils manifest serious nitrogen, phosphorous and potassium depletions. The loss in soil fertility is estimated at 22kg/ha, 2.5kg/ha and 15kg/ha for N, P and K, respectively (Sanchez, 2002). However, N and P deficiencies are the major soil fertility constraints to maize production in Malawi (Akinnifesi et al., 2007). Of all the nutrients required by maize plants, nitrogen is the most limiting nutrient (Phiri et al., 1999). In maize, nitrogen is important for plant metabolism, protein synthesis and contributes greatly to grain production and protein quantity (Machado and Fernandes, 2001). Improving soil fertility can increase maize yield from an average of 1300 kg ha^{-1} to as high as 6000–7000 $kgha^{-1}$ (Zambezi, 1993). To maintain soil fertility therefore, a more encompassing approach is required, such as the efficient use of chemical and organic inputs, crop rotations and use of nutrient efficient cultivars (CIMMYT, 2000). Maize varieties that efficiently use nitrogen under low N soil conditions can contribute towards sustainable agriculture (Presterl et al., 2002).

1.4.5 Diseases and Pests

1.4.5.1 Diseases

Due to variability in environmental conditions, maize crop is prone to attacks by a wide range of diseases. The effect of diseases are noted through reduced grain yield, poor grain quality, poor feeding value and production of toxic animal feed (Ngwira, 2001). Of the many diseases affecting maize production in Malawi, Maize Streak Virus (MSV), Grey Leaf Spot (GLS) and leaf blight are of major concern (Ngwira, 2001). MSV and leaf blight can cause grain yield losses of up 70% (CIMMYT, 2000; Ngwira, 2001), while GLS can reduce grain yield by 30% (CIMMYT, 2000).

1.4.5.2 Postharvest insect pests

The sustainability of food sufficiency at household level in Malawi is further threatened by huge postharvest losses due to grain damage by insect pests, such as larger grain borer (LGB) and maize weevil (MW). Postharvest losses due to MW and LGB erode net gain in maize production. For instance, LGB can cause maize yield losses ranging from 5–40% (Paliani and Muwalo, 2001; Ching'oma, 2009). Without chemical application, postharvest losses due to LGB can range between 40-100% (Denning et al., 2009). Maize weevil is another important pest in developing countries with yield losses ranging from 5 to 15% (Tigar et al., 1994). Yield losses of more than 80% have been reported in untreated grain in storage facilities (Pingali and Pandey, 2001). However, other publications by APHLIS (2015) showed that between 2003 and 2014, Africa experienced an average of 18% in postharvest weight losses of maize.

1.5 The larger grain borer (*Prostephanus truncatus* Horn)

1.5.1 Origin and distribution

The larger grain borer (LGB) *Prostephanus truncatus* (Horn) (Coleoptera:Bostrichidae) is also known as powder post beetles (Booth et al., 1990).The origin of LGB is traced back to meso-America (Hodges, 1994). LGB is now wide spread in many areas, notably in the New world, Israel, Iraq, Central America, Thailand and East, West and Sub-Saharan Africa (Hill et al., 2002; Nansen and Meikle, 2002). Larger grain borer was accidentally introduced in East Africa (Tanzania) in 1970's where it caused a lot of devastation on maize (Golob and Hodges, 1981). The pest has now spread to many countries in Africa causing havoc on dried maize and cassava, threatening food security in the affected countries (Dunstan and Magazini, 1981; Farrell and Schulten, 2002). Incidences of LGB have been reported in Benin, Burkina Faso, Burundi, Ghana, Guinea Conakry, Kenya, Mozambique, Namibia, Niger, Nigeria, Rwanda, South Africa, Tanzania, Togo, Uganda, Zambia, Zimbabwe and Malawi (Tyler and Boxal, 1984; Mushi, 1990; Nyagwaya et al., 2010; Tefera et al., 2010). In Malawi, LGB was first observed in Karonga district which borders Tanzania in 1991. Its presence has now been detected in 27 of the 28 political districts in the country and has become a major storage pest of maize in Malawi (Binder, 1992; Paliani and Muwalo, 2001; Ching'oma, 2009; Singano et al., 2009; Kamanula et al., 2011).

1.5.2 Ecology, morphology and reproduction of larger grain borer

Larger grain borer is found in diverse habitats and ecologies (Hodges, 2002). Hill et al. (2002) categorized the ecology of *Prostephanus truncatus* into, ecology outside the storage system and ecology within the storage system. Ecology outside the storage system includes forests, woody frames, grain storage facilities, dry timber, green timber, sap wood and forest branches (Hill et al., 2002). Sixteen tree species belonging to the groups Leguminosea, Burseracea and Anacardiaceae are alternative hosts of LGB. The pest prefers young soft wood to old wood (Nang'ayo et al., 1993). Within the storage system, LGB associates with other insect pests that destroy maize, such as predators, parasitoids and ecto-parasites (Hill et al., 2002). The presence of *P .truncatus* has also been reported in stored cassava roots (Hodges et al., 1985). The morphology of LGB is characterised by deflexed head with well-built mandibles and cylindrical body protected by pronotum that give the insect excavation abilities (LI, 1988). LGB has a body length of 2 to 3.5 mm and a width of between 1 to 1.5 mm. The pest is able to reproduce on maize grain and cobs, dry cassava and other stored-products. Females can lay five to eight eggs in each oviposition chamber and 300 eggs can be produced in its entire lifespan (Tefera et al., 2010).

1.5.3 Control of the larger grain borer

Various methods have been employed to contain LGB. These methods include, the use of insecticides, pesticidal plants, biological control, integrated pest management (IPM) and host resistance (Paliani and Muwalo, 2001; Adda et al., 2002; Farrell and Schulten, 2002; Golob, 2002; Ching'oma, 2009; Kasambala, 2009).

1.5.3.1 Use of insecticides

The control of LGB has depended heavily on the use of insecticides mainly organophosphates. Organophosphates such as pirimiphos-methyl, fenitrothion, permethrin and bromophos dilute dust have been used in Tanzania (Golob, 2002). In a trial that was conducted at Tumbi Research Station in Tanzania, only Pirimiphos-methyl was found to be more effective against LGB (Golob et al., 1983). In Togo, a combination of organophosphates with synthetic pyrethroid has been used to control LGB (Golob, 1988). Actellic super which is the mixture of 1.6% Pirimiphos-methyl and 0.3% permethrin has been adopted as an effective chemical against larger grain borer (Farrell and Schulten, 2002). The use of Actellic super by smallholder farmers has been documented in a number of African countries (Kimenju and De Groote, 2010). For example, in Tanzania, Actellic super is overwhelmingly being used by smallholder farmers with an adoption rate of 93% (Kaliba et al., 1998). In Malawi, farmers use Methacrifos 2P, bifenthrin and Actellic super to control LGB (Paliani and Muwalo, 2001; Ching'oma, 2009; Kasambala, 2009). Even though the use of chemical control has largely been effective in mitigating the devastating effects of LGB, there is a possibility of the pest developing resistance to the insecticides due to misuse. For instance, after permethrin was used for 4 years in Tanzania in the form of dust, an increase in adult survival of *P. truncutus* was observed in maize (Golob, 2002). Due to the increasing occurrence of insecticide resistance, possibility of environmental damage, grain contamination and costs, there is need to look for alternative methods to protect maize against LGB (Golob, 2002; Ahmed and Yusuf, 2007; Singano et al., 2009).

1.5.3.2 Use of Pesticidal plants

The use of pesticidal plants by local farmers has been reported in countries such as Malawi, Zambia and Kenya. Kamanula et al. (2011) reported the use of *Tephrosia vogelii*, Fabaceae, neem, tobacco, pepper and vernonia by smallholder farmers in Malawi and Zambia. These plants have been used to preserve maize grain and beans. Other reports from Kenya revealed the use of leaf dust of *Tephrosia vogelii* in maize grain to control the infestation and spread of storage pests (Ogendo et al., 2004).

1.5.3.3 Use of natural enemies (biological agents)

The use of natural enemies has been one of the key strategies in controlling larger grain borer. One of such biological agent is *Teretrius nigrescens* Lewis. *T. nigrescens* Lewis (Coleoptera: Histeridae) is natural predator of larger grain borer (Paliani and Muwalo, 2001) as it is attracted by aggregation pheromone produced by LGB (Rees et al., 1990). The larvae and adults of *T.nigrescens* feed on eggs and larvae of LGB (Rees, 1987). The predatory effect of *T.nigrescens Lewis* on LGB population has been investigated. In a study to assess the impact of *Teretrius nigrescens* on *Prostephanus truncatus* and losses in traditional maize stores in Southern Togo, Richter et al. (1997) reported a decrease in LGB infestation after the introduction of *T. nigrescens*. This was attributed to the fast multiplication of the predator within a short time after release. Rees (1991) reported LGB infestation reduction by 83% after introduction of the natural enemy. Since 1990, *T. nigrescens* has been deployed in selected sites in Malawi (Paliani and Muwalo, 2001). Although, there were reports of reduction in numbers of LGB after introduction of *T.nigrescens*, (Paliani and Muwalo, 2001), the strategy has not been very successful, as the population of the insect pest is on the increase. New infestations have been found in forest reserves that act as reservoirs for secondary infections on field plants (Ching'oma, 2009). Hence, the need to develop a more robust system that could effectively contain the spread of LGB in Malawi, and that system should incorporate host resistance.

1.5.3.4 Integrated Pest Management (IPM)

The unexpected presence of LGB in Malawi led to the deployment of an IPM strategy that included, awareness programmes, field and storage facility inspections, deployment of traps, chemical applications and use of *T. nigrescens* (Paliani and Muwalo, 2001).The use of a storage system that integrates improved variety with higher yield and moderately good husk cover characteristics has been effective in reducing insect pest infestation in Togo (Adda et al., 2002). The use of post-harvest insect resistance maize with *Teretrius nigrescens* has been investigated. The combination of the biological agent with both resistant and susceptible maize grains showed significant differences in progeny number, grain weight loss and frass production. Without the biological agent, susceptible genotypes suffered significant damages than resistant genotypes (Bergvinson and Garcïa-Lara, 2011).

1.5.3.5 Host resistance

The use of host resistance has been reported as the easiest, the most safe, effective, and economical way of controlling insect pests on stored grain (Ahmed and Yusuf, 2007). Host insect resistance provides farmers with the opportunity to benefit much from farming by minimizing costs of synthetic insecticides (Smith, 1994). Screening and evaluation for insect resistant materials is a first step in developing cultivars that can minimize damage caused by insect pests (Ahmed and Yusuf, 2007). The screening of resistant material necessitates artificially infesting storage grains with insect pest, assessing levels of oviposition, and insect development (Ahmed and Yusuf, 2007). For grain, anti-biosis and non-preference are the most important forms of resistance against storage pests (Derera et al., 2000). The numbers of progenies emerging during incubation, percent grain damage, grain weight loss and grain physical characteristics have been used to determine levels of resistance against LGB among genotypes (Kasambala, 2009). Grain characteristics, such as grain moisture, grain hardness, vitreous endosperm and nutritional factors play a significant role in LGB development and behaviour (Arnason et al., 1992).

The variability in LGB resistance exists among maize materials. In Kenya, differences in resistance to LGB were observed among landraces along the coastal region (Ndiso et al., 2007). Mwololo et al. (2010) reported significant differences in grain damage, amount of flour, number of dead and live insects among Kenyan genotypes. In Benin, Meikle et al. (1998) reported the existence of resistance among maize varieties due to husk cover other than with grain characteristics. Kumar (2002) identified 19 landraces from the Caribbean with high resistance to LGB after a series of infestation, selection and inbreeding. The resistance observed in these landraces was attributed to antibiosis especially within the S_3 progenies (Kumar, 2002).

1.6 Maize weevil (*Sitophilus zeamais* Motschulsky)

1.6.1 Importance, morphology and reproduction of maize weevil

Maize weevil (MW) is an important pest in developing countries with yield losses ranging from 5 to 80% (Tigar et al., 1994; Pingali and Pandey, 2001). Pest infestation starts in the field and is carried over to the storage facilities (CIMMYT, 2000; Dhliwayo and Pixley, 2003; Demissie et al., 2008). Maize weevil belongs to the order Coleoptera and family Curculionidae. The pest has a body size of between 2.4 to 4.5m (Tefera et al., 2010). The body is mostly reddish brown, dark brown or black in colour. Maize weevil has a pre-ovipositing of three days and females can lay eggs up to four eggs in a kernel. Adult maize weevil feeds and lives between four to five months (Tefera et al., 2010).

1.6.2 Control of maize weevil

Different control measures have been employed to manage maize weevil especially in storage. These methods include, sun drying of maize grain, use of plant leaves, flowers, seeds and powder extracts mixed with grain, use of synthetic chemicals and host resistance (CIMMYT, 2001; Nukenine, 2010).

1.6.3 Use of plant material

Plant powders from *Nicotiana tobacum*, *Allium sativa* and *Zingiber Officinale* have been effective in controlling maize weevil on maize grain (Danjumma et al., 2009). Plant spices, such as *Piper guineense, Afromomum meleguate, Xylopia aethiopica and Tetrapleura tetrapterra* have also been reported to be effective in controlling maize weevil (Udo, 2005). Demissie et al. (2008) reported high mortality rates of maize weevil due to silicosec and wood ash. Plant extracts, such as *Angustifolia* Ch*, Laurus nobilis* L, *Rosmarimus officianalis* L and *Thymus* have been used in controlling weevils, but their commercial application depends on obtaining adequate amount of essential oils (Rozman et al., 2007). Laboratory evaluations of ethanolic extracts revealed high levels of toxicity against maize weevil in *Cupressus arizonica*, *Ocimum gratissimum* and *Eucalyptus grandis* leaves (Akob and Ewete, 2009).

1.6.4 Chemical control

Use of chemicals has been the major method of controlling maize weevil (Rozman et al., 2007). Synthetic chemical insecticides, such as pyrethroid, organophosphates and gaseous fumigants have been applied to control MW (Udo, 2005; Abebe et al., 2009; Pereira et al., 2009; Nukenine, 2010; Kamanula et al., 2011). However, the use of insecticides to control of maize weevil is being threatened by development of maize weevil resistance (Fragoso et al., 2005; Pereira et al., 2009) and the chemical products are also mostly beyond the reach of smallholder farmers (Dhliwayo and Pixley, 2003).

1.6.5 Breeding for resistance against maize weevil

The initial breeding focus for weevil resistance has been on husk cover. The role of husk cover in controlling maize weevil has been investigated (Meikle et al., 1998; Demissie et al., 2008). Differences in resistance among genotypes have been observed due to the size of the husk cover. Genotypes with good husk cover extension showed low numbers of weevils and damaged ears. Husk cover extension and tightness were the most important parameters in maize resistance to maize weevil in the field (Demissie et al., 2008). Meikle et al. (1998) reported negative association between the susceptibility of varieties to maize weevil and husk cover extension in the field. The combination of husk cover extension and grain flintiness can improve resistance to maize weevil (Kim and Kossou, 2003). Harder seeds tend to be more resistant than soft seeds (Tongjura et al., 2010). Makate (2010) reported a positive correlation between susceptibility of genotypes with moisture content and seed weight. The variation in results obtained by researchers when assessing traits and factors responsible for grain resistance against maize weevil could be due to genotypic differences and differences in environmental conditions under which the research work was carried out. This implies that a holistic approach (multi-trait approach) and standardisation of assessment procedures must be employed when devising a breeding strategy for the control of maize weevil.

1.6.6 Genetic basis for weevil resistance

Additive gene action, dominance gene action and maternal effects play a role in maize weevil resistance (Derera et al., 2000; Kim and Kossou, 2003). In a study to screen F_2 hybrids, commercial hybrids and popcorn for resistance against maize weevil, Derera et al. (2000) reported significant maternal effects on weevil emergence and susceptibility. Both GCA and SCA were significant in determining susceptibility index, weevil emergence and grain weight loss. Kim and Kossou (2003) evaluated maize cultivars and crosses between inbred lines. The results showed significant variation for weevil attack, general combining ability, and specific combining ability. Both additive and non-additive gene actions contributed significantly to maize weevil resistance among the genotypes. Dhliwayo and Pixley (2003) reported improved resistance against maize weevil through divergent selection in six maize populations due to additive gene action. Masasa et al., (2013) reported significant differences in susceptibility of local maize varieties to maize weevil in Zimbabwe. Significant differences were observed for the number of damaged grains, grain weight loss and weevil mortality but no significant differences were observed for weevil progeny emergence, fecundity and Dobie Index of Susceptibility. Parameters such as progeny emergence, grain weight loss, median development period and Dobie susceptibility index were found to be heritable. Significant differences in genotypic variation, general combining ability, and specific combining ability were also reported among lines and hybrids for grain weight loss and emerged F_1 weevils (Dari et al., 2010). Both additive and non-additive gene actions were responsible for resistance observed in the genotypes. Dhliwayo et al. (2005) reported significant SCA, GCA and reciprocal effects in F_1 weevils emerging from F_2 grain in 14 Southern African maize inbred lines and weevil resistance was controlled by additive gene action only.

1.7 Participatory Rural Appraisal (PRA) and Surveys

The incorporation of farmers' knowledge and opinions in breeding programmes is of paramount importance. Participatory approaches and methods provide enabling environment for farmers to share ideas on issues affecting their wellbeing (Chambers, 1992). PRA tools and surveys help to bring out issues that may not be priotised by researchers, scientists and policy makers but are important to smallholder farmers (CIMMYT, 2001). PRA has been effective in narrowing the information gap between researchers and farmers to reach a common consensus on issues affecting farmers. Miti (2007) successfully used PRA to obtain farmers’ preferences in selecting maize crop cultivars. Fisher and Mazunda (2011) used PRA tools to assess adoption of modern varieties in Malawi and reported that farmers still use both landraces and locally adapted varieties in their fields. Information from both farmers and researchers is critical in research and technology development. Incorporation of farmers’ views and knowledge may increase acceptability of varieties by farmers (Mukanga et al., 2011).

1.8 Genetic diversity

Genetic diversity is the basic component of the biological and species diversity (Yao et al., 2007) critical for the sustainability of plant and crop productivity (Jarvis and Hodgkin, 2005). Determination of genetic diversity involves analysis of variation among individuals or populations (Jarvis and Hodgkin, 2005). The variation is measured by the number of polymorphic genes, number of alleles for each polymorphic gene and the number of genes per individual that are polymorphic (Magorokosho, 2006). Genetic markers have been used to explore variation that exists among individuals or populations. The genetic markers can be morphological, biochemical or molecular (Jones et al., 1997; Collard et al., 2005; Magorokosho, 2006). Morphological markers represent phenotypic traits, such as flower colour, seed shape among other traits, biochemical markers are markers that use electrophoresis and staining to identify variation, and molecular markers are genetic markers that utilize variation within the DNA structure (Jones et al., 1997; Collard et al., 2005).

Phenotypic and molecular characterisation has revealed the extreme diversity of maize plant. Sadly, its potential in breeding programmes has been underutilized due to failure to identify variation within maize germplasm, especially among landraces and lines (Tanksley and McCouch, 1997). Studies have been conducted to identify genetic diversity in maize landraces using both morphological and molecular markers. Magorokosho (2006) explored maize diversity in Malawi, Zambia and Zimbabwe using morphological markers and reported that Open pollinated varieties (OPV) and landraces grown by farmers in these three countries contain substantial variation. It is worth noting that landraces remain the main good source of genetic diversity and unique alleles not present in OPVs (Warburton et al., 2008).

Specific molecular markers have been applied in diversity studies, such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPDS) and Simple Sequence Repeats (SSRs). Comparatively, the SSRs are mostly applied in diversity studies because they are co-dominant, simple to deploy, transferable between populations, locus specific, and multi-allelic (Powell et al., 1996; McCouch et al., 1997; Collard et al., 2005; Magorokosho, 2006). Use of SSR markers to quantify genetic diversity in maize has been reported (Betra'n et al., 2003; Reif et al., 2004; Reif et al., 2005; Magorokosho, 2006). Magorokosho (2006) reported high levels of diversity between landraces and commercial varieties collected from Southern Africa, USA and CIMMYT. Betra'n et al. (2003) successfully used SSR markers to assess genetic diversity in tropical maize under-stress and non-stress environments. Reif et al. (2004) and Reif et al. (2005) deployed SSR markers to determine levels of genetic diversity within CIMMYT materials and European maize landraces, respectively.

1.9 Genotype x Environment Interaction (GEI)

Yield potential and stability of genotypes are some of the important factors considered when selecting genotypes for particular environments (Yan and Hunt, 1998; Mendes et al., 2012). Differences in environmental and climatic conditions affect yields of maize mainly due to genotype and environment interaction (GIE) (Grada and Ciulca, 2013). GIE is the differential reaction of genotypes to environmental factors, such as soil nutrients, light, pests, diseases and physical injury (Yan and Kang, 2003; Mekonnen and Mohammed, 2009). GEI complicates selection of superior genotypes in target environments (Yan and Hunt, 1998; Yan and Kang, 2003; Mekonnen and Mohammed, 2009). Significant GEI affects heritability of traits, adaptability of genotypes, ranking of genotypes and selection of superior genotypes across environments (Yan and Hunt, 1998).

The performance of genotypes across environments is assessed through Multiple Environment Trial (MET). MET refers to multiple testing on genotypes in one or more environments (Yan and Hunt, 1998). Data on the performance of cultivars across environments aids in selection of superior genotypes (Setimela et al., 2007). Different methods are available for determining yield, GEI and stability of genotypes across environments. The use of Analysis of Variance (ANOVA), Linear regression, GGE Biplot, Additive Main Effects and Multiplicative Interaction (AMMI) and Residual maximum likelihood (REML) in yield analysis has been reported (Finlay and Wilkinson, 1963, Gauch, 1992; Maa'li, 2008; Miranda et al., 2009; Payne et al., 2009; Nzuve et al., 2013). ANOVA shows main effects only without GEI (Miranda et al., 2009). Finlay and Wilkinson (1963) reported the use of regression on mean model, where GEI is obtained through the variation for yield potential of different genotypes to the change of the environment. This is represented as $Y_{ge} = \mu + \alpha_{g+} y_g\beta_e + \theta_{ge} + \mathcal{E}_{ge}$, where; Y_{ge} is the measure GEI for yield, μ is the overall mean, y_g is the yield sensitivity of the genotype g to the environmental alteration, θ_{ge} is part of GEI not accounted for by the regression line, and β_e is a measure of the environment. GGE biplot and AMMI use both ANOVA and PCA to provide information about individual genotypes, environments and the interaction between genotypes and environments (Gauch, 1992; Maa'li, 2008; Miranda et al., 2009; Nzuve et al., 2013). The following AMMI model has been used in GEI analysis: $Y = \mu + G_i + E_j + \sum_{k=1}^{n} \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$, Where, Yij is the average response of genotype i in environment j, μ is the general mean, G_i is the genotype effect, E_j is the environment effect, GE_{ij} was modelled in the way that λ_k is the square root of the kth eigenvalue of the matrices (GE)(GE)' and (GE)'(GE) (from non-null equal eigenvalues), γ_{ik} is the ith element (related to genotype i) of the kth auto vector of (GE) (GE)', αjk is the jth element (related to environment j) of the kth auto vector of (GE)'(GE), ρij is the residual not explained by principal components used, and εij is the associated error (Balestre et al., 2009). REML is an efficient yield analysis tool (Payne et al., 2009). REML analyses more than one source of error variation, and its use on unbalanced designs has been recommended (Payne et al., 2009). Residual maximum likelihood manipulates both random and fixed factors affecting yield as follows (O'Neil, 2010): Yield = mean + fixed effects + random effects.

1.10 Conclusion

Through the review of literature, it has been established that maize remains the main food crop in Malawi. The net gain in maize production is being curtailed by post–harvest loses due to larger grain borer and maize weevil. These losses are threatening food security at household level. Measures for controlling storage pests are available but are inadequate to address the problem of storage insect pests in Malawi. Incorporation of host resistance could improve the efficiency of the strategies to control storage pests. Variation for insect resistance exists among maize materials which can be exploited in breeding for insect resistance. But the variation has not been fully utilised in maize breeding programmes. The review of literature has not established any published reports indicating any released productive hybrids that have fully incorporated genes for resistant to larger grain borer and maize weevil. Both additive and non–additive actions are responsible for determining weevil resistance, but nothing is documented on the type of gene action responsible for resistance against larger grain borer in maize genotypes. It has further been established that incorporation of farmers' knowledge and opinions in breeding programmes can lead to high adoption rate of maize varieties. But this has generally been overlooked or ignored by researchers when designing breeding programmes. Maize is one of the diverse crops in the world but its potential in breeding programmes has been underutilized due to inability to identify variation within local varieties and landraces. Local maize varieties and landraces could provide good sources of materials for breeding. The yield potential of genotypes is affected by significant GEI which influences heritability of traits, adaptability of genotypes, ranking of genotypes and selection of superior genotypes across environments. This calls for the multi-location testing of maize varieties.

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Chapter 2

Assessment of farmers' perceptions on maize production constraints, trait preference and storability of local maize varieties in Central Malawi

Abstract

The major shift in hybrid maize seed production from semi-flint varieties to dent varieties ushered in high yielding maize hybrids in Malawi. Despite the yield advantage that the hybrids have over local varieties, smallholder farmers still cling to their own local varieties seemingly due to their superior storability. A farmer perception assessment was conducted at Msitu, Ngwangwa and Chinguluwe Extension Planning Areas (EPA) in Mchinji, Lilongwe and Salima districts, respectively, in 2012. The objectives of the assessment were to understand farmers' perception on maize production constraints and storability of local maize varieties, to identify critical traits used to select varieties for planting and to develop selection criteria for insect resistant maize materials for future breeding programmes. The assessment was carried out on 210 farmers using semi-structured questionnaire, focus group discussions, direct matrix, transect walks and key informants. Farmers continue to grow both hybrids and local varieties. Hybrids were cultivated mainly because of their high yield potential and early maturity, while local maize varieties were grown for good tolerance to pests and diseases, large grain size, large cob size, good yields under low soil fertility, white colour, superior poundability, drought tolerance and high storability than hybrids. Conspicuously missing on their preferred traits was taste which has been generally regarded as one of the important attributes in local varieties. The major maize production constraints were lack of fertilizer, low soil fertility, pests, and lack of high quality seeds. Farmers identified grain hardness, grain size, grain colour, poundability and grain texture as the main characteristics used to select maize varieties tolerant to maize weevil and larger grain borer. Farmer requirements should be incorporated in the conventional breeding programmes in Malawi. Storability and other traits should be bred in hybrids that are preferred by farmers.

Keywords: Breeding, insect resistance, landraces, maize storability, participatory rural appraisal

2.1 Introduction

Malawi has experienced a major shift in maize seed production from semi-flint varieties to dent varieties especially by multinational seeds companies (Gilbert and Jones, 2012). In addition, the Government of Malawi has been providing largely hybrid seeds and improved open pollinated varieties (OPV) to farmers through its subsidy programme to improve food sufficiency at national level (Denning et al., 2009). The shift in maize seed production coupled with the large distribution of hybrid seeds and improved OPVs has ushered in high yielding varieties but highly susceptible to storage pests, such as larger grain borer (LGB) (*Prostephanus truncatus* Horn) and maize weevil (MW) (*Sitophilus zeamais* Motschulsky) (Gilbert and Jones, 2012). LGB and MW have been identified as the major storage pests of maize in Malawi (Binder, 1992; Ching'oma, 2009; Kamanula et al., 2011). Unfortunately, the majority of smallholder farmers in Malawi still use traditional methods and structures of keeping grain (Figure 2.1).

Figure 2.1: Maize storage structure in Chinguluwe Extension Planning Area (EPA), Salima district

Under such conditions, maize grain is more vulnerable to serious attacks from rodents, birds, micro-organisms and insect pests (Nukenine, 2010; World Bank, 2011). According to the Ministry of Agriculture (2012), postharvest losses account for more than 12% reduction in national maize yield output every year and insect damage contributes significantly to that loss. Postharvest losses caused by the insect pests are hindering the translation of the current national levels in maize production to food sufficiency at household level.

In the past, postharvest losses or storability have been recognised as an important factor in famers' decision making process on the type of maize seeds to grow, but its significance has largely been ignored (Gilbelt and Jones, 2012). In cases where storability has been recognised as an important issue, the focus has been on chemical control (Ching'oma, 2009; Gilbert and Jones, 2012). However, not much attention has been put on improving host resistance among maize varieties in Malawi. Despite the yielding advantage that hybrid varieties have over OPVs, and many researchers and scientists advocating for increased adoption of hybrid seeds, many farmers still cling to their landraces and locally adapted varieties (Fisher and Mazunda, 2011). In this regard, farmers' and researchers' views differ on preferences, choices and criteria for selection of maize varieties (Ouma and De Grote, 2011). Hence, there is need to assess and understand farmers' perceptions on maize production constraints, trait preference, storability of local varieties and determine the implication of farmers' perceptions on development of insect resistant maize varieties in Malawi.

Participatory rural appraisal (PRA) approaches and methods have enabled local people to brainstorm and share ideas on many topical issues (Chambers, 1992). PRA tools, such as focus group discussions help to bring out issues that are not apparent to researchers, scientists and policy makers but are important to smallholder farmers (CIMMYT, 2001). For example, selection of varieties by farmers involves use of many traits, some of which may be perceived as insignificant by researchers and may not be prioritized by breeders when developing breeding programmes. In addition, production constraints faced by farmers in their respective ecological zones dictates their preferences (Derera et al., 2006; Holden and Lunduka, 2010). The importance of PRA in obtaining information from farmers needs not to be overemphasized. For instance, Miti (2007) effectively used PRA to obtain farmers' preferences in selecting maize crop cultivars in Zambia, Fisher and Mazunda (2011) used PRA tools to assess adoption of modern varieties in Malawi and report that farmers plant both local maize varieties and maize hybrids. Information from both farmers and researchers is critical in research and technology development. Incorporation of farmers' views and knowledge may increase acceptability of varieties by farmers (Mukanga et al., 2011).

2.2 Study objectives

The objectives of the study were therefore to:

1. Understand farmers' perception on maize production constraints and storability of local maize varieties.

2. Identify critical traits used by farmers when selecting suitable varieties for planting.

3. Develop selection criteria for insect resistant maize materials for future breeding programmes.

2.2 Methodology

2.2.1 Study areas

The study was conducted in Lilongwe, Mchinji and Salima districts in the Central region of Malawi in 2012. The three districts belong to three different Agricultural Development Divisions (ADD), namely Lilongwe, Kasungu and Salima, respectively (Fig 2.2).

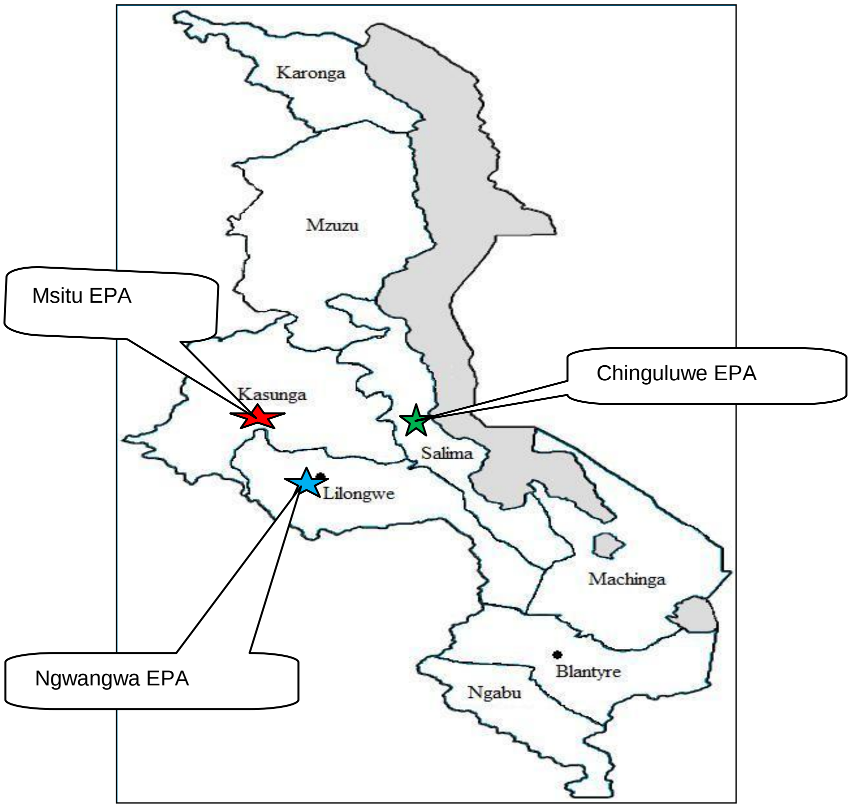

Figure 2.2: Eight (8) Agricultural Development Divisions (ADD) in Malawi with study areas indicated by stars

Source: IFPRI (2010) modified

From each district, an Extension Planning Area (EPA) was selected for the assessment. In Lilongwe, the study was conducted in villages surrounding Ngwangwa EPA (S 13^0 52.156’ E

033^0 40.767’), while villages surrounding Chinguluwe EPA (S 13^0 41.269’ E 034^0 23.834’) and Msitu EPA (S 13^0 57.646’ E 033’ 19.235’) were selected for Salima and Mchinji districts, respectively. EPAs were purposefully selected for their agricultural activities and maize is predominantly grown by farmers in these areas. Selection of villages was at random using farming family registers kept by the Agricultural Extension Officers.

2.2.2 Data collection

Five PRA tools were used in data collection, namely semi-structured questionnaire (Appendix 2.1) focus group discussions, transect walks, direct matrix and key informants.

2.2.2.1 Semi-structured Interviews

Fourteen (14) villages were selected in Ngwangwa EPA and 49 households were sampled, six villages were chosen from Msitu EPA with a total of 42 households sampled and in Chinguluwe EPA, six villages were selected and 64 households used. The households were selected based on their farming records and their active participation in agricultural activities in their respective EPAs as indicated by the Agricultural Extension Officers (Table 2.1).

Table 2.1: Study areas indicating name of village, EPA, District and ADD

Village	EPA	District	ADD	Village	EPA	District	ADD
S4 North	Chinguluwe	Salima	Salima	Chizululu	Ngwangwa	Lilongwe	Lilongwe
Kalembo				Ng'ombe			
Kalala				Kamkwende			
Kadala				Kalimbakatha			
Thengolimeta				Malango			
Chisomba				Kangunje			
Muyeso	Msitu	Mchinji	Kasungu	Chirombo			
Potazina				Khola			
Mweso				Tsokalofanana			
Chiutsi				Jambo			
Chophola				Kafulatira			
Ovilisoni				Akanike			
Zanje	Ngwangwa	Lilongwe	Lilongwe	Kaluma			

EPA= Extension Planning Area, ADD= Agricultural Development Division

The semi-structured questionnaire was used to collect demographic information of the respondents, such as sex, EPA, village , district, region, family headship, marital status, age, education, source of income, and production factors, such as type of farming, farm size, crops grown, maize production levels, storage losses, storage pests, seed preference, seed source, type of storage facilities, level of knowledge on post-harvest losses, level of interaction between the farmer and agricultural extension officer, pest control measures, key traits for identifying storage pest tolerant varieties, traits preference on maize crop in general, important traits for selecting local varieties and constraints to maize production.

2.2.2.2 Direct matrix and transect walks

Direct matrix was used for ranking of preferred traits by farmers, maize production constraints and storage pests. Transect walks were used to collect information on storage facilities within the villages.

2.2.2.3 Focus group discussion

Twenty (20) individuals were involved in focus group discussions in Ngwangwa EPA, 15 and 20 people in Msitu and Chinguluwe, respectively (Fig 2.3). One focus group discussion was conducted in each EPA.

Figure 2.3: Focus groups (Top-Ngwangwa EPA, Lilongwe ADD, Bottom-Msitu EPA, Kasungu ADD)

The main focus of the discussion was to gather in-depth information from the respondents on various topical issues, such as cropping system, important crops, farming problems, source of seeds, important storage pests and control measures, knowledge about local varieties that have resistance to storage pests and grain loss experience due to storage pests, sources of income, and production constraints.

2.2.2.4 Key informants and secondary information

Agricultural Extension Officers and Chiefs were used as key informants to get important information, such as cultural values, demographic and social issues before conducting interviews, and focus group discussions. Secondary information was obtained through reports and publications on line. In total, 210 respondents were involved in the assessment, 55 of which were used for focus group discussions, 155 respondents were interviewed using a semi-structured questionnaire.

2.2.3 Data analysis

Data collected was analysed using the Statistical Package for Social Scientists (SPSS) version 16 (2007). Chi-square analysis was applied on interaction between farmers and agricultural extension workers, and farmers' knowledge on crop storage problems. Results from the analysis were presented in tables and graphs.

2.3 Results

2.3.1 Demographic characteristics of the households

The respondents were predominantly females (52%), married (91.6%), young adults (21-35), with primary education (73.5%) and engaged in farming (78.7%) (Table 2.2).

Table 2.2: Demographic characteristics of respondents

Sex	%	Marital status	%	Age range	%	Level of Education	%	Source of income	%
Female	52	Married	91.6	15-20 years	5.8	Never	13.5	Farming	78.7
Male	48	Single	1.9	21-35 years	47	Primary	73.5	Farming & business	17.4
		Divorced	1.9	36-45 years	17.4	Secondary	9	Farming & employment	3.9
		Separation	0.6	46-55 years	12.3	Tertiary	0		
		Widowed	3.9	above 55 years	17.5	Adult literacy	4		

The households engaged in different farming practices, with 57% of the respondents engaged in production of field crops and 25% combined field crops and horticulture (Figure 2.3). About 85% of the households had less than two acres of farm land while 15% had between two and five acres of farm land.

Table 2.3: Percentages of respondents involved in different farming practices

Farming practices	Percentage
Field crops only	57
Field crops + livestock	6
Field crops + poultry	3
Field crops + horticulture	25
Field crops + Livestock + horticulture	2
Field crops + Livestock + poultry	2
Field crops+ livestock + poultry + horticulture	2
Not sure	3

Analysis of Variance on the demographic characteristics showed that marital status, age, income sources and education level were not significantly different among the EPAs, while significant differences ($p \leq 0.05$).were observed for the type of farming, farm sizes, and type of crops grown in the three EPAs.

2.3.2 Most important crops grown by farmers

Maize, cassava, groundnuts, cotton, rice, sweet potato and pigeon peas were the most important crops in Chinguluwe EPA. In Msitu, respondents mentioned maize, soybeans, common beans, groundnuts and tobacco, while farmers in Ngwangwa EPA indicated maize, groundnuts, tobacco, sweet potato, soybeans, and common beans as the most important crops

(Table 2.4). However, maize and groundnuts were the only crops mentioned in all the three EPAs.

Table 2.4: Crops grown in each EPA in order of importance

Rank	Chunguluwe EPA	Msitu EPA	Ngwangwa EPA
1	Maize	Maize	Maize
2	Cassava	Soybeans	Groundnuts
3	Groundnuts	Common beans	Tobacco
4	Cotton	Groundnuts	Sweet potato
5	Rice	Tobacco	Soybeans
6	Sweet potato		Common beans
7	Pigeon peas		

2.3.3 Maize production at household level

About 56.8% of the households produced enough maize to feed their families for the whole year but only 33.3% of these households realised surplus maize. The majority of the respondents fell into two major categories, those that produced between 6 and 10 bags (50 kg) of maize/year and more than 20 bags/ year (Fig 2.4). However, food sufficiency for the whole year at a household depended on the family size.

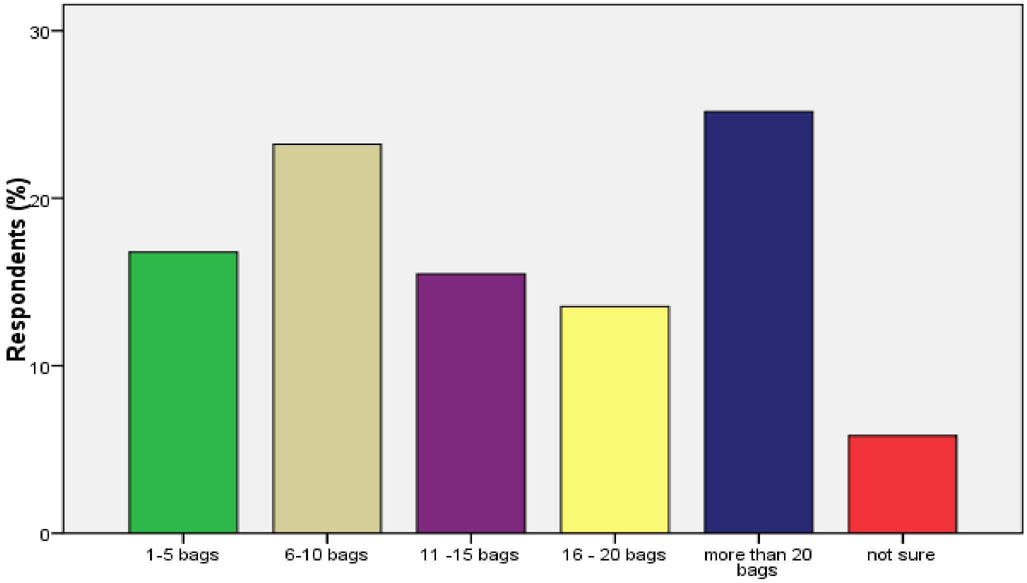

Figure 2.4: Number of maize bags (50 kg) produced per household

2.3.4 Maize production constraints

There were differences on farmers' perceptions on maize production constraints among the three EPAs. In general, the most frequently mentioned maize production constraints were lack of fertilizer, low soil fertility, pests, lack of good seeds, and drought (Figure 2.5).

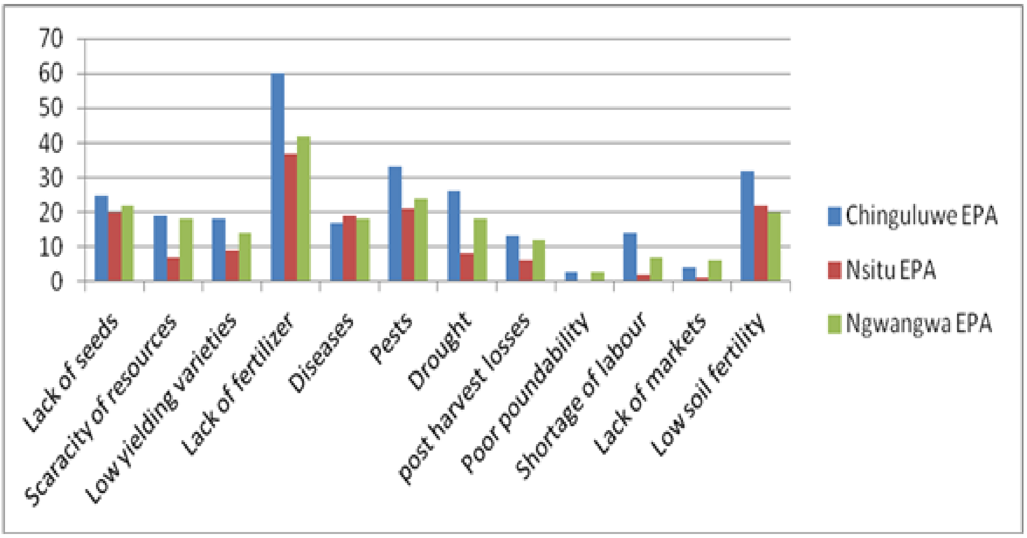

Figure 2.5: Maize production constraints as indicated by farmers in the 3 Extension Planning Areas (EPA)

2.3.5 Storage facilities, yield losses and control measures

Thirty eight percent (38%) of the households used traditional structures to store maize grain, 34.8% combined traditional structures and bags, 27.1% use bags. Farmers had experienced maize losses in storage ranging from 0 to 100 %, with most respondents reporting losses of 25% and 50% of total production within six months after harvesting (Table 2.5).

Table 2.5: Number of respondents reporting yield losses in maize storage facilities

Yield loss	Number of respondents	Percentage
100%	3	1.9
75%	10	6.5
50%	54	34.8
25%	43	27.7
0%	8	5.2
not sure	37	23.9
	155	100

Larger grain borer, maize weevil and rodents were reported as the most common storage pests. Farmers use different control measures to protect their harvest from insect pest attacks, such as insecticides, general sanitation, use of tolerant varieties, grain processing, and early harvesting. The use of synthetic pesticides, such as actellic dust, is the single most commonly used control measure (used by 52.3% of the farmers). However, most of the times farmers used different combinations of control measures to protect maize grain which was a form of Integrated Pest Management.

2.3.6 Interaction between farmers and agricultural extension workers

Fifty four percent (54%) of the households had an excellent interaction with the Agricultural extension officers on storage related problems, 29% discussed storage problems in passing, while 17% indicated that they had never discussed any storage problems with their extension officers. Highly significant differences ($p<0.001$) for interaction levels between farmers and extension officers on storage problems were observed among the EPAs (Table 2.6).

Table 2.6: Level of interaction between farmers and extension workers on storage related problems

Name of EPA	**Excellent**	**Good**	**Fair**	**Poor**	**None**	**Total**
Chinguluwe	33	11	7	11	2	**64**
Msitu	15	11	7	9	0	**42**
Ngwangwa	6	9	5	14	15	**49**
Total	**54**	**31**	**19**	**34**	**17**	**155**

Note: Figures in the table are absolute numbers
Pearson Chi-square = 41.459

2.3.7 Type and source of maize seeds

Most respondents used hybrid seeds (59%), followed by those that combined hybrids and open pollinated varieties (OPV) (32%) and 9% used OPVs only. Farmers got their seeds mainly through recycling, Government subsidy programme, and from commercial seed companies.

2.3.8 Farmers’ perception on important maize characteristics

For maize crop in general, grain size, yield, cob size, poundability, resistance to pests and diseases, storability, and drought tolerance were perceived as the most important attributes by the respondents. However, preferences for maize attributes were significantly different between the EPAs ($p \leq 0.01$). Hybrids were cultivated mainly because of their high yield potential and early maturity than local varieties, while local maize varieties were grown due to good tolerance to pests and diseases, large grain size, large cob size, good yields under low soil fertility, white color, superior poundability, drought tolerance and high storability than hybrids. Preference on traits was generally the same in all the three EPAs (Fig 2.6).

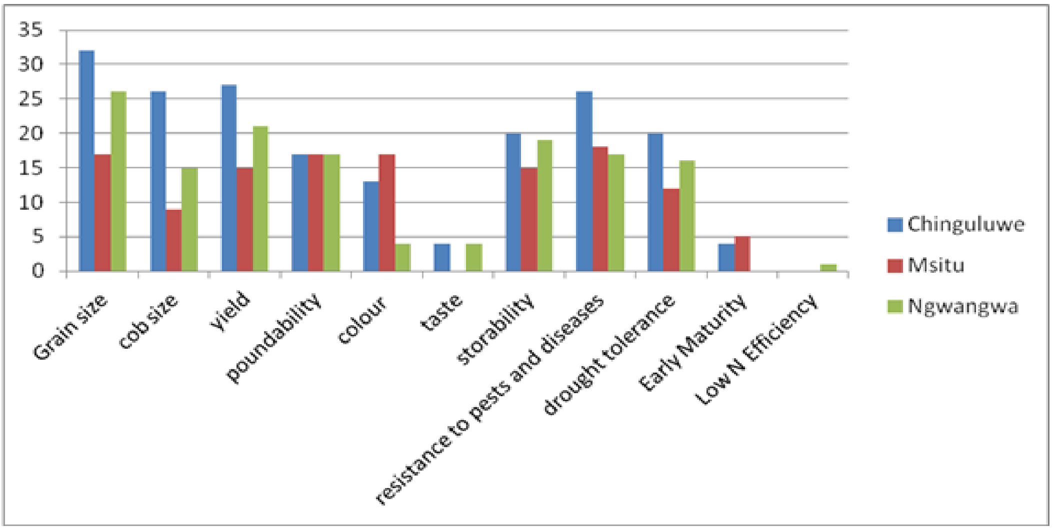

Figure 2.6: Important attributes for selecting local maize varieties

2.3.9 Prioritization on the most important characteristics for local varieties by farmers

During the focus group discussions, respondents were divided into two groups based on gender. One group comprised of males and the other group was made up of females to prioritize on the most important characteristics for local varieties using a scale of 1-8, with 1 being the most important maize characteristic and 8 the least important. Differences in prioritization appeared among the EPAs and between the groups. Yield featured highly as the most important attribute (Table 2.7). In Ngwangwa EPA, the group was predominantly male (only one female), as such gender differences did not apply. The ranking was therefore done by males. Using spearman’s rho, correlation in the rankings between males and females in each EPA were significant. For instance, in Chunguluwe the correlation in ranking between sex was significant ($p<0.01$) with a correlation coefficient of 0.857**, while at Msitu the correlation was significant ($p<0.05$) with a correlation coefficient of 0.810*.

Table 2.7: Ranking of traits in local varieties by sex

	Extension Planning Area (EPA)				
	Ngwangwa	**Chinguluwe**		**Msitu**	
Trait	**Males**	**Males**	**Females**	**Males**	**Females**
Grain size	5	5	5	1	3
Cob size	6	3	4	2	2
Yield	1	1	1	3	1
Poundability	4	4	2	4	4
Color	7	8	7	5	5
Taste	8	7	6	6	8
Storability	3	6	8	7	7
Pest and disease resistance	2	2	3	8	6

Scale 1-8, 1= most important, 8= least important

2.3.10 Traits used by farmers to identify storage pest tolerant varieties

Farmers identified grain hardness, grain size, grain color, poundability and grain texture as the main characteristics used to identify maize varieties that are tolerant to storage pests especially maize weevil and larger grain borer. Surprisingly, 60% of respondents indicated that they would rather get slightly low yielding varieties with high levels of resistance to storage pests than high yielding varieties that are highly susceptible to storage pests.

2.4 Discussion

The distribution between males and females in the sample followed the national trend where approximately 51% of the population are women and 49% are me (NSO, 2008). Most of the farming households were male headed which is typical of Malawi society. Most farmers were largely young (21-35 years), having attended mainly primary school education. This could probably be attributed to high numbers of primary school drop outs. For instance, in 2007, primary school dropout in Malawi was estimated at 65% (Sabates et al., 2011). Some of these pupils end up engaging themselves in farming activities. Though there were differences on the type of crops grown in the areas, maize and groundnuts were the most common crops grown.

About 58% of households produce enough maize for the whole year, few produce surplus maize (18%), slightly lower than national average of 20% (FAOSTAT, 2008). Therefore, there is need for an urgent intervention to reduce the post-harvest losses that farmer’s incur in storage, as it increases food deficit.

Lack of fertilizer, low soil fertility, pests, lack of quality seeds and drought were the main constraints to maize production. Lack of fertilizer and seeds can ably be handled by the government by making farm inputs affordable to smallholder farmers. The problem of pests and diseases, low soil fertility and drought need the intervention of researchers and breeders to provide a long term solution. This could be achieved through the development of maize varieties that are pest resistant, drought tolerant and nutrient utilization-efficient.

Farmers mostly use hybrid maize seeds, followed by those that combine hybrid and local varieties. This concurs with Fisher and Mazunda (2011) who reported that farmers in Malawi use hybrid seeds but they also keep their local varieties. It is worth noting that farmers in these areas have specific hybrids they like, such as DK33, DK 9089, SC 403 and Njovu among a host of varieties available on the market. Farmers however, lamented that most of the maize hybrids are susceptible to storage pests and easily rot, as such they would only opt to increase the acreage of hybrid maize only if they can afford the purchase of pesticides, such as Actellic. Otherwise they would opt for local varieties. This therefore means that farmers have different perceptions from researchers on yield potential, production constraints and resistance to postharvest grain pests in local varieties. In that case, provision of high yielding insect resistant varieties would offer a solution to the quagmire farmers face in making decisions on maize varieties to cultivate. Farmers further complained that most hybrids especially those from multinational seed companies do not stay long on the market despite their preference. They believe that these companies do not serve the interests of the farmers but just making profits out of them.

Farmers were aware of the need to have maize varieties that are tolerant to insect pests and of the existing resistance variation among varieties especially between maize hybrid and locally varieties. Gilbert and Jones (2012) reported that farmers are aware of the large postharvest losses in the improved varieties as compared to local varieties. The use of traditional grain storage structures, bags and a combination of traditional structures and bags to keep maize was common. For example, soon after harvesting, farmers keep their maize with husks in traditional structures, when maize cobs are completely dry, cobs are shelled and grains are stored in bags. Losses of maize in storage are attributed to LGB, maize weevil and rodents. About 24% of the farmers were not sure of the yield losses experienced in their households due to storage pests simply because they never bothered to quantify maize losses incurred in their storage facilities. Others claimed that they did not have enough maize to last long enough to observe grain losses in storage.

In general, grain size, yield, cob size, poundability, resistance to pests and diseases, storability and drought tolerance were the most desired traits in maize. The results agreed with Holden and Lunduka (2010) who reported that farmers in Malawi use a wide range of traits for selecting maize materials for planting. Specifically, high yielding and early maturity were the main reasons for farmers opting for maize hybrids, while good tolerance to pests and diseases, large grain size, high storability, and superior poundability were some of the main reasons for farmers choosing local varieties. Interestingly, farmers in this study did not perceive taste in local varieties as an important trait. This could signal a significant shift in farmers' perception on important traits for opting local maize varieties.

Fisher and Mazunda (2011) reported that storage, high poundability, high flour-grain ratio, and good taste are the key characteristics that farmers look for in local varieties. Holden and Lunduka (2010) also reported storability, poundability, taste, and high flour–grain ratio as farmer preferred traits. Reports from other countries, such as Zambia indicate storability, recyclability, good flour quality, high yielding, readily availability of seed and lack of cash as some of the reasons farmers opt for local varieties (Miti, 2007). Mukanga et al. (2011) reported that farmers look for high yielding, drought tolerance, early maturing, resistance to storage pests and husk cover in opting local varieties and landraces.

Storability, grain hardness, grain size, grain colour, poundability and grain texture were the main maize attributes that farmers use for selecting maize varieties with resistance to MW and LGB Interestingly, grain hardness and other physical grain characteristics have been reported to confer resistance to storage pest damage caused by LGB and MW (Arnason et al., 1992; Kasambala, 2009).

2.5 Breeding perspective

Taking into consideration the wide range of attributes that farmers use when choosing varieties for planting, selection of a large breeding population is a prerequisite when developing maize varieties for small holder farmers. Breeding for insect pest resistant maize varieties should focus on yield and other biophysical grain characteristics, such as grain hardness, grain size, grain colour, poundability and grain texture. Maize breeders should also consider incorporation of other important traits such as drought tolerance, pest and diseases resistance and cob size that were perceived as critical by famers. Since farmers tend to keep their own local seeds, apart from developing hybrids, breeding initiatives should also focus on developing improved open pollinated varieties.

2.6 Conclusion

Farmers in Malawi still cultivate both hybrid and local varieties and use a wide range of traits to select desirable maize varieties for planting. Farmers generally perceive yield as the most important trait in maize varieties. However, under certain circumstances, such as when the hybrid varieties are very susceptible to storage pests and have no resources to buy pesticides, they would opt to grow local varieties. To increase the chances of adoption of varieties by farmers, as many traits as practically possible should be incorporated in the selection index. Breeding for insect resistant maize varieties should focus at developing both hybrids and improved OPVs. Therefore, farmer requirements should be incorporated in the conventional breeding programmes in Malawi. Storability and other traits should be bred in hybrids that are preferred by farmers.

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Chapter 3

Phenotypic and molecular genetic diversity of local maize varieties in Malawi

Abstract

Breeding for storage insect pest resistance in maize is an important breeding initiative in Malawi. Identification of existing genetic diversity among local maize varieties is fundamental in exploring parents for such breeding programmes. The objective of the study was to determine genetic marker diversity of the potential breeding sources for use in introgressing larger grain borer and maize weevil resistance genes in farmer-preferred local varieties. Sixty eight (68) local maize varieties were characterised for genetic diversity using 15 phenotypic markers and 41 SSR markers. Local maize varieties showed significant variation ($P<0.05$) for plant height, ear placement, kernel colour, kernel size, kernel type, days to tassel, days to silking, ear damage, 1000 kernel weight, number of kernel rows. The observed variation in the local varieties was mainly due to 1000 kernel weight, plant height and ear placement. Using phenotypic data, the local varieties were grouped into eight clusters. SSR markers revealed 97.56% polymorphism among the loci. A total of 165 alleles were detected, with a range of 2-9 alleles and an average of four (4) alleles per locus. Gene diversity (H_e) ranged from 0.0298 to 0.7905, with a mean of 0.5115. Heterozygosity (H_o) ranged from 0-1, with a mean of 0.5233. Polymorphism Information Content (PIC) ranged from 0.094 to 0.7565 and showed a mean of 0.4548. A total of 303 allele pairs were obtained, ranging from 2-17 allele pairs per locus. The frequency of major alleles ranged from 0.2540 to 0.9848. The furthest genetic distance was between varieties 206 and local 2 (0.9001) and the shortest genetic distance was between varieties 203 and 811 (0.2189). Based on SRR marker data, the local varieties were grouped into ten clusters. Local maize varieties expressed substantial levels of genetic diversity both at phenotypic and molecular levels. The expressed variation provides an opportunity to explore local maize varieties for useful levels of resistance to maize weevil and larger grain borer.

Key words: Genetic diversity, local maize varieties, phenotypic markers, SSR markers

3.1 Introduction

Phenotypic and molecular characterization plays a crucial role in crop improvement through the identification of variation of individuals and/or populations (Jarvis and Hodgkin, 2005). Variation is important in plant breeding for the identification of cultivars, selection of parents, introgressing of genes into a population and development of new hybrids (Li et al., 2002; Xia et al., 2004; Jarvis and Hodgkin, 2005; Magorokosho, 2006). Genetic diversity within a population is measured by the number of polymorphic genes, number of alleles for each polymorphic gene and the number of genes per individual that are polymorphic (Magorokosho, 2006).

Genetic markers have been employed to characterize materials for genetic diversity and have revealed existing variation among individuals or populations (Jarvis and Hodgkin, 2005). These genetic markers can be phenotypic, biochemical and molecular in nature (Jones et al., 1997). Based on phenotypic and molecular markers, maize has been identified as one of the most diverse crops in the world (Buckler et al., 2006) exhibiting high levels of genetic diversity (Jaric et al., 2010). Because of the wide variation that exists in maize, it is widely grown in different environments across the globe (Shah et al., 2010).

Maize (*Zea mays* L.) is widely grown in Malawi (Ngwira, 2001; Denning et al., 2009). However, its potential yield is compromised by insect pest damage in storage especially maize weevil (MW) and larger grain borer (LGB) (Ching'oma, 2009; Kamanula et al., 2011). This therefore, dictates the need to search for maize materials with useful resistance to such storage pests. A wide genetic diversity among these materials is prerequisite for successful implementation of such breeding initiatives. Breeding for storage insect pest resistance in maize is possible though little progress has been made in identifying genetic resistance of maize grain to storage insects (Derera et al., 2000). For instance, maize has not been fully exploited in breeding programmes especially landraces due to underutilisation of available genetic variation (Warburton et al., 2008). Landraces can be a good source of resistance (Mwololo et al., 2012). Identification of existing genetic diversity among local maize varieties in Malawi would be a starting point in the exploration and exploitation of maize materials for storage insect pest resistant breeding programme. The identified resistant varieties could be used to introgress LGB and MW resistance genes in farmer-preferred local varieties or develop new maize populations resistant to maize weevil and larger grain borer.

Studies have been conducted to identify genetic diversity in maize populations using phenotypic and molecular markers. Warburton et al. (2008) assessed genetic diversity among maize landraces at molecular level and reported the uniqueness of maize landraces as source of new alleles not present in introduced open pollinated varieties. Magorokosho (2006) explored maize diversity in maize varieties from Malawi, Zambia, Zimbabwe, USA and CIMMYT using both phenotypic and molecular markers. According to Magorokosho (2006) open pollinated varieties and landraces grown by farmers in these countries have substantial variation and contain unique traits not present in improved varieties. Reif et al. (2004, 2005) successfully determined the levels of genetic diversity within CIMMYT materials and European maize landraces using SSR markers. Apart from the diversity work done by Magorokosho (2006) that revealed genetic diversity among maize varieties in Malawi, Zambia and Zimbabwe, no comprehensive work has been done or documented in Malawi to reveal the extent of genetic diversity that exists in local maize varieties tailored for a specific maize breeding programme such as storage insect pest resistance screening. In addition to maize hybrids, farmers continue to grow local maize varieties (Fisher and Mazunda, 2011) partly due to their storability. Hence the need to determine genetic marker diversity of the potential breeding sources for use in introgressing LGB and MW resistance genes in farmer-preferred local varieties and the development of new insect resistant maize populations.

3.2 Study objectives

The objectives of the study were to:

a. Assess genetic diversity in local maize varieties using phenotypic markers.

b. Quantify genetic diversity in local maize varieties using SSR markers.

3.3 Materials and methods

3.3.1 Phenotypic diversity analysis of local maize varieties

3.3.1.1 Plant materials and planting

Sixty eight (68) local maize varieties were collected from the National Gene Bank (65) and smallholder farmers (3) in Malawi (Table 3.1).

Table 3.1: List of local maize varieties and origin

Variety	District	Longitude	Latitude	Altitude	Variety	District	Longitude	Latitude	Altitude
172	Nkhatabay	34° 03´	11° 38´	650 m	2027	Lilongwe	34° 04´	14° 02´	131.5m
243	Mzimba	33° 32´	12° 05´	45m	289	Karonga	33° 44´	9° 45´	
322	Rumphi	33° 54´	11° 12´	38m	1786	Dedza			
250	Mzimba	33° 20´	12° 14´	38.4m	699	Zomba	36° 26´	15° 40´	
1772	Ntcheu	34° 45´	15° 01´	91.9m	2872	Likoma	34° 44´	12° 02´	55m
740	Balaka	34° 54´	15° 15´		Local 1	Dowa			
787	Machinga	35°32´	14° 52´		164	Nkhatabay	34° 14´	11° 35´	510m
3414	Zomba	35° 04´	15° 31´	51.8m	1992	Dedza	34° 25´	14° 18´	158.9m
3411	Zomba	35° 11´	15° 23´		725	Balaka	35° 00´	14° 55´	
629	Thyolo	35° 12´	15° 09´	880m	148	Mzimba	35° 44´	11° 18´	1150m
163	Nkhatabay	33° 57´	11° 43´	1300m	206	Mzimba	33° 27´	11° 57´	1200m
1795	Dowa	34° 16´	13° 42´	65.4m	315	Mzimba	33° 26´	11° 15´	1100m
218	Mzimba	33° 20´	11° 53´	37.4m	1845	Ntchisi	33° 52´	13° 22´	141.1m
696	Zomba	35° 21´	15° 34´		260	Chitipa	33° 41´	10° 20´	
199	Mzimba	33° 37´	11° 56´	1410m	2012	Lilongwe	33° 58´	14° 09´	131.5m
410	Chikwawa	34° 41´	16° 22´		445	Chikwawa			
752	Balaka	34° 55´	15° 03´		249	Mzimba	33° 29´	12° 13´	1300m
332	Mzimba	33° 54´	11° 12´	1180m	741	Balaka	34° 54´	15° 11´	
145	Mzimba	33° 45´	11° 26´	1200m	193	Mzimba	33° 36´	11° 54´	1350m
2017	Lilongwe	33° 58´	14° 09´	131.5m	811	Mangochi	35° 33´	14° 40´	
310	Mzimba	33° 36´	11° 17´	1140m	1983	Dedza	34° 24´	14° 21´	163.8m
139	Mzimba				226	Mzimba	33° 27´	11° 41´	1210m
569	Chiradzulu	35° 18´	15° 57´	710m	1915	Kasungu	33° 23´	12° 47´	98.5m
736	Balaka	35° 03´	14° 58´		Local 2	Lilongwe			
303	Mzimba	33° 38´	10° 52´	1120m	1850	Dowa	33° 46´	13° 28´	136.4m
292	Karonga	33° 50´	9° 58´	600m	403	Nsanje	35° 15´	16° 27´	1350m
240	Mzimba	33° 26´	11° 23´	1120m	Knjnj	Blantyre			
386	Nsanje	35° 10´	17° 05´		3243	Mzimba			
750	Balaka	34° 53´			2862	Karonga	34° 02´	10° 09´	52.6m
3244	Mzimba				783	Machinga	35° 32´	14° 55´	
203	Mzimba	33° 32´	11° 53´	1260m	539	Phalombe	35° 44´	15° 40´	710m
1857	Dowa	33° 25´	13° 25´	119.16m	637	Thyolo	35° 15´	16° 23´	240m
584	Chiradzulu	35° 08´	15° 33´	955m	1892	Mchinji	33° 50´	13° 57´	127.4m
297	Karonga	33° 58´	10° 03´	520m	154	Nkhatabay	33° 58´	11° 43´	1000m

The varieties were planted at Chitedze Research Station and Chimoto during the 2011/2012 and 2013/2014 growing seasons, respectively. The two locations belong to two different agro-ecological zones. Field planting was done using the Alpha lattice design (10 blocks and 6/7 entries per block) with 3 replicates. Each replicate was 10 m in width and 124 m in length, giving a total field area of approximately 3720m^2. One unplanted ridge separated the rows and 4 unplanted ridges separated the blocks. One seed was planted at 25 cm between planting stations and 75 cm between rows. A 10 m row represented a plot, translating to approximately 40 plants per plot and 120 plants in total per variety. Hybrid maize “DK8053” was used in guard rows. As a standard practice in Malawi, basal application of fertilizer was done using NPK (23.21.0+4S) and top dressing was done using Urea (46% N) fertilizer at 100kg/ha. Maize fields were weeded twice and an insecticide “Karate” (lambda-cyhalothrin) was applied to control termites.

3.3.1.2 Data collection

Data was collected based on phenotypic descriptors associated with grain characteristics and some important agronomic descriptors. The characteristics measured included, plant height (measured from ground level to the base of the tassel after milking stage), ear placement (from ground level to the node bearing the upper most ear after milking stage), kernel colour, days to tasselling (number of days from sowing to when 50% of the plants had shed pollen), days to silking (number of days from sowing to when silks had emerged on 50% of the plants) kernel type, husk extension, ear damage, kernel row arrangement (using the upper most ear), number of kernel rows (number of kernel rows were determined in the central part of the uppermost ear), kernel colour, kernel size, number of ears per plant, 1000 kernel weight (g), number of tassel branches and yield.

3.3.1.3 Statistical analysis

Data collected was analysed in GenStat Release 14 (Payne et al., 2011). Analysis of Variance (ANOVA) was applied to observe variation among phenotypic traits. Correlation analysis was used to assess relationships between phenotypic traits. Principal component analysis was employed to identify phenotypic traits that significantly contributed to the phenotypic variation observed in the local maize varieties. Cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) was applied to identify group formations among maize varieties. Broad-sense heritability was calculated based on the ANOVA as follows (Hallauer and Miranda, 1988):

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_\epsilon^2 / r}$$

H^2 = Broad -sense heritability

σ_g^2 = Mean sum of square for varieties

σ_ε^2 = Mean sum of square for error r = Replication

3.3.2 Molecular diversity of local maize varieties using SSR markers

3.3.2.1 Plant materials and SSR markers

Seeds from sixty seven (67) maize varieties (Table 3.1) were sent to BecA hub in Kenya for genotyping services. Each genotype was represented by 15 seeds (plants). Seeds were planted in the green house at BecA and three weeks after germination, bulked leaf tissues were harvested from all 15 plants for each variety. Forty one (41) markers which have been used in maize analysis before were picked for the analysis (Table 3.2).

Table 3.2: List of 41 SSR markers used for molecular diversity analysis of local maize varieties

Marker	Motif	Forward_Primer	Reverse_Primer	Annealing_Tm
nc130	AGC	gCACATgAAgATCCTgCTgA	TgTggATgACggTgATgC	54
nc133	GTGTC	AATCAAACACACACCTTgCg	gCAAgggAATAAggTgACgA	
phi014	GGC	ggACCTCATCggCAACAA	CCTCgCTgCTTCgTTCTTATC	
phi029	AGCG	TTgTCTTTCTTCCTCCACAAgCAgCgAA	ATTTCCAgTTgCCACCgACgAAgAACTT	56
phi031	GTAC	gCAACAggTTACATgAgCTgACgA	CCAgCgTgCTgTTCCAgTAgTT	60
phi034	3bp	TAgCgACAggATggCCTCTTCT	ggggAgCACgCCTTCgTTCT	62
phi041	AGCC	TTggCTCCCAgCgCCgCAAA	gATCCAgAgCgATTTgACggCA	56
phi046	ACGC	ATCTCgCgAACgTgTgCAgATTCT	TCgATCTTTCCCggAACTCTgAC	60
phi056	CCG	ACTTgCTTgCCTgCCgTTAC	CgCACACCACTTCCCAgAA	56
phi062	ACG	CCAACCCgCTAggCTACTTCAA	ATgCCATgCgTTCgCTCTgTATC	56
phi063	TATC	ggCggCggTgCTggTAg	CAgCTAgCCgCTAgATATACgCT	
phi065	CACTT	AgggACAAATACgTggAgACACAg	CgATCTgCACAAAgTggAgTAgTC	
phi069	GAC	AgACACCgCCgTggTCgTC	AgTCCggCTCCACCTCCTTC	
phi072	AAAC	ACCgTgCATgATTAATTTCTCCAgCCTT	gACAgCgCgCAAATggATTgAACT	56
phi075	CT	ggAggAgctCACCggCgCATAA	AAAggTTACTggACAAATATgC	54
phi076	GAGCGG	TTCTTCCgCggCTTCAATTTgACC	gCATCAggACCCgCAgAgTC	
phi079	CATCT	TggTgCTCgTTgCCAAATCTACgA	gCAgTggTggTTTCgAACAgACAA	
phi084	GAA	AgAAggAATCCgATCCATCCAAgC	CACCCgTACTTgAggAAAACCC	54
phi085	AACGC	AgCAgAACggCAAgggCTACT	TTTggCACACCACgACgA	
phi090	ATATC	CTACCTATCCAAgCgATggggA	CgTgCAAATAATTCCCCgTgggA	
phi093	AGCT	AgTgCgTCAgCTTCATCgCCTACAAg	AggCCATgCATgCTTgCAACAATggATACA	
phi102228	AAGC	ATTCCgACgCAATCAACA	TTCATCTCCTCCAggAgCCTT	54
phi108411	AGCT	CgTCCCTTggATTTCgAC	CgTACgggACCTgTCAACAA	
phi112	AG	TgCCCTgCAggTTCACATTgAgT	AggAgTACgCTTggATgCTCTTC	
phi114	GCCT	CCgAgACCgTCAAgACCATCAA	AgCTCCAAACgATTCTgAACTCgC	60
phi123	AAAG	ggAgACgAggTgCTACTTCTTCAA	TgTggCTgAggCTAggAATCTC	
phi127	AGAC	ATATgCATTgCCTggAACTggAAggA	AATTCAAACACgCCTCCCgAgTgT	
phi227562	ACC	TgATAAAgCTCAgCCACAAgg	ATCTCggCTACggCCAgA	56
phi299852	AGC	gATgTgggTgCTACgAgCC	AgATCTCggAgCTCggCTA	
phi308707	AGC	gCAACAAgATCCAgCCgAT	gTCgCCCTCATATgACCTTC	54
phi331888	AAG	TTgCgCAAgTTTgTAgCTg	ACTgAACCgCATgCCAAC	
phi374118	ACC	TACCCggACATggTTgAgC	TgAAgggTgTCCTTCCgAT	56
phi96100	ACCT	AggAggACCCCAACTCCTg	TTgCACgAgCCATCgTAT	56
umc1161	GCTGGG	ggTACCgCTACTgCTTgTTACTgC	gCTCgCTgTTggTAgCAAgTTTTA	56
umc1266	CAG	CACAggTAAAAgTAAACgCACACg	CTC gTCATTTTCAACgTCCTCTTT	
umc1304	TCGA	CATgCAgCTCTCCAAATTAAATCC	gCCAACTAgAACTACTgCTgCTCC	
umc1367	CGA	TggACgATCTgCTTCTTCAgg	gAAggCTTCTTCCTCgAgTAggTC	62
umc1545	AAGA	gAAAACTgCATCAACAACAAgCTg	ATTggTTggTTCTTgCTTCCATTA	
umc1917	CTG	ACTTCCACTTCACCAgCCTTTTC	ggAAAgAAgAgCCgCTTggT	52
umc2047	GACT	gACAgACATTCCTCgCTACCTgAT	CTgCTAgCTACCAAACATTCCgAT	
umc2250	ACG	ACAggTCACAgATgTTCATCCAgg	CTCgACTggATCgCCTCCTC	58

3.3.2.1.1 Harvesting of plant tissues

Plant tissues were harvested using a well labelled 96-well box containing one stainless steel ball in each tube. Tubes were placed in ice bucket filled with liquid nitrogen for cooling. A 96–well grid worksheet was positioned in the same way as the plate and labelled accordingly as tissues were being harvested. Approximately 1.2 cm^2 of leaf tissue was placed into each tube.

3.3.2.1.2 DNA extraction

DNA was extracted using a modified CTAB procedure (BecA hub laboratory protocol, Kenya) as follows. The freeze-dried leaf sample (at least 0.01g lyophilized tissue) was crushed into fine powder using GenoGrinder-2000 at a speed of 500 strokes per minute for four minutes. Tubes were spun down for about two to three minutes to bring down the tissues into the bottom of the tube. Freshly prepared modified CTAB extraction buffer (600 ul) was added and ground for two minutes. The samples were incubated at 65^{0}C water bath for 30 minutes with continuous gentle shaking. Tubes were inverted once every ten minutes to homogenize the tissue with the extraction buffer then removed from the water bath and allowed to cool for five to ten minutes in fume hood. Tubes were again centrifuged at 3500 rpm for ten minutes at 15^{0}C. An aqueous phase (500 ul) was transferred into new tubes. Chloroform: isoamylalcohol (24:1) (400 ul) was added into the side of the tubes. The contents were mixed with gentle continuous shaking for 30 minutes at room temperature then centrifuged at 3500 rpm for ten minutes. The aqueous layer was transferred to fresh strip tubes and the chloroform: isoamylalcohol wash was repeated. The upper aqueous layer (400 ul) was transferred into fresh strip tubes and 300 ul of 100% cold isopropanol stored at -20^{0}C was added. The contents were mixed gently in the tubes for five minutes to precipitate the nucleic acid and kept frozen over night at -20^{0}C. The tubes were left on the bench for five to ten minutes, while being gently inverted for about 50x until whitish substance floated. The contents were then centrifuged at 3500 rpm for 30 minutes to form pellets at the bottom of the tube. The supernatant were discarded. About 400 ul of 70% ethanol was added into the tubes and gently inverted to let the pellet float for ease of washing, then centrifuged for 15 minutes. Ethanol was discarded by decantation. The pellet was washed with 200 ul of 70% ethanol and centrifuged for 15 minutes. Ethanol was discarded by decantation. The pellet was allowed to air dry for one hour until ethanol evaporated. A 10mM Tris-HCL at ph 8.3 (150 ul) was added into the tubes and incubated for about 45 minutes at 45^{0}C water bath with gentle tapping every ten minutes. RNase (3 ul) was added after the pellets have completely dissolved. The RNase was spun down with the centrifuge at 3500 rpm for one to two minutes and incubated at 37^{0}C water bath for three hours. The samples were kept in fridge at 4^{0}C awaiting further analysis.

3.3.2.1.3 Quality control and normalization of DNA samples

About 2ul of DNA was loaded in a 0.8% agarose gel and electrophoresed at 120 volts/hour to check the overall sample quality. Most of the samples were found to be of good quality with intact DNA. The concentration and quality were further determined by OD reading using a nanodrop ND-8000. The concentrations were used to guide the normalisation of each sample at a concentration of 50ng/ul. In addition, the ratio 260/280 was provided by the nanodrop revealing purity of the samples. The ratio of most samples was 1.8 to 2.0 within the eptable range for subsequent analysis.

3.3.2.1.4 PCR procedure

PCR reaction conditioning for amplification of DNA was implemented using buffer (10x), MgCl2 (10mM), dNTPs (2.5mM), 1.0 pmoles/ul of primer (F&R), TaqDNA polymerase (5.0U/ul), water, and DNA (50ng/ul). A six step thermal cycler programme was implemented (Table 3.3).

Table 3.3: PCR reaction conditioning for maize DNA sequencing

Components	**Stock concentration**	**One reaction in 10ul**
Buffer	10X	1.0 ul
MgCl2	10mM	0.8ul
dNTPs	2.5mM	0.8ul
Primer F & R	1.0 pmoles/ul	0.2ul
TaqDNA polymerase	5.0 U/ul	0.075ul
H2O		4.725ul
DNA	50 ng/ul	1.0ul
Final volume		10ul
Thermal cycler programme 1. 94^{0}C x 3 minutes 2. 94^{0}C x 30 seconds 3.52^{0}C-60^{0}C x 1 minute for 35 cycles 4. 72^{0}C x 2.0 minutes 5. 72^{0}C x 10 minutes 6. 4^{0}C hold		

3.3.2.1.5 DNA fragment analysis procedure

Approximately 1.0 ml of HIDI-formamide was pipetted into 1.5 ml eppendorf tube. About 12.0 ul of LIZ-500 size standard was added and mixed by vortexing. An aliquot of 9 ul was mixed into each of 96 well plates. PCR products (1.2 ul) were added and denatured at 95^0C then quickly cooled in ice for five minutes.

3.3.2.1.6 Fragment analysis

The PCR products were ran and detected on capillary system ABI-3730 and ABI-3130 using the LIZ500 as internal size standard.

3.3.2.1.7 Data analysis and output

The data from markers was captured using the genescan collection software (Applied bios stems) and the fragments analysed using the gene mapper software version 4.1 (Applied biosystems). A total of 2675 data points were achieved out of the expected 2747 data points giving an overall success rate of 97.4%. The data was compiled into a spread sheet as a standard genemapper output file. The output file was composed of sample ID and marker to identify each genotype. Ned(Y) Pet R) 6-FAM (B) and Vic (G) were used as reference dyes. The sizes for each detected allele were indicated in base pairs. Parameters considered for data quality were indicated in the peak height and genotyping quality (GQ) columns of the excel file (Table 3.4). The lower peaks were verified manually and discarded where necessary. Statistical analysis of data was done using Power marker, version 3.25 (Liu and Muse, 2005) and Popgene, version 1.32 (Yen and Yan, 2002).

Table 3.4: Partial marker data output

Sample ID	Marker	Dye	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	Peak Area 1	Peak Area 2	GQ
811	phi10228	Y	121	121	120.97	120.97	32576	32576	239086	239086	0.1735
1850	phi10228	Y	121	125	120.95	125.19	17340	3283	99053	19563	1
LOCAL1	phi10228	Y	121	125	121.19	125.18	15639	20681	92146	110788	0.1405
811	nc130	G	139	139	138.44	138.44	17832	17832	109431	109431	1
1850	nc130	G	139	139	138.43	138.43	15446	15446	89523	89523	0.3945
LOCAL1	nc130	G	139	141	138.43	141.46	1991	3172	11274	18338	0.6245
811	phi029	B	148	152	147.3	151.78	930	222	6634	1578	1
1850	phi029	B	148	148	147.18	147.18	390	390	2821	2821	1
LOCAL1	phi029	B	148	152	147.07	151.72	868	852	5297	5215	0.3123

3.4 Results

3.4.1 Phenotypic diversity of local maize varieties at Chitedze Research Station

Maize varieties showed significant variation for plant height, ear placement, kernel colour, kernel size, kernel weight, kernel type, days to tasselling, days to silking, ear damage, number of kernel rows ($P<0.05$). No significant variation was observed for husk cover, number of ears per plant, number of tassel branches and kernel row arrangement. Broad sense heritability (H^2) ranged from 0.69 for kernel role arrangement to 0.94 for number of kernel rows (Table 3.5).

Table 3.5: Analysis of variance (ANOVA) for phenotypic traits at Chitedze Research Station

			Trait													
SOV	**df**		**PH**	**EPL**	**KC**	**KS**	**KT**	**DT**	**KW**	**DS**	**KR**	**ED**	**HC**	**EP**	**KRA**	**TB**
variety	67	**MS**	1518.7*	1066*.1	0.1669*	0.1828*	0.16194*	14.44*	7516*	22.791*	2.6376*	0.03473	0.2371	0.03984	0.03646	14.35
Block	9	**MS**	1315.5	926.8	0.09086	0.05999	0.07485	5.91	2986	18.386	1.0396	0.0159	0.2362	0.07843	0.0548	8.7
Residual	127	**MS**	328.2	429.3	0.01948	0.04265	0.04334	12.77	1998	8.391	0.4733	0.01461	0.1919	0.03429	0.04817	11.08
Total	203	**MS**	764.9	661.6	0.07132	0.08969	0.08388	13.02	3863	13.587	1.2127	0.02131	0.2088	0.03807	0.0446	12.05
		R^2	73	59	83	70	68	39	68	61	76	57	43	44	32	42
		CV (%)	8.73	19.46	11.66	9.6	8.67	4.8	13.1	3.78	6.79	48.48	6.4	16.13	15.62	20.63
		SE	18.12	20.72	0.1396	0.2065	0.2082	3.574	44.7	2.897	0.688	0.1209	0.438	0.1852	0.2195	3.329
		H^2	0.93	0.88	0.96	0.93	0.91	0.77	0.92	0.89	0.94	0.88	0.79	0.78	0.69	0.79

Days_to_silking (DS), Ear damage (ED), Ear placements (EPL), Husk cover (HC), Kernel size (KS), Number_of_ears_plant (EP), Number_of_tassel_branches (TB), Plant_hieght (PH), days_to_tassel (DT), kernel colour (KC), number_of_kernels_rows (KR), kernel type (KT), kernel weight (KW), kernel_role_arrangement (KRA). Note: Sg* = significant at $p<0.05$

Plant height

Maize varieties showed significant differences ($P<0.05$) for plant height. The following varieties were the tallest, 297(260.5 cm), 1915 (242.8 cm), 206 (242.3 cm), 303 (234.8 cm) and 164 9 (233.9 cm). The shortest varieties were 2872 (174.3 cm), 193 (172.9 cm), 3243 (161.7 cm), 569 (159.6 cm) and 2862 (107 cm) (Appendix 3.1).

Kernel colour

Significant differences ($p<0.05$) were observed for kernel colour. Maize varieties revealed white, orange, red, pink and variegated kernels (Figure 3.1).

Figure 3.1: Local maize varieties showing variation in grain colour

Ear damage

Significant differences ($p<0.05$) were observed for ear damage. Varieties 289, 240, 403, 164, local 1 were less susceptible to ear damage, while varieties 2862, 2872, 315, 584 and 2027 were more susceptible to ear damage (Appendix 3.1).

Kernel size

Significant differences (p<0.05) were observed for kernel size. The following varieties showed large kernel sizes, local 1, 240, 1892, 154 and 303. On the other hand, varieties 3244, 445, 3243, 569 and 2862 showed the smallest kernel size (Appendix 3.1).

Days to tassel

Significant differences (p<0.05) were observed for days to tasselling. The early tasselling varieties were 629 (72), 811 (71), 3411 (71), kanjerenjere (71) and local 2 (70), while 410 (81), 1795 (80), 154 (79), 218 (79) and 332 (79) were the late tasseling varieties (Appendix 3.1).

Days to silking

Significant differences (p<0.05) were observed for days to silking. Varieties 445 (73), local 2 (73), 1983 (73), 3243 (72) and 2863 (62) produced silks early, while varieties 740 (82), 1772 (81), 240 (81), 139 (80) and 279 (80) started producing silks late (Appendix 3.1).

Kernel type

Significant differences (p<0.05) were observed for grain hardness among the varieties. Varieties, such as 629, 303, 226, 322 and 260 were semi-flint, while varieties, such as 2862, local 1, 3243, 410 and 3244 were dent (Appendix 3.1).

Kernel rows

Significant differences (p<0.05) were observed for number of kernel rows. The following varieties had the highest number of kernel rows, 2872 (12), 206 (12) 2012 (12) 172 (12) and 3244 (12), while varieties 1845 (9) 410(8), 243 (8), 629 (8) and local 1 had the lowest number of kernel rows (Appendix 3.1).

Ear placement

Significant differences (p<0.05) were observed for ear placement. Varieties 584 (179.6 cm), 297 (138.3 cm), 206 (128.6cm), 164 (126.8 cm) and 203 (126.4 cm) showed higher ear placements, while 736 (84.6 cm), 696 (83.5 cm), 2872 (67.1 cm), 3243 (62.8 cm) and 2862 (23.4cm) showed the lowest ear placements (Appendix 3.1).

1000 Kernel weight

Significant differences ($p<0.05$) were observed for kernel weight. Local 1 (465g), 1857 (438g), 240 (413g), 1845 (412g) and 206 (404g) showed high grain weights. Varieties 445 (263g), 403 (258g), 410 (258g), 369 (213g) and 2862 (140g) showed the lowest grain weights (Appendix 3.1).

3.4.1.2 Yield assessment of local maize varieties

No significant differences were observed for yield among the local maize varieties (Table 3.6).

Table 3.6: Analysis of variance for yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block	9	4.4453	0.4939	2.7	0.011
Variety	66	13.3588	0.2024	1.11	0.354
Residual	54	9.8895	0.1831		
Total	129	27.6936	0.2147		

CV (%) =46, sed = 0.4765, H^2 = 0.76

3.4.1.3 Correlation analysis among phenotypic traits

Significant correlations were observed for the phenotypic traits. For example, positive and significant correlations were observed between plant height and kernel weight (0.41), plant height and kernel size (0.43), kernel size and kernel weight (0.72). Negative but significant associations were obtained between yield with 1000 kernel weight (-0.29), and ear damage with plant height (-0.29) (Table 3.7)

Table 3.7: Correlation between phenotypic traits among local maize varieties

KW															
EPL	0.16	-													
ED	-0.19*	-0.02	-												
HC	0.14	0.05	-0.11	-											
KRA	-0.04	-0.04	0.08	0.16	-										
KT	-0.11	-0.1	-0.04	-0.08	-0.01										
EP	0.07	0.01	-0.16	0	0.02	-0.04	-								
TB	0.13	0.16	-0.14	-0.04	-0.12	0.05	-0.01	-							
KRA	-0.16	-0.08	0.15	-0.07	-0.19*	-0.11	0.1	0.12	-						
PH	0.41***	0.54***	-0.29***	0.08	-0.09	-0.08	0.15	0.24**	0.03	-					
DT	-0.7	-0.08	-0.03	0.1	0.16	0.05	0.07	-0.16	-0.08	-0.09					
YD	-0.29***	-0.05	0.11	-0.22*	-0.1	-0.01	0.13	-0.09	0.22*	-0.18*	-0.06	-			
KC	-0.11	-0.15	-0.01	-0.05	0.1	0.26***	0.17	-0.05	0.1	-0.12	-0.04	0.10***	-		
KS	0.72***	0.19*	-0.14	0.11	-0.03	-0.19*	0.07	0.14	-0.17	0.43***	0.01	-0.31	-0.27**	-	
DS	0.02	0.09	-0.09	-0.16	0.08	0.03	-0.11	0.20*	-0.04	0.07	0.11	0.01	-0.09	0.1	-
	KW	**EPL**	**ED**	**HC**	**KRA**	**KT**	**EP**	**TB**	**KRA**	**PH**	**DT**	**YD**	**KC**	**KS**	**DS**

Days_to_silking (DS), Ear damage (ED), Ear placements (EPL), Husk cover (HC), Kernel size (KS), Number_of_ears_plant (EP), Number_of_tassel_branches (TB), Plant_hieght (PH), days_to_tassel (DT), kernel colour (KC), number_of_kernels_rows (KR), kernel type (KT), kernel weight (KW), kernel_role_arrangement (KRA), Yield (YD) . Note: Correlation coefficients with * were significantly correlated p<0.05, ** significantly correlated p<0.01 and *** significantly correlated at p<0.001

3.4.1.4 Principal component analysis

Four principal components accounted for 99.57% of the observed variation. However, the first two principal components accounted for 94.98% of the observed variation (Table 3.8)

Table 3.8: Variation within the local maize varieties as explained by principal component analysis

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
%1000 kernel weight	0.95475	-0.28628	-0.07963	0.0051	0.00624	0.00762	0.00435	0.00096
Ear Placement	0.15407	0.70644	-0.69057	-0.00083	0.01662	0.00379	0.00506	-0.00002
Ear damage	-0.00063	-0.00077	-0.00213	-0.00766	0.00482	-0.00112	0.00405	0.03801
Husk cover	0.00073	-0.00023	0.00023	0.00437	0.01684	0.01744	-0.01984	-0.99248
Kernel row arrangement	0.00005	-0.00088	-0.00057	0.00837	-0.00318	0.00391	-0.02567	-0.10482
Kernel type	-0.00081	-0.00155	-0.00004	-0.00043	-0.00046	0.00153	-0.04753	0.03606
No of ears plant	0.00038	0.00057	0.00217	0.00308	0.00088	0.00398	0.01736	0.00136
No of tassel branches	0.00999	0.02746	0.0165	-0.15127	-0.71109	0.68076	-0.08241	0.00139
Number of kernel rows	-0.00221	0.00383	0.00942	-0.04793	-0.06678	0.0402	0.99355	-0.02105
Plant height cm	0.25357	0.64608	0.71842	-0.01815	0.04111	-0.00909	-0.00656	0.00076
Days to Tasseling	-0.0083	0.00381	0.01074	0.82848	0.28111	0.48205	0.03967	0.0168
Kernel colour	-0.0004	-0.00085	0.00086	-0.00169	-0.00386	-0.00283	0.02736	-0.01484
Kernel size	0.00358	0.00111	0.00139	0.0103	-0.00523	0.00571	-0.00165	0.00143
Days to Silking	0.0151	0.02785	0.01284	0.53649	-0.63915	-0.54958	0.00461	-0.01719
Percent variation	**77.9**	**17.08**	**4.33**	**0.26**	**0.24**	**0.17**	**0.02**	**0**

The variation observed in PC1 was largely as a result of kernel weight, ear placements and plant height. The variation in PC2 was mainly due to ear placements and plant height. Plant height, ear placement were again responsible for the variation accounted for in PC3 and days to tasselling and days to silking contributed significantly to the variation observed in PC4.

The plot of the varieties using the first two principal components depicted maize varieties concentrating between -100 and 100 on the Y axis and between -150 and 100 on the X axis. Local varieties 2862 and 1857 were outliers on the left and right hand side of the plot, respectively (Figure 3.2). Data on phenotypic traits showed that genotype 2862 (outlier) had the lowest mean plant height (107 cm), lowest ear placement (62.46) and smallest kernel weight, while 1847 had one of the highest kernel weight (Appendix 3.1).

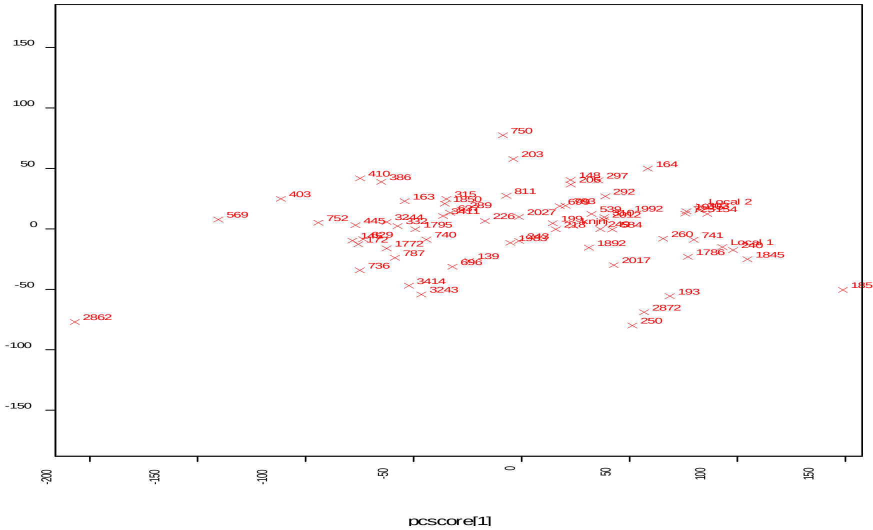

Figure 3.2: Distribution of varieties based on 1st and 2nd principal components

3.4.1.5 Cluster analysis using phenotypic data

Cluster analysis based on the phenotypic data revealed 8 groups. The composition of each group was as follows, Group 1 had 2 varieties, Group 2 (15 varieties), Group 3 (11), Group 4 (5), Group 5 (4) Group 6 (8), Group 7 (11) and Group 8 had 10 varieties. However, local 1 and variety 322 were singletons (Figure 3.3).

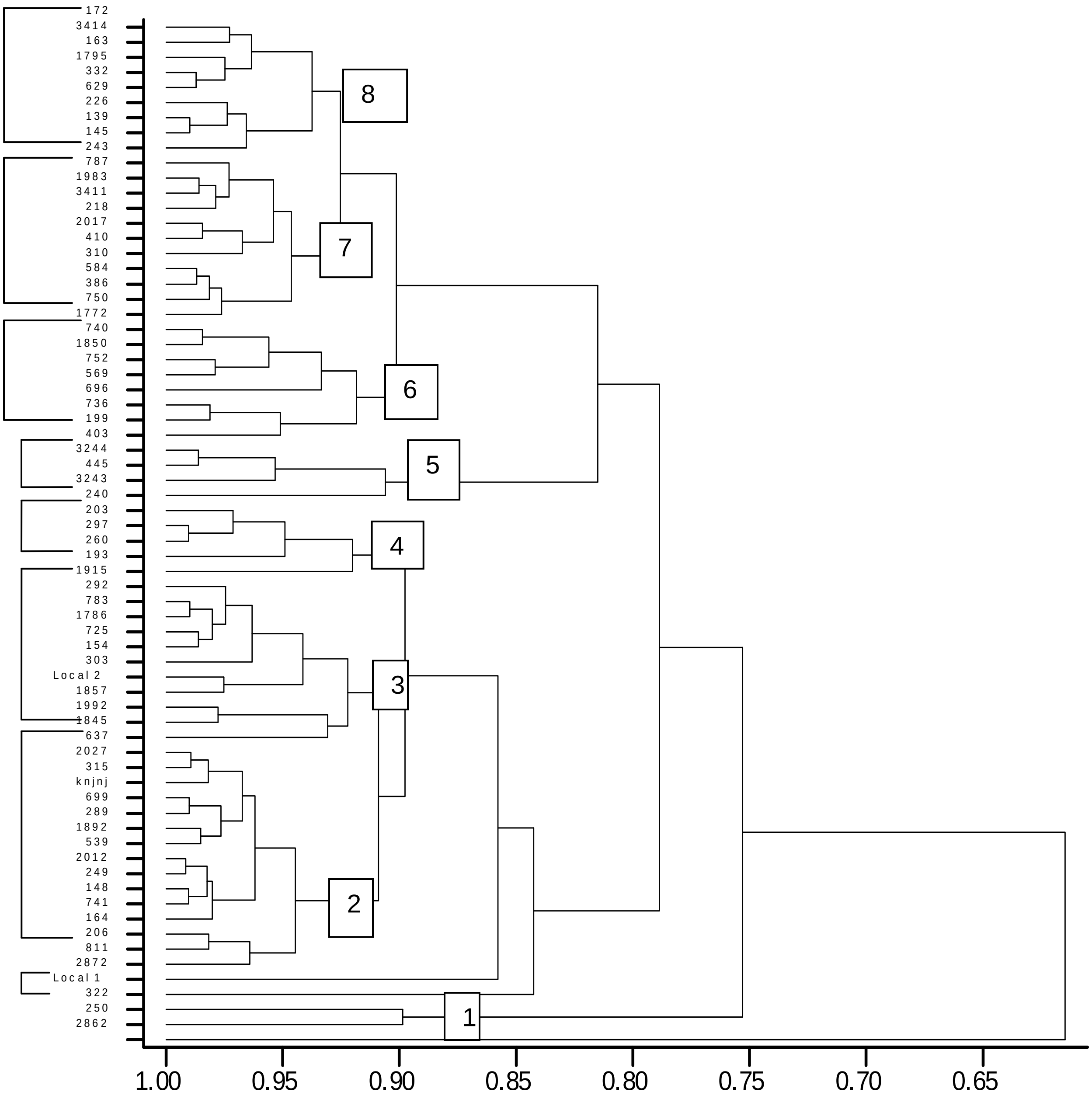

Figure 3.3: Cluster analysis of maize varieties using the unweighted pair-group method with arithmetic averages (UPGMA) based on phenotypic data.

3.4.1.6 Phenotypic diversity of local maize varieties at Chimoto

Significant differences ($p<0.05$) were observed for levels of ear damage. Maize varieties did not show any significant differences for plant height, ear placement, kernel colour, kernel size, kernel weight, kernel type, days to tasselling, days to silking, number of kernel rows, husk cover, number of ears per plant, number of tassel branches and kernel row arrangement.

3.4.2 Molecular diversity of maize varieties using SSR markers

3.4.2.1 Loci polymorphism and number of alleles

SSR markers revealed that 40 loci were polymorphic and 0ne (1) locus was monomorphic, representing 97.56% polymorphism. A total of 165 alleles were detected, ranging between 2-9 alleles and an average of 4 alleles per locus. The least number of alleles (2) were obtained from loci phi046, phi014, phi062, phi112, phi090, phi034, umc1266 and umc2047. The largest number of alleles (9) was found on locus phi079 (Table 3.9).

3.4.2.2 Gene diversity (H_e) and Heterozygosity (H_o)

Gene diversity ranged from 0.0298 to 0.7905, with an average of 0.5115. The largest numbers of polymorphic alleles were observed on locus phi227562, while locus phi112 was monomorphic with 2 alleles. Observed heterozygosity ranged from 0-1, with a mean of 0.5233. Locus phi112 was homozygous (0). Locus umc2250 had the most observed heterozygous alleles, with a value of 1(Table 3.9).

3.4.2.3 Polymorphism Information Content (PIC)

Polymorphism Information Content (PIC) ranged between 0.0294 and 0.7565, with a mean PIC value of 0.4548. Loci phi112 and phi227562 had the lowest and largest PICs, respectively. Nine (9) loci had PIC values of more than 0.6 (Table 3.9).

3.4.2.4 Number of allele pairs (genotypes) and major allele frequency

A total of 303 genotypes were observed with a mean of 7.39 genotypes per locus. The largest number of genotypes were observed on locus umc1545 (17) and the lowest number of genotypes (2) were found on loci phi062, phi112, umc2250 and phi090. The major allele frequencies ranged from 0.2540 to 0.9848 and a mean frequency of 0.5966. The most frequent major alleles were from locus phi112, while the less frequent major alleles were from locus phi227562 (Table 3.9)

Table 3.9: Molecular diversity among local maize varieties

Marker	Major allele frequency	No of Genotypes	Observations.	No of Alleles	Gene Diversity	Heterozygosity	PIC
phi10228	0.6538	7	65	4	0.5292	0.5077	0.4911
nc130	0.6	4	65	3	0.5334	0.7538	0.4552
nc133	0.8636	5	66	3	0.2407	0.197	0.2207
phi227562	0.254	15	63	5	0.7905	0.6508	0.7568
phi029	0.7368	6	57	3	0.4038	0.3684	0.3459
phi031	0.5	9	66	5	0.6748	0.8939	0.6337
phi041	0.4091	13	55	5	0.738	0.4364	0.7002
phi046	0.6591	3	66	2	0.4494	0.4697	0.3484
phi056	0.4167	10	66	5	0.7052	0.7424	0.6548
phi062	0.9621	2	66	2	0.0729	0.0758	0.0702
phi065	0.7045	6	66	4	0.4576	0.5455	0.4114
phi072	0.447	10	66	5	0.6374	0.7121	0.5668
phi075	0.5303	7	66	4	0.5859	0.6818	0.5083
phi076	0.4621	7	66	4	0.5821	0.8939	0.4929
phi079	0.5077	12	65	9	0.6233	0.3692	0.5599
phi084	0.5682	4	66	3	0.5258	0.5303	0.4275
phi112	0.9848	2	66	2	0.0298	0	0.0294
phi114	0.3594	12	64	5	0.7295	0.6719	0.6812
phi123	0.4615	6	65	3	0.6401	0.4154	0.5673
phi2998852	0.3281	15	64	6	0.755	0.7656	0.7167
phi308707	0.6172	6	64	3	0.5406	0.5313	0.4786
phi331888	0.4844	8	64	5	0.5519	0.5	0.4513
phi374118	0.4615	9	65	5	0.616	0.7077	0.5396
phi96100	0.4167	12	66	7	0.6969	0.7576	0.6452
umc1161	0.6923	12	65	6	0.4946	0.4	0.4691
umc1304	0.6429	7	63	4	0.4897	0.3492	0.4092
umc1367	0.8125	5	64	4	0.3168	0.3438	0.2863
umc1545	0.4375	17	56	7	0.7296	0.7857	0.6953
umc1917	0.7727	7	66	5	0.3773	0.3788	0.3466
umc2250	0.5	2	65	3	0.5149	1	0.3973
phi014	0.5859	3	64	2	0.4852	0.6094	0.3675
phi034	0.75	3	66	2	0.375	0.4394	0.3047
phi063	0.4688	8	64	4	0.5983	0.6563	0.5147
phi069	0.375	10	60	4	0.7113	0.4333	0.6574
phi085	0.5565	13	62	5	0.6351	0.4677	0.5989
phi090	0.9242	2	66	2	0.14	0.1515	0.1302
phi093	0.6563	5	64	3	0.4896	0.5625	0.4202
phi108411	0.7955	4	66	3	0.3449	0.3485	0.3165
phi127	0.5985	9	66	5	0.5731	0.6061	0.5217
umc1266	0.9524	3	63	2	0.0907	0.0635	0.0866
umc2047	0.553	3	66	2	0.4944	0.6818	0.3722
Mean	**0.5966**	**7.3902**	**64.2439**	**4.0244**	**0.5115**	**0.5233**	**0.4548**

3.4.2.5 Rare and common alleles within local maize varieties

Alleles, such as 242, 267, 279,136, 161, 171, 171, and 178 were less frequent, while other alleles, such as 154, 162, 134, 142 and 113 were more frequent within the maize population (varieties) (Table 3.10).

Table 3.10: Some rare and common alleles within local maize varieties

Marker	Allele	Count	Frequency	Variance	SD	status
phi056	242	1	0.0076	5.652E-05	0.0075	Rare
phi96100	267	1	0.0076	5.652E-05	0.0075	Rare
phi96100	279	1	0.0076	5.652E-05	0.0075	Rare
umc1917	136	1	0.0076	5.652E-05	0.0075	Rare
phi079	161	1	0.0077	5.826E-05	0.0076	Rare
phi079	171	1	0.0077	5.826E-05	0.0076	Rare
phi079	178	1	0.0077	5.826E-05	0.0076	Rare
phi079	195	1	0.0077	5.826E-05	0.0076	Rare
phi374118	219	1	0.0077	5.826E-05	0.0076	Rare
umc1367	156	1	0.0078	6.008E-05	0.0078	Rare
phi065	147	2	0.0152	0.0001113	0.0106	Rare
phi072	161	2	0.0152	0.0001113	0.0106	Rare
phi075	211	2	0.0152	0.0001113	0.0106	Rare
phi112	160	2	0.0152	0.0002261	0.015	Rare
phi127	127	2	0.0152	0.0001113	0.0106	Rare
phi079	179	2	0.0154	0.0001147	0.0107	Rare
umc1161	137	2	0.0154	0.0001147	0.0107	Rare
umc2250	53	2	0.0154	0.0001147	0.0107	Rare
phi114	170	2	0.0156	0.0001183	0.0109	Rare
phi331888	129	2	0.0156	0.0001183	0.0109	Rare
phi331888	134	2	0.0156	0.0001183	0.0109	Rare
phi063	181	2	0.0156	0.0001183	0.0109	Rare
umc1304	128	2	0.0159	0.000248	0.0157	Rare
umc1545	67	2	0.0179	0.0001537	0.0124	Rare
nc130	139	78	0.6000	0.0009704	0.0312	common
phi308707	131	79	0.6172	0.0019217	0.0438	common
umc1304	132	81	0.6429	0.0023216	0.0482	common
phi10228	121	85	0.6538	0.0016477	0.0406	common
phi093	288	84	0.6563	0.0015717	0.0396	common
phi046	60	87	0.6591	0.0016252	0.0403	common
umc1161	143	90	0.6923	0.0019754	0.0444	common
phi065	131	93	0.7045	0.0011452	0.0338	common
phi029	148	84	0.7368	0.0018629	0.0432	common
phi034	98	99	0.7500	0.0011765	0.0343	common
umc1917	130	102	0.7727	0.0012835	0.0358	common
phi108411	122	105	0.7955	0.0011452	0.0338	common
umc1367	160	104	0.8125	0.0010376	0.0322	common
nc133	113	114	0.8636	0.0010957	0.0331	common
phi090	142	122	0.9242	0.000487	0.0221	common
umc1266	134	120	0.9524	0.0004679	0.0216	common
phi062	162	127	0.9621	0.0002652	0.0163	common
phi112	154	130	0.9848	0.0002261	0.015	common

3.4.2.6 Genetic distances

The furthest genetic distance was between varieties 206 and local 2 (0.9001) and the shortest genetic distance was between varieties 203 and 811 (0.2189) (Table 3.11). Full genetic distance matrix in appendix 3.2.

Table 3.11: Partial genetic distance matrix for the local maize varieties based on SSR marker data

203	0.219	0.3015	0.2538	0.3815	0.2513	0.4408	0.4004	0.3629	0.3082	0.4221	0.3885
750	0.357	0.2636	0.3576	0.3549	0.4181	0.3616	0.3093	0.4143	0.331	0.4896	0.3251
699	0.472	0.36	0.5195	0.3683	0.4581	0.4408	0.3234	0.5108	0.4095	0.435	0.5079
696	0.446	0.3973	0.4613	0.3804	0.4053	0.3881	0.5384	0.5216	0.4215	0.6609	0.4783
193	0.32	0.3071	0.3397	0.4255	0.2252	0.331	0.3781	0.4051	0.4002	0.439	0.4055
249	0.437	0.4894	0.3523	0.4918	0.4994	0.3319	0.4965	0.5815	0.4917	0.5435	0.5214
kjnj	0.524	0.4008	0.4487	0.4595	0.3841	0.2769	0.492	0.5328	0.4876	0.4996	0.4784
297	0.359	0.2796	0.3681	0.3885	0.2957	0.3474	0.3692	0.4608	0.4166	0.5217	0.4219
163	0.499	0.3721	0.4631	0.5401	0.3927	0.586	0.4984	0.4964	0.5682	0.5447	0.522
629	0.425	0.3775	0.4744	0.3328	0.4447	0.492	0.5058	0.6351	0.4609	0.4008	0.4407
260	0.427	0.3393	0.391	0.5596	0.3604	0.4513	0.3961	0.4803	0.4773	0.5748	0.506
164	0.497	0.4602	0.4883	0.3054	0.5827	0.3881	0.4804	0.5805	0.3578	0.4346	0.452
3244	0.586	0.5331	0.551	0.3985	0.4886	0.5572	0.5153	0.5277	0.4399	0.7704	0.6604
local2	0.56	0.5081	0.5103	0.6215	0.547	0.6351	0.5027	0.6674	0.5557	0.9001	0.4601
2012	0.413	0.2791	0.4237	0.3092	0.4079	0.519	0.3051	0.519	0.4478	0.4126	0.4531
243	0.357	0.2656	0.3433	0.3637	0.414	0.4096	0.332	0.4768	0.3661	0.4053	0.3018
1983	0.337	0.4221	0.4229	0.4943	0.3677	0.3382	0.3358	0.4011	0.4504	0.4747	0.4665
226	0.379	0.4846	0.3416	0.4329	0.3751	0.4507	0.4236	0.3579	0.5506	0.4986	0.4525
154	0.495	0.4713	0.4208	0.4997	0.5209	0.4503	0.5044	0.5174	0.4858	0.6384	0.4253
410	0.47	0.3282	0.5124	0.4855	0.3454	0.49	0.3363	0.6045	0.3818	0.5105	0.4009
3414	0.31	0.2414	0.3082	0.2904	0.3536	0.3616	0.3093	0.4009	0.306	0.3702	0.4002
1772	0.317	0.2714	0.3721	0.3675	0.3565	0.3275	0.3364	0.4394	0.4348	0.5136	0.4013
	811	**1850**	**Local 1**	**303**	**199**	**386**	**250**	**740**	**445**	**206**	**1786**

3.4.2.7 Cluster analysis using SSR markers

Cluster analysis of maize varieties using the unweighted pair-group method with arithmetic averages (UPGMA) based on molecular data revealed 10 clusters. Cluster 1 had 2 varieties, Cluster 2 (5), Cluster 3 (7), Cluster 4 (8), Cluster 5(15), Cluster 6 (11), Cluster 7(9), Cluster 8(3), Cluster 9 (2) and Cluster 10 (2). Varieties 1772 and 163 were singletons. The closest genetic distance was between clusters 6 (203) and 3 (811), while the furthest genetic distance was between clusters 9 (206) and 1(local 2) (Figure 3.4).

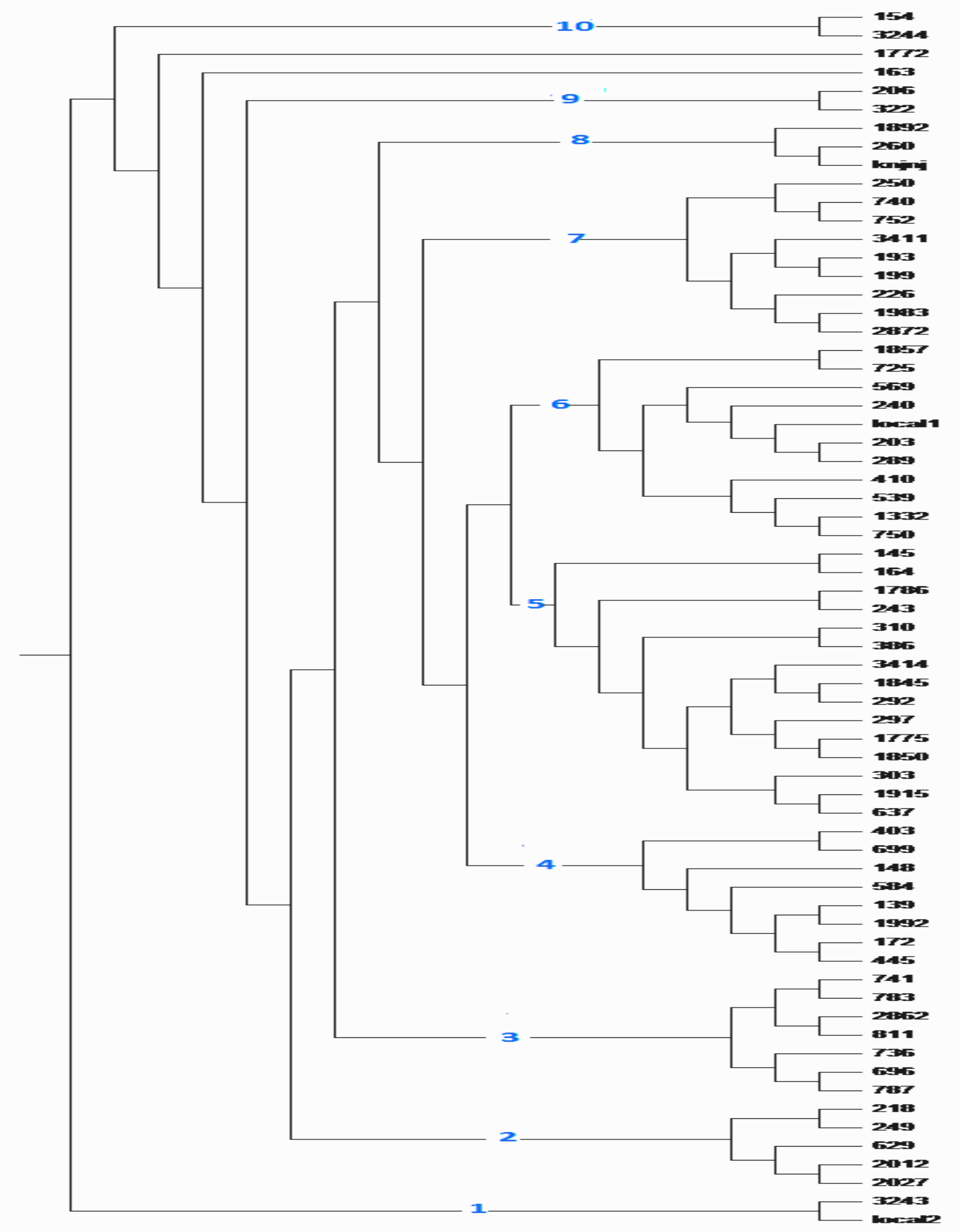

Figure 3.4 : Cluster analysis of maize varieties using Rogers (1972) the unweighted pair-group method with arithmetic averages (UPGMA) based on SSR data

3.4.2.8 Comparison of clusters based on phenotypic data and SSR data

The clusters formed by phenotypic and SSR data were not similar. However, some varieties appeared together in the same clusters for both phenotypic data and SSR data. For example, using phenotypic data, varieties 249, 2012 and 2017 were in group 2, based on SSR data, the varieties appeared together in cluster 2. The origin of the varieties and the clusters developed by the two data sets did not show any obvious pattern. The only notable pattern for SSR data were clusters 3 and 9. In cluster 3, maize varieties were predominantly from districts in the east of the country, except for 1 variety (2862) which originated from the north. Varieties in cluster 9 all came from districts in the north of the country (Table 3.12).

Table 3.12: Comparison between phenotypic data and SSR data clusters and origin

Variety	SSR Clusters	Phenotypic Clusters	District	Region	Variety	SRR Clusters	Phenotypic Clusters	District	Region
3243	**1**	5	Mzimba	North	1850	**5**	6	Dowa	Centre
Local 2		3	Lilongwe	Centre	303		3	Mzimba	North
218	2	7	Mzimba	North	1915		3	Kasungu	Centre
249		2	Mzimba	North	637		2	Thyolo	South
629		8	Thyolo	South	1857	**6**	3	Dowa	Centre
2012		2	Lilongwe	Centre	725		3	Balaka	East
2027		2	Lilongwe	Central	569		6	Chiradzulu	East
741	**3**	2	Balaka	East	240		5	Mzimba	North
783		3	Machinga	East	Local 1		None	Dowa	Centre
2862		1	Karonga	North	203		4	Mzimba	North
736		6	Balaka	East	289		2	Karonga	North
696		6	Zomba	East	410		7	Chikwawa	South
811		2	Mangochi	East	539		2	Phalombe	South
787		7	Machinga	East	750		7	Balaka	East
403	**4**	6	Nsanje	South	332		8	Mzimba	North
148		2	Mzimba	North	250	**7**	1	Mzimba	North
584		7	Chiradzulu	East	740		6	Balaka	East
139		8	Mzimba	North	752		6	Balaka	East
1992		3	Dedza	Central	3411		7	Zomba	East
172		8	Nkhatabay	north	193		4	Mzimba	North
445		5	Chikwawa	South	199		6	Mzimba	North
699		2	Zomba	East	226		8	Mzimba	North
145	**5**	8	Mzimba	North	1983		7	Dedza	Centre
164		2	Nkhatabay	North	2872		2	Likoma	North
1786		3	Dedza	Central	1892	**8**	2	Mchinji	Centre
243		8	Mzimba	North	260		4	Chitipa	North
310		7	Mzimba	North	Knjnj		2	Blantyre	South
386		7	Nsanje	South	206	**9**	2	Mzimba	North
3414		8	Zomba	East	322		none	Rumphi	North
1845		3	Ntchisi	Centre	154	**10**	3	Nkhatabay	North
292		3	Karonga	North	3244		5	Mzimba	North
297		4	Karonga	North	1772	none	7	Ntcheu	Centre
1795		8	Dowa	Centre	163	none	8	Nkhatabay	North

3.4.2.9 Number of migrants

The proportion of migrants among populations using private alleles was at 0.333 Nm, with a mean frequency of 0.5909 (Table 3.13).

Table 3.13: Number of migrants within maize population

Number of populations detected	67
Number of loci detected	41
Mean sample size	0.999234
Mean frequency of private alleles	0.590909
Number of migrants	0.333262

3.5 Discussion

3.5.1 Phenotypic diversity of local maize varieties

At Chitedze Research Station, local maize varieties showed significant differences for plant height, ear placement, kernel colour, kernel size, kernel type, tasselling days, silking days, ear damage, and number of kernel rows. However, no significant differences were observed for yield, husk cover, number of ears per plant, number of tassel branches and kernel row arrangement. Chitedze Research Station experienced normal season in 2011/2012 planting season. At Chimoto, maize varieties did not show any significant differences for all phenotypic traits except for ear damage. The 2013/2014 growing season was characterised frequent dry spells. As such phenotypic makers were affected by the change in environmental conditions (Jones et al., 1997; Collard et al., 2005; Antwi et al., 2012). Magorokosho (2006) found significant variation for number of ears per plant, number of kernel rows per ear, weight of 1000 kernels, days to silking, days to tassel, plant height, ear placements, kernel arrangement, number of primary tassels, cob colour. No significant differences were observed for kernel texture, husk cover and kernel size among landraces and local varieties from Malawi, Zambia and Zimbabwe. Rivella and Tracy (1995) found significant variation for plant height, tassel size but no significant variation for ear and kernel related characteristics in sweet corn land races. Significant variation for days to tassel, days to silking, plant height, ear placements, number of ears per plant among landraces were reported by Antwi et al. (2012). Bige and Lorenzoni (2007) reported high significant variation for silking date, ear height, number of kernel rows and kernel shape among Angola landraces.

Significant correlations were obtained among the phenotypic traits and yield. This was in agreement with Antwi et al. (2012) who reported significant correlations between kernel characteristics with yield. Magorokosho (2006) reported strong correlations among phenotypic markers within landraces and open pollinated maize varieties. The knowledge about these associations is critical when selecting traits of interests in maize germplasm (Bocanski et al., 2009).

Principal component analysis revealed that the variation among the varieties was largely due to kernel weight, plant height and ear placement. For example, the observed variation in PC1 was largely as a result of kernel weight, ear placement and plant height. The variation in PC2 was mainly due to ear placement and plant height. Plant height and ear placement were again responsible for the variation accounted for in PC3 and days to tasselling and days to silking contributed significantly to the variation observed in PC4. This implies that during selection of maize materials for breeding purposes, plant height, ear placement, 1000 kernel weight, days to tassel and days to silking will have significant influence on the outcome of the breeding population. In addition, the broad-sense heritability values for these important traits were relatively high. For example, plant height had a broad-sense heritability of 0.93, 1000 kernel weight (0.92), days to silking (0.89), ear placement (0.88) and days to tassel (0.77). Plant height, ear height, days to tassel, 1000-seed weight and number kernel rows are important in the expression of genetic variation among maize varieties (Jaric´ et al., 2010; Khaldun and Sanda, 2012). The use of Principal components has been reported. For instance, Khavari et al. (2011) used principal component analysis to study variability in new corn hybrids. The Principal component analysis efficiently identified factors that were contributing significantly to the observed phenotypic variation in sweet corn maize.

Cluster analysis categorized maize varieties into eight distinct groups. The clusters were mainly influenced by variation for plant height, ear placement, kernel weight, days to tassel and days to silking. Apart from ear damage, kernel size and kernel colour, all the phenotypic traits that showed significant variation among the varieties, such as kernel weight, plant height, ear placement, days to tassel, number of kernel rows have been recommended for clustering of maize (Sanchez et al., 1993; Magorokosho, 2006). This implies that clusters as shown in Figure 3.3 were a reflection of the possible phenotypic similarities and differences within the clusters and between clusters at Chitedze Research Station.

3.5.2 Molecular diversity of local maize varieties

SSR markers revealed the existence of genetic variation among local maize varieties in Malawi. The existing variation has been demonstrated through high polymorphism among the loci (97.56%), high gene diversity (0.5115), high heterozygosity (0.5233), larger number of genotypes (303), large genetic distances between varieties (0.9001) and highly informative Polymorphism Information Content (PIC) (0.0294-0.7565). These measurements of molecular variation have been used in genetic diversity analysis for maize genotypes and have revealed the existence of molecular variation in different maize materials (Xia et al., 2004; Choukan and Warburton, 2005; Magorokosho, 2006; Legesse et al., 2007; Yu et al., 2007; Wende et al., 2013, Mafu et al., 2014). For instance, using number of alleles within maize germplasm, Legesse (2007) reported a total of 104 alleles, with an average of 3.85 alleles per locus within CIMMYT inbred lines, Xia et al. (2004) reported 566 alleles, with an average of 7.2 alleles per locus among CIMMYT inbred lines. Magorokosho (2006) reported a total of 214 alleles, with a mean of 9.3 alleles and a range of 4–7 alleles in 108 varieties collected from USA, Malawi, Zambia and Zimbabwe. Reif et al. (2006) reported a total of 196 alleles with 7.84 alleles per locus among Mexican varieties. Choukan and Warburton (2005) reported 194 alleles with an average of 4.5 alleles per locus on Iranian and CIMMYT materials. Wende et al. (2013) reported 108 alleles, with allelic range of 1-11 among 20 medium to late maturing tropical maize inbred lines. Mafu et al. (2014) reported the presence of 94 alleles, ranging from 1-9 among 25 inbred lines tailored towards the development of Maize Streak Virus (MSV) resistant hybrids.

Differences in gene diversity and heterozygosity among maize germplasm from different geographic areas appear to be common. For example, Magorokosho (2006) reported gene diversity of 0.652 among USA, Malawi, Zambia, and Zimbabwe varieties, Legesse et al. (2007) reported a gene diversity of 0.59 among African maize inbred lines. The reported diversity figures were slightly higher than those found among maize varieties in the present study (0.5115). This can be attributed to small geographic collection (Malawi) from which the current materials were collected and materials were all open pollinated varieties. Materials reported by other authors (Magorokosho, 2006; Legesse et al., 2007) were a collection from a large geographical area and from different countries, with some of the materials being inbred lines, including tropical and temperate germplasm. The results further showed that the mean heterozygosity among the local maize varieties was 0.5233. This was also indicative of the presence of gene diversity among the varieties (Halliburton, 2004). In contrast, Yu et al. (2007) reported lower heterozygosity among Chinese lines (<0.2).

A wide range of Polymorphism Information Content (PIC) values (0.0294-0.7565) and nine markers showing PIC values >0.6 demonstrated the efficacy of the markers to discriminate local maize varieties based on DNA (Legesse et al., 2007; Wende et al., 2013; Mafu et al., 2014).). Wende et al. (2013) reported correlations between PIC values and number of alleles. Large PIC values were associated with high numbers of alleles. Although in the present study, the locus with the largest PIC value was not linked with the highest number of alleles, but the smallest PIC values were associated with the least numbers of alleles. Speculatively, the difference could be due to type of maize materials used (inbred lines versus open pollinated varieties).

The average number of individuals migrating between population (varieties) per generation was relatively low <1 Nm. This implied that gene flow among the varieties was low (Wolf and Soltis, 1992). Hence, the study results showed some alleles that were rare and in low frequencies among the local varieties. This was possible because, a large proportion of the varieties used in the study have been kept in isolation at the Malawi Gene Bank for long time. This led to reduced selection, no gene flow and no genetic drift among the varieties. As reported by Warburton et al. (2008) genetic diversity can be reduced through genetic drift and selection within the population. This provides an opportunity to find unique and distinct varieties for developing new maize populations.

Phenotypic and molecular data did not form similar clusters. However, some varieties appeared together for both phenotypic and SSR clusters. Both cluster systems produced a large number of clusters, eight and ten clusters for phenotypic and molecular data, respectively. This was an indication of diversity among the local maize varieties. Clustering of varieties into groups in association with origin did not reveal any obvious pattern. However, for SSR marker data, in cluster 3, all but one variety came from districts from the eastern part of Malawi and in cluster 9, all varieties came from the northern region. This showed that there was a high probability of these varieties sharing similar alleles. The clustering of maize materials has been instrumental in understanding the pedigree and origin of maize materials for possible use in breeding programmes, such as Maize Streak Virus resistance breeding (Mafu et al., 2014) and selection for grain yield (Wende et al., 2013). The identified clusters could be valuable information when conducting further evaluations on local maize varieties for resistance against maize weevil and larger grain borer. The clusters may point to similarity in some genes within clusters which may help in selection of materials for evaluations.

3.6 Conclusion

Analysis of variance, cluster analysis, principal component analysis and SSR marker analysis revealed that genetic diversity exists among local maize varieties grown in Malawi. Phenotypically, plant height, ear placement and kernel weight were largely responsible for the observed phenotypic variation. SSR markers revealed high genetic variation through high polymorphism, high gene diversity, high heterozygosity, larger number of genotypes, large genetic distances between varieties and highly informative Polymorphism Information Content (PIC). The expressed variation provides evidence of diversity for exploiting local maize varieties in Malawi for maize weevil and larger grain borer resistance screening.

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Chapter 4

Variation in levels of resistance against maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (*Prostephanus truncatus* Horn) among local maize varieties in Malawi

Abstract

Maize weevil (*Sitophilus zeamais* Motschulsky) and larger grain borer (*Prostephanus truncatus* Horn) are the most important grain storage pests in Malawi. Farmers in the country continue to cultivate local maize varieties because of their perceived tolerance to larger grain borer (LGB) and maize weevil (MW), among other factors. The objectives of the study were to determine levels of LGB and MW resistance among local maize varieties and to identify local maize varieties that can be exploited for LGB and maize weevil resistance breeding. Sixty eight (68) local maize varieties were assessed for MW and LGB resistance using fecundity, grain damage (%), grain weight loss (%) and flour weight. Against maize weevil, maize varieties showed significant differences for adult mortality, median development period, grain damage (%) and number of F_1 progenies. About 14.5% of the varieties were resistant, 21.7% moderately resistant, 24.6% moderately susceptible, 23.2% susceptible and 16% highly susceptible. Maize varieties denoted as 1772, 1983, 1992, 3243, 3244, 750 and 752 showed high resistance to maize weevil. For larger grain borer, significant differences were observed among maize varieties for insect mortality, total number of insects, grain damage (%), weight loss (%) and flour weight. All maize varieties were susceptible to larger grain borer. However, varieties 1992, 2012, and 1983, representing 5% of the entire maize populations had reasonable levels of resistance against LGB. Of interest were local varieties 1992 and 1983 that also showed high levels of resistance to maize weevil. Substantial variation for resistance against MW exists among local varieties. The resistance can be exploited to develop new populations or improve resistance in productive maize populations. For LGB resistance, recurrent selection should be used to increase frequency of resistant genes in the identified varieties.

Keywords: larger grain borer, maize breeding, maize weevil, insect resistance, storage pests, insect resistance variation

4.1 Introduction

Maize (*Zea mais* L) is an important staple food crop in Malawi. However, postharvest losses due to storage insect pests are becoming a serious challenge to food security at household level in the country (Denning et al., 2009). Maize weevil (MW) (*Sitophilus zeamais* Motschulsky) and larger grain borer (LGB) (*Prostephanus truncatus* Horn) are the most important post-harvest pests in Malawi (Makoka, 2008; Singano et al., 2009; Kamanula et al., 2011). Yield losses ranging from 5% to 80% caused by maize weevil have been reported (Tigar et al., 1994; Pingali and Pandey, 2001; Dhliwayo et al., 2005). Larger gain borer is prevalent in Africa and is negatively affecting maize production (Tefera et al., 2011). For instance, about 1.2% of household grain losses of maize in Malawi were reportedly due to LGB (APHLIS, 2015) and from 1995 to 2001, weight loss of stored maize due to the pest increased from 5 to 16% (Denning et al., 2009; Singano et al., 2009).

The management of the two insect pests has relied heavily on the use of chemical compounds, such as Actellic Super dust (Dhliwayo and Pixley, 2003; Ching'oma, 2009). Unfortunately, the use of insecticides to control insect pests such as maize weevil and larger grain borer is being threatened by development of insect resistance (Golob, 2002; Fragoso et al., 2005; Pereira et al., 2009). In addition, these chemical products are generally costly to smallholder farmers (Dhliwayo and Pixley, 2003).

However, host resistance can be integrated into the pest management system and could provide a durable means of resistance to pest damage (Smith, 1994). Unfortunately, host resistance has largely been overlooked in Malawi, mainly due to the promotion of pesticide use against storage pests.

Understanding the variation for resistance that may exist among genotypes is an important step in breeding for durable pest resistance (Mwololo et al., 2010). Differential reaction of genotypes to insect pests can be exploited for breeding purposes (Kitaw et al., 2001). For example, resistant varieties can be combined with other control measures, such as metal silos to protect grains from LGB and MW (Tefera et al., 2011). Thee combination of the biological agent with both resistant and susceptible maize grains increases maize resistance to storage pests through reduced progeny numbers, grain weight loss and frass production (Bergvinson and Garcïa-Lara, 2011).

Genetic variation for resistance against the storage pests has been observed. Variable and useful maize weevil resistance has been reported by Kim and Kossou (2003) in both open pollinated and hybrid cultivars of maize in Africa. Derera et al. (2000) reported variation for resistance against maize weevil among maize genotypes sampled from Southern, Eastern and Western Africa. The existence of weevil resistance variation was also reported among Mexican landraces by Arnason et al. (1992). Abebe et al. (2009) reported variability in resistance against maize weevil in improved maize varieties in Ethiopia. The results showed a decrease in number of F_1 progenies, low seed damage and low seed weight loss among resistant genotypes. For larger grain borer resistance, Ndiso et al. (2007) reported variation for resistance to LGB among maize varieties in Kenya. In Malawi, variation in susceptibility among maize varieties against LGB was reported by Kasambala (2009). Kumar (2002) reported some 19 landraces from the Caribbean which showed resistance to LGB. The observed variation for resistance among the varieties was due to mechanical and biochemical factors, such as phenolic compounds that provide both mechanical resistance and antibiosis in maize grain (Arnason et al., 1992; Derera et al., 2000; Kumar, 2002; García-Lara et al., 2004).

Considering huge grain losses emanating from storage insect pests in Malawi, exploration for variation in maize resistance against maize weevil and larger grain borer among different local maize varieties would be an important step in identifying resistant varieties. The identified resistant varieties could be used for the development of insect resistant maize populations and for improvement of resistance in productive maize populations in Malawi.

4.2 Study objectives

The objectives of the study were:

1. To determine levels of larger grain borer and maize weevil resistance among local maize varieties in Malawi.
2. To identify maize varieties that can be exploited for larger grain borer and maize weevil resistance breeding.

4.3 Materials and methods

4.3.1 Plant materials

Sixty eight (68) local maize varieties were collected from the National Gene Bank and smallholder farmers, in Malawi. The list included 1 commercial hybrid and 1 local landrace (Kanjerenjere) with known resistance against maize weevil and larger grain borer as standard checks for susceptibility and resistance, respectively (list of varieties in chapter 4, Table 4.1).

4.3.2 Planting and experimental design

The maize varieties were planted at Chitedze Research Station during the 2011/2012 growing season using the Alpha lattice design (10 incomplete blocks, each with 6 or 7 entries) and three replicates. Each replicate was 10 m wide and 124 m long, giving a total area of approximately 3720 m^2. One seed was planted per station using 25 cm spacing between plants and 75 cm between rows. The hybrid maize variety “DK 8053” was used in guard rows. Full-sib mating was done for each variety. As a standard practice in Malawi, basal application of fertilizer was done using NPK (23:21:0 +4S) and top dressing was done using Urea (46% N) fertilizers at the rate of 100kg/ha. The fields were weeded twice and Karate (lambda-cyhalothrin) was applied to control termites. At maturity, cobs were harvested and dried at 12-13% moisture content for resistance evaluations in the laboratory.

4.3.3 Rearing of larger grain borer and maize weevil

The rearing of LGB and MW was done at Chitedze Research Station (crop storage facilities) according to the procedures outlined by CIMMYT (Tefera et al., 2010). Unsexed pests were reared in a controlled environment at 28± 1^oC, 65±5% RH, with a 12h: 12h light: dark regime to minimize fluctuations in temperature and relative humidity and promote insect survival (Haines, 1991). The LGB and MW were cultured on susceptible mixed maize grain in sealed but ventilated glass jars. All precautionary measures were taken to exclude other insects from contaminating the cultures. The emergences of new adults were carefully monitored to ensure that insects were of the same generation.

4.3.4 Evaluations of maize varieties for maize weevil and LGB resistance

Maize varieties were evaluated for maize weevil and larger grain borer resistance under lab conditions (controlled environment) using Complete Randomised Block Design (CRBD) with four replications. About 1 kg maize grains from each variety were collected for testing. Grains were fumigated with phostoxin tablets for seven days to avoid carry over insects from the field. One hundred (100) grams of grain were sampled from each of the 1 kg maize grains and placed into jars. Forty-five (45) unsexed adult beetles (7-15 days old) were infested on 100 g of grain and kept inside 250 ml plastic jars for maize weevil and in 400 ml glass jars for LGB (Fig 4.1). A commercial maize hybrid variety 'DK8053' and a local variety 'Kanjerenjere' were used as standard checks for susceptibility and resistance, respectively.

Figure 4.1: Maize samples in plastic containers (250 mls) bottom and glass jars (400 mls) top with insect pests for resistance screening against MW and LGB, respectively

4.4 Data collected

4.4.1 Measurements for maize weevil resistance

The following parameters were used for measuring weevil resistance among the varieties: **Adult mortality** was determined 10 days after infestation; both live and dead insects were counted and discarded. Insects were separated from maize materials using sieves. The **F_1 progenies** were recorded 21 days after the 10 day ovipositioning, the recording was done every 3 days, until no more insects were expected. The **F_1 progeny mortality** was assessed by separating dead progenies from the total number of F_1 progenies. **Damaged and undamaged grains** were counted based on 100 grains randomly selected from each jar. Based on percent grain damage, resistance among maize varieties were conveniently categorised as follows, highly resistant (0%), resistant (≤2%), moderately resistant (2.1-2.9%), moderately susceptible (3-3.9%), susceptible (4-4.9%) and highly susceptible (≥5%). **Susceptibility index** was determined using the susceptibility index developed by Dobie (1974): DSI = $[\mathrm{Log}_e\, Y/t] \times 100$; where DSI = Dobie susceptibility Index, Y = total number of progenies emerging from the treatment, t = median development period (number of days from the middle of the oviposition (day 5) period to the emergence of 50% of the F_1 progeny (Derera et al., 2000). However, where zero or 1 maize weevil emerged, the maximum median development period was calculated based on the last day of insect counting. The values calculated were assigned resistance/susceptibility categories as follows, highly resistant (0), resistant (≤2), moderately resistant (2.1-2.9), moderately susceptible (3-3.9), susceptible (4-4.9) and highly susceptible (≥5). High susceptibility index signified that the maize varieties were susceptible and low susceptibility index meant, maize varieties were resistant. For comparison purposes, **grain weight loss** was also calculated using the damaged and undamaged grains (CIMMYT protocol, Boxall 2002) as follows: Weight loss (%) = $\{(Wu \times Nd) - (Wd \times Nu)/ Wu \times (ND + Nu)\} \times 100$; where Wu= weight of undamaged seed, NU= number of undamaged seeds, Wd = Weight of damaged seed Nd = number of damaged seed. The following categories were used to determine resistance based on grain weight loss: Resistant (grain weight loss ≤ 2%), moderately resistant (grain weight loss between 2.1% and 4%), moderately susceptible (grain weight loss between 4.1 and 6%), susceptible (grain weight loss of between 6.1% and 8%), highly susceptible (grain weight loss ≥8.1%).

4.4.2 Measurements for Larger grain borer resistance

Due to the peak in lab activities at Chitedze crop storage laboratory, a different resistance screening methodology (CIMMYT Protocol) was adopted for LGB that does not require collection of data every 3 days as outlined in section 4.4.1. Collection of data on resistant parameters was done 90 days after infestation. For LGB, the following resistance parameters were collected, total number of insects, insect mortality, grain damage and weight loss and flour weight. **Insect total number of insects** was determined by a total count of both live and dead insects, **insect mortality** was assessed by separating dead insects from the total number of insects. **Percent grain damage** and **Grain weight loss** were determined as indicated in section (4.4.1). **Weight of flour** produced in the jars due to insect damage was separated from insects and maize using sieves and measurements were taken using an electronic weighing balance.

4.5 Data analysis

Data collected on flour weight (g), grain damage (%), grain weight loss (%), number of insects was transformed using log (base e) to normalize variance before subjecting it to the analysis of variance (ANOVA) and correlation analysis in GenStat (Payne et al., 2011). Broad-sense heritability was calculated based on ANOVA as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_\varepsilon^2/r}$$

H^2 = Broad -sense heritability

σ_g^2 = Mean sum of square for varieties

σ_ε^2 = Mean sum of square for error

r = Replication

4.6 Results

4.6.1 Response of maize varieties to maize weevil infestation

Maize varieties showed significant differences for adult mortality, median development period, grain damage (%) and total F_1 progenies, while F_1 progeny mortality and grain weight loss did not show any significant differences. Broad-sense heritability (H^2) among the parameters ranged from 0.84 for grain weight loss (%) to 0.92 for adult mortality (Table 4.1).

Table 4.1: Analysis of Variance (ANOVA) for grain resistance related parameters for maize weevil

			Resistance parameters					
SOV	**Df**		**Adult mortality**	**F_1 progenies**	**MDP**	**Grain damage (%)**	**Weight loss (%)**	**F_1 progeny mortality**
Variety	68	**MS**	0.4756**	0.275**	0.033*	0.2753**	0.1424	0.2462
Block	3	**MS**	0.2461	0.1754	0.0332	0.0931	0.1232	0.165
Residual	204	**MS**	0.1684	0.1418	0.0238	0.156	0.1101	0.1803
Total	275	**MS**	0.2452	0.1751	0.02614	0.1848	0.1186	0.1964
		CV (%)	12.8	22.4	4.3	27.6	35.3	37.1
		lsd (0.05)	0.5721	0.2663	0.2149	0.2093	0.4748	0.3003
		SED	0.2902	0.525	0.109	0.5506	0.2407	0.592
		H^2	0.92	0.89	0.85	0.88	0.84	0.85

Sg** = significant at $P<0.001$, Sg*= significant at $P<0.05$

4.6.1.1 Adult mortality

Highly significant differences ($p<0.001$) for adult mortality were observed among the varieties. The following varieties showed the highest adult mortality numbers: 148, 3244, 2862, 445, 249 and Kenjerenjere (resistant check). Three varieties, namely 148, 3244, and 249 had mean insect mortality numbers of 39.25, 42.5 and 40.75, respectively. These varieties performed better than the resistant check (38.50) (Table 4.2). The variation for adult mortality was normally distributed. The majority of the varieties experienced moderate numbers of adult mortality (Figure 4.2).

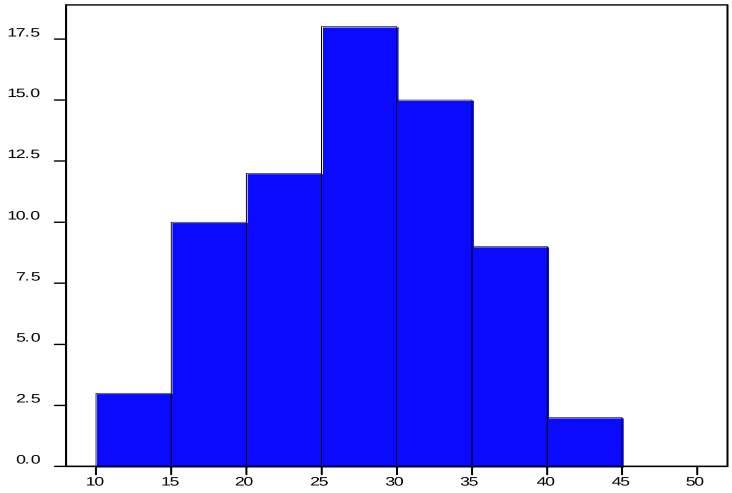

Figure 4.2: Distribution of variation for adult mortality among local maze varieties

4.6.1.2 Total number of F_1 progenies

Highly significant differences ($p<0.001$) were observed for total number of F_1 progenies among maize varieties. Maize varieties 1992, 1772, 3243, 3244, and 403 had the lowest mean number of F_1 progenies (<1). These varieties outperformed the resistant check “Kanjerenjere,” which had a mean value of 2.0 (Table 4.2). Most of the local varieties experienced moderate to lower numbers of F_1 progenies (Figure 4.3).

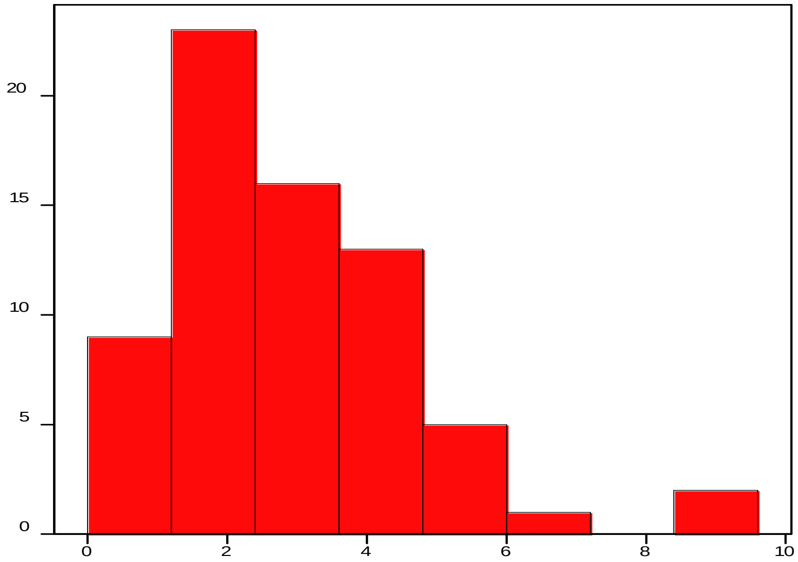

Figure 4.3: Distribution of variation for F_1 progenies among local maize varieties

4.6.1.3 Median Development Period (MDP)

Significant differences ($p<0.05$) were observed among the varieties for the median development period. Varieties 148, 315, 3243, 1992, and 3244 had the longest median development period compared to the resistant check "kanjerenjere" (Table 4.2).The majority of F_1 progenies took moderate to short periods of time to reach the 50% threshold from the middle of oviposition, in the majority of the varieties (Figure 4.4)

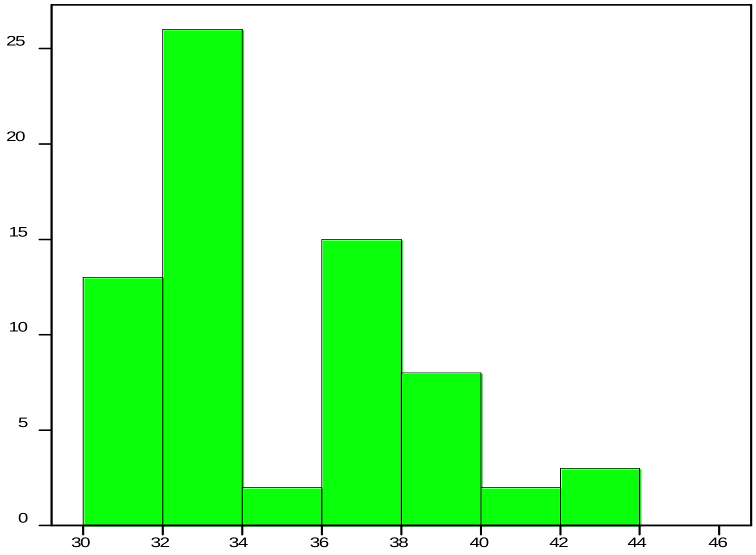

Figure 4.4: Distribution of variation for MDP among local maize varieties

4.6.1.4 Percent grain damage

Highly significant differences ($p<0.001$) for percent grain damage were observed among maize varieties. Varieties 3244, 2012, 445, 250 and 218 had values ≤ 1%. These values were better than the resistant check (2.5) (Table 4.2 and appendix 4.1). The majority of the varieties experienced moderate to little grain damage from maize weevil (Figure 4.5).

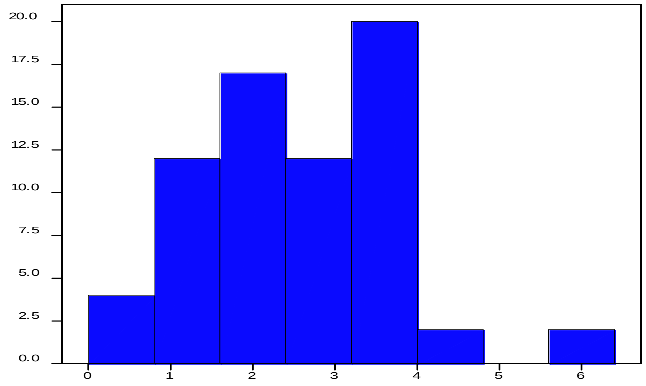

Figure 4.5: Distribution of variation for percent grain damage among local maize varieties

4.6.1.5 Percent grain weight loss

Using percent grain weight loss as an indicator of resistance among the varieties, 9% of the varieties were resistant, 35.3% moderately resistant, 38.2 % moderately susceptible, 16% susceptible and 1.4 % highly susceptible. Varieties 148, 322, 1772, 445, 386 and 218 experienced less grain weight loss (%) (Table 4.2). The grain weight loss (%) due to insects feeding was moderate among most of the varieties. However, few varieties experienced very low grain weight loss (Figure 4.6).

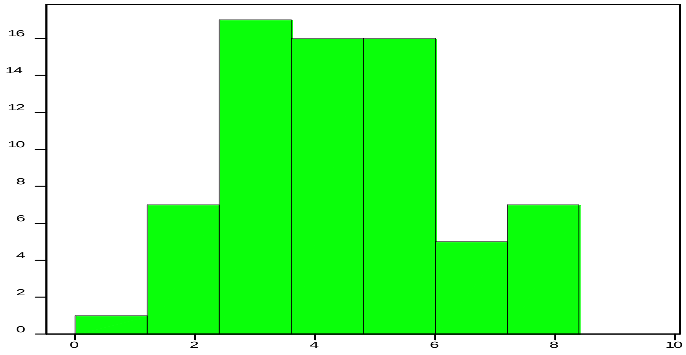

Figure 4.6: Distribution of variation for percent weight loss among local maize varieties

4.6.1.6 Dobie index of susceptibility (DSI)

Using Dobie index of susceptibility, 14.5% of the varieties were resistant, 21.7% were moderately resistant, 24.6% moderately susceptible, 23.2% susceptible and 16% highly susceptible (Figure 4.7). The most promising varieties were 1772, 1992, 811, 699, 249, 403, 1995, 240, 3243, 1983, 750, 752 and 3244. These varieties had values <2 which were lower than the resistant check “kanjerenjere” (2.9) (Table 4.2).

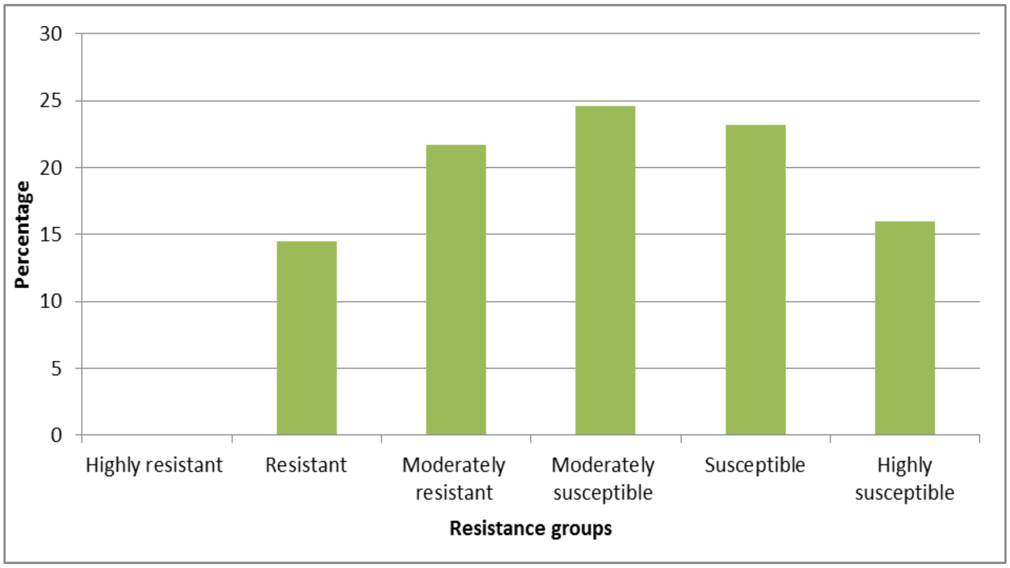

Figure 4.7: Grouping of local maize varieties into maize weevil resistance groups using DIS

Distribution of variation for DIS among the varieties was normal, which means, most the varieties were moderately resistant and moderately susceptible. Few varieties were highly susceptible and highly resistant as shown by left and right tails of the histogram, respectively (Figure 4.8).

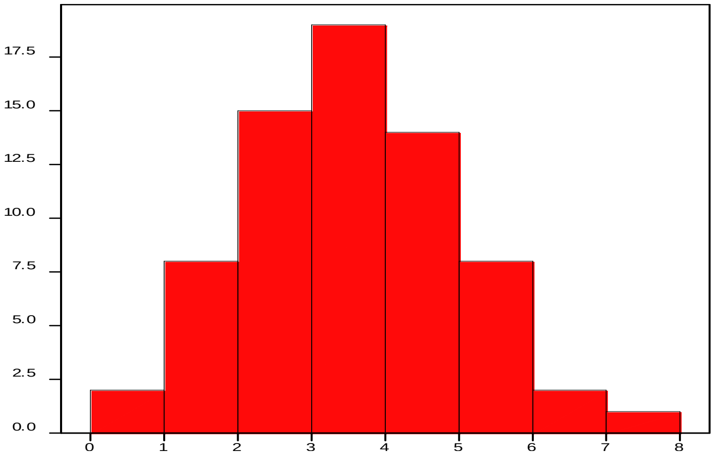

Figure 4.8: Distribution of variation for DIS among local maize varieties

Table 4.2: Table of means for grain resistance parameters against maize weevil

Variety	Adult mortality	Total F1 progenies	MDP	Grain damage (%)	Grain weight loss (%)	DSI	Resistance status	Yield (tons/ha)
139	27.5	3.25	36.5	3.8	5.3	3.92	moderately susceptible	2.32
145	22.0	3.75	33.3	3.5	7.9	4.33	susceptible	3.43
148	39.2	1.75	39.8	2.0	1.8	2.55	moderately resistant	1.51
154	11.3	4.75	34.0	3.8	4.0	4.89	susceptible	2.77
163	23.0	4.00	30.0	3.8	4.4	5.19	highly susceptible	4.15
164	17.8	9.25	30.0	2.8	5.2	7.67	highly susceptible	2.19
172	26.0	1.75	36.0	1.8	6.0	2.71	moderately resistant	1.80
1772	32.0	0.25	43.0	1.3	1.4	0.40	resistant	3.76
1786	16.3	3.75	30.8	1.5	5.8	4.95	susceptible	4.81
1795	30.5	2.75	33.0	3.3	7.9	3.97	moderately susceptible	3.49
1845	25.5	2.25	30.8	3.0	3.4	3.80	moderately susceptible	1.93
1850	33.0	2.25	36.5	1.5	2.9	3.05	moderately susceptible	1.70
1857	29.5	3.75	33.3	3.3	5.3	4.27	susceptible	1.29
1892	17.3	6.50	30.8	2.3	3.7	6.50	highly susceptible	2.80
260	24.3	3.50	33.3	3.0	4.0	4.39	susceptible	2.41
2862	36.0	1.75	36.5	2.8	7.0	2.88	moderately resistant	
2872	20.3	3.75	33.3	3.5	8.0	4.67	susceptible	2.49
289	26.8	2.25	39.8	2.8	4.2	2.54	moderately resistant	2.43
297	20.3	2.00	36.5	2.3	4.1	2.81	moderately resistant	1.54
303	32.8	1.75	34.0	2.8	3.1	2.90	moderately resistant	0.26
310	21.3	2.75	33.3	2.5	3.4	4.05	susceptible	1.02
315	26.8	1.75	39.8	3.3	8.2	2.30	moderately resistant	2.35
322	32.0	2.00	36.5	1.8	1.9	2.68	moderately resistant	1.74
3243	23.3	0.75	43.0	2.3	4.6	1.21	resistant	2.85
3244	42.5	0.75	39.8	0.8	3.4	1.16	resistant	3.91
332	28.5	3.25	30.8	3.3	5.8	4.43	susceptible	2.44
3411	26.5	2.75	36.5	4.0	4.1	3.64	moderately susceptible	2.60
3414	20.3	5.00	30.8	4.0	5.7	5.80	highly susceptible	2.30
386	25.5	3.25	33.3	1.0	1.6	4.04	susceptible	1.60
403	23.5	0.75	40.5	2.3	3.7	1.24	resistant	2.49
410	16.0	4.50	34.0	5.8	5.2	4.35	susceptible	1.65
445	36.0	2.50	33.5	0.3	0.0	3.81	moderately susceptible	3.81
Dk8053	1.07	5.00	30.8	4.8	5.4	5.38	highly susceptible	
Kanjnj	38.5	2.00	33.3	2.5	8.0	2.90	moderately resistant	2.31
local1	30.3	2.75	33.3	2.3	3.2	3.81	moderately susceptible	1.72
193	19.3	2.75	33.3	2.0	4.1	3.69	moderately susceptible	2.79
1983	35.8	2.00	36.5	2.3	3.2	3.06	moderately susceptible	3.16
199	31.5	1.75	36.5	1.3	2.4	2.88	moderately resistant	2.22
1992	39.0	0.5	43.0	2.0	3.5	0.81	resistant	2.88
1995	31.5	1.25	39.8	5.8	7.6	1.49	resistant	
2012	29.0	1.75	33.3	0.5	2.5	2.99	moderately resistant	4.57
2017	33.3	4.00	33.3	2.8	5.7	4.73	susceptible	1.01
2027	29.5	4.00	30.1	4.0	6.0	5.19	highly susceptible	3.17
203	30.3	2.75	33.3	4.5	7.5	4.05	susceptible	2.42
206	34.8	1.75	34.0	3.5	4.0	2.88	moderately resistant	2.39
218	29.8	2.75	36.5	0.5	1.7	3.64	moderately susceptible	0.99
226	24.0	4.25	36.5	2.0	4.9	3.63	moderately susceptible	2.06
240	27.3	0.75	39.8	3.0	2.5	1.32	resistant	1.40
243	18.3	2.75	33.3	1.8	3.8	4.00	susceptible	2.61
249	40.8	0.75	40.5	1.8	2.3	1.24	resistant	2.20
250	27.0	2.75	33.3	1.0	3.1	4.00	susceptible	1.79
292	12.0	5.50	30.0	4.0	6.5	5.97	highly susceptible	2.76
539	31.5	4.75	30.8	2.8	2.8	5.24	highly susceptible	1.35
569	27.8	2.25	33.3	2.0	6.5	3.41	moderately susceptible	4.09
584	14.8	5.25	31.5	3.5	5.4	5.72	highly susceptible	2.13
629	21.8	4.25	37.3	3.8	6.0	4.03	susceptible	2.41
637	20.0	2.50	36.5	1.5	3.7	3.45	moderately susceptible	2.58
696	18.5	3.75	34.0	3.8	4.9	4.38	susceptible	2.24
699	37.8	0.75	33.3	1.5	3.5	1.24	resistant	2.29

Table 4.2…continued

Variety	Adult mortality	Total F1 progenies	MDP	Grain damage (%)	Grain weight loss (%)	DSI	Resistance status	Yield (tons/ha)
725	26.0	3.25	34.8	2.3	3.7	3.63	moderately susceptible	1.48
736	23.5	5.25	30.8	3.3	4.9	5.73	highly susceptible	2.45
740	25.5	8.50	34.0	3.3	6.7	6.28	highly susceptible	3.84
741	40.0	2.00	36.5	1.8	4.2	2.68	moderately resistant	1.81
750	29.0	1.50	39.8	4.0	6.9	2.15	moderately resistant	3.52
752	17.5	2.25	33.3	1.5	3.0	3.32	moderately susceptible	4.18
783	31.5	2.00	36.5	2.5	5.8	3.12	moderately susceptible	2.81
787	36.2	1.75	36.5	1.5	2.5	2.66	moderately resistant	1.46
811	34.0	0.75	39.8	1.3	3.0	1.32	resistant	1.71
local2	34.0	2.00	33.3	1.5	3.6	3.17	moderately susceptible	2.66
mean	**28.63**	**2.76**	**35.01**	**2.47**	**4.41**	**3.44**		
H^2	**0.92**	**0.89**	**0.85**	**0.88**	**0.85**			

DSI= Dobie index of susceptibility, MDP= Median Development Period

4.6.1.7 Correlation analysis among resistance parameters and yield

Correlation analysis showed significant relationships among different resistance parameters. Importantly, highly significant ($p<0.001$) and positive correlations were observed between percent grain damage and percent grain weight loss (0.637), and between percent grain damage and total number of F_1 progenies (0.4299). Negative but highly significant correlations ($p<0.001$) were observed between median development period and DSI (-0.8312), and between median development period and total number of F_1 progenies (-0.6572) Yield potential among the local varieties did not show any significant differences (Chapter 3, Table 3.7). Correlation between yield and weevil resistance parameters was not significant (Table 4.3).

Table 4.3: Correlation among parameters for measuring maize weevil resistance and yield

Adult mortality	1	-						
DSI	2	-0.5923***	-					
Grain damage_%	3	-0.4056***	0.4258***	-				
MDP	4	0.4108***	-0.8312***	-0.2678*	-			
Total F1 progenies	5	-0.5755***	0.9369***	0.4299***	-0.6572**	-		
Weight loss %	6	-0.2937*	0.3395**	0.637***	-0.2478*	0.3418**	-	
Yield tons ha	7	-0.1266	0.0368	-0.0654	-0.0582	0.0337	0.206	-
		1	2	3	4	5	6	7

Note: Correlation coefficients with * were significantly correlated $p<0.05$, ** significantly correlated $p<0.01$ and *** significantly correlated at $p<0.001$

4.6.2 Response of maize varieties to larger grain borer infestation

Significant differences in response of maize varieties to larger grain borer were observed for insect mortality, total number of insects, grain damage (%), flour weight (g) and grain weight loss (%). Broad-sense heritability (H^2) ranged from 0.65 for grain weight loss to 0.97 for flour weight (Table 4.4).

Table 4.4: Analysis of Variance (ANOVA) for grain resistance related parameters against larger grain borer

SOV	df		Insect mortality	Total number of insects	Flour weight	Grain damage (%)	Grain weight loss (%)
variety	66	**MS**	0.131**	0.078*	0.212**	0.451**	0.051**
Block	3	**MS**	0.627	0.175	0.162	0.068	0.349
Residual	198	**MS**	0.09	0.047	0.027	0.108	0.108
Total	267	**MS**	0.106	0.056	0.074	0.193	0.168
		CV (%)	9.2	5.5	8.4	11.7	13.4
		lsd (0.05)	0.4195	0.3021	0.229	0.459	0.462
		SED	0.2127	0.1532	0.116	0.233	0.234
		H^2	0.85	0.87	0.97	0.94	0.65

Sg** = significant at P<0.001, sg*= significant at P<0.01

4.6.2.1 Total number of insects

Maize varieties showed significant differences (p<0.01) for total number of insects. Varieties 172, 164, 699, 410, and 322 experienced the lowest number of insects than the resistant check (42.75) (Table 4.5). The majority of the varieties experienced moderate number of insects, while very few varieties had high and lower numbers of insects as shown by the right and left tails of the histogram, respectively (Figure 4.9).

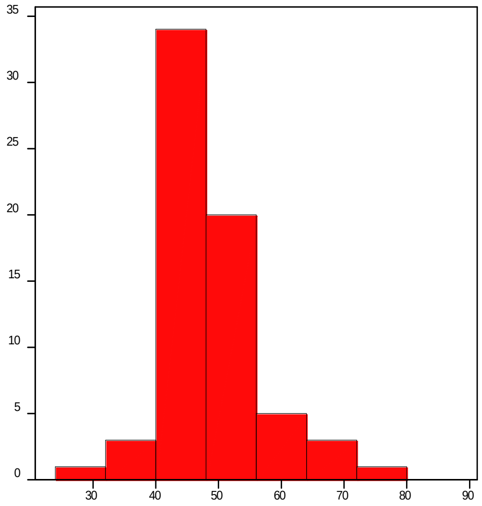

Figure 4.9: Distribution of variation for total number of insects among local maize varieties

4.6.2.2 Insect mortality

Significant differences ($P<0.05$) for insect mortality were observed among varieties. Varieties 1992 (33.5), 445 (36.5), 1983 (41.5), 292 (38.08) and 154 (41.25) had the highest number of dead insects and outperformed the resistant check "Kanjerenjere" (23) (Table 4.5). The majority of the varieties had medium to lower number of dead insects. However, few varieties experienced extremely high number of dead insects (Figure 4.10).

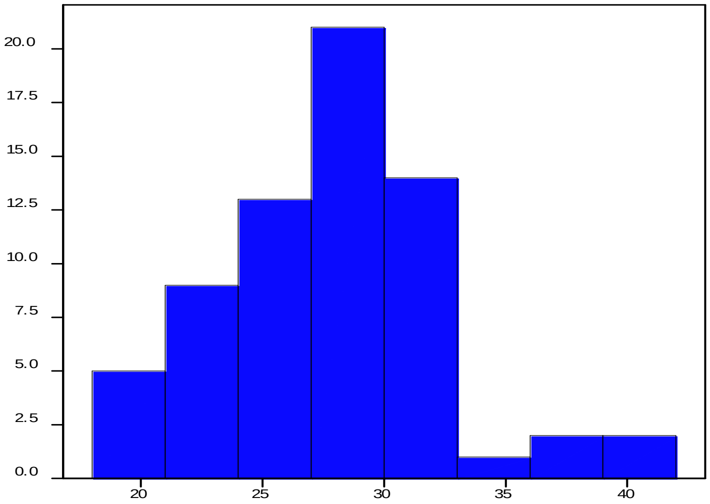

Figure 4.10: Distribution of variation for insect mortality among local varieties

4.6.2.3 Flour weight (g)

Highly significant difference (p<0.001) for flour weights were observed among the varieties. The best performers with the least amount of flour produced were varieties 1983 (0.95g), and 1992 (1.8g). Resistant and susceptible checks had 3.225g and 4.225g of flour produced, respectively. The worse performers with high amount of flour were varieties 304 (8.5g), 154 (8.1g), 1957 (7.95g), 260 (7.82g) and 310 (7.6g) (Table 4.5). The maize varieties produced moderate to high amount of flour as shown by the skewness to the right of the distribution of variation for flour weight (Figure 4.11).

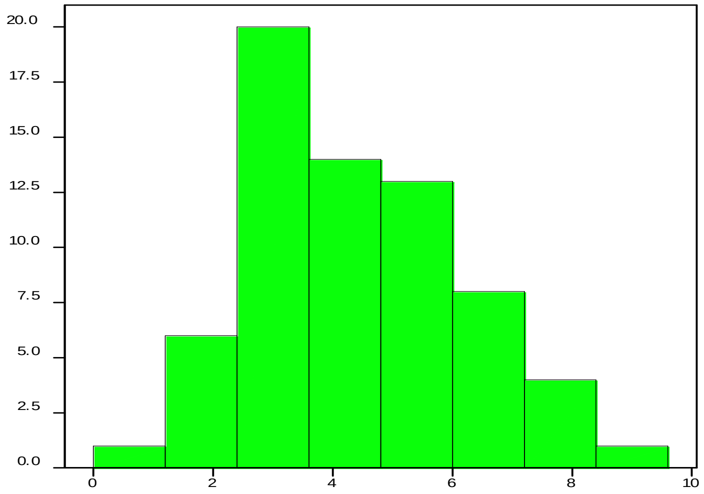

Figure 4.11: Distribution of variation for flour weight among local varieties

4.6.2.4 Grain damage (%)

High significant differences (p<0.001) for percent grain damage were observed among the varieties. Varieties 1983, 1992, and 2012 experienced the least grain damage ranging from 5-6.75%, while the resistant and susceptible checks had 10.75% and 13.50% grain damage (%), respectively (Table 4.5 and Appendix 4.1). Maize varieties experienced largely moderate grain damage, with exceptions of few varieties that experienced high grain damage (Figure 4.12).

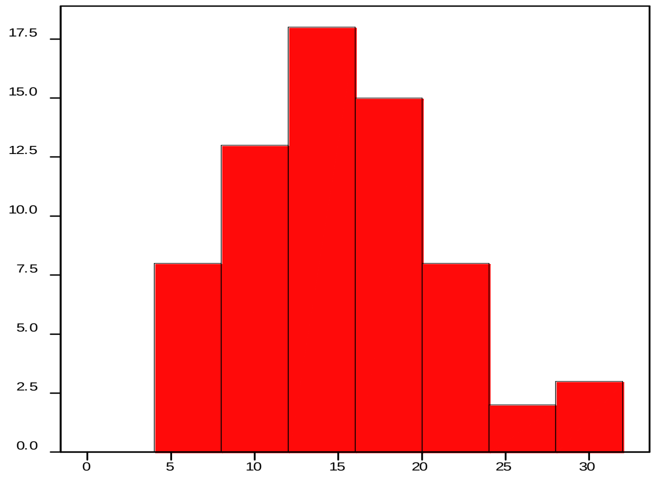

Figure 4.12: Distribution of variation for grain damage (%) among local varieties

4.6.2.5 Grain weight loss (%)

Percent grain weight loss showed highly significant differences (p<0.001) among the varieties. Despite showing significant differences, percent grain weight loss as a measure of resistance determined that all the varieties were highly susceptible to LGB. However, a good number of varieties such as 1983 (10.64%), 1850 (13.33%), 1992 (12.93%), 2012 (12.01%), 386 (11.89%) and 2017 (13.37%) performed better than the resistant control (kanjerenjere) (15.62%). Varieties, such as 310, 260, 292, 303, and 154 performed worse than the susceptible commercial hybrid (DK8053) (Table 4.5). Most varieties experienced low to moderate weight losses except for a few susceptible varieties that had high grain weight loss (Figure 4.13).

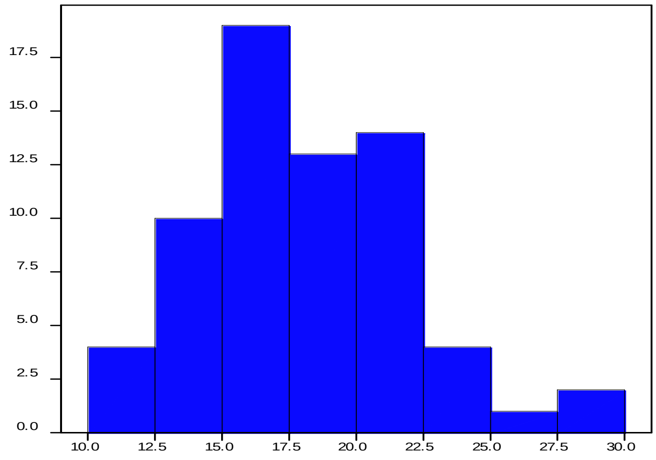

Figure 4.13: Distribution of variation for grain weight loss (%) among local maize varieties

Table 4.5: Table of means for grain resistance parameters against larger grain borer

Variety	Total insect number	Insect mortality	Grain damage (%)	Grain weight loss (%)	Flour weight (g)	Yield (tons/ha)
139	47.5	25.3	18.0	20.4	5.5	2.32
145	51.3	30.3	18.3	19.3	5.3	3.43
148	54.0	29.3	16.3	17.5	6.3	1.51
154	71.8	41.3	25.0	23.6	8.1	2.77
163	43.0	23.8	14.3	19.8	4.1	4.15
164	39.8	18.8	20.8	21.5	4.7	2.19
172	40.5	24.0	13.0	17.4	3.5	1.80
1772	51.8	29.8	22.8	21.5	4.1	3.76
1786	48.0	21.8	17.8	19.6	4.6	4.81
1795	43.8	26.8	13.3	17.9	3.1	3.49
1845	66.5	30.3	18.3	20.6	6.1	1.93
1850	40.5	30.0	7.8	13.3	2.3	1.70
1857	79.3	31.0	22.5	23.1	8.0	1.29
1892	54.5	23.5	20.8	21.0	6.3	2.80
1915	48.5	29.0	12.8	16.9	3.4	
193	47.8	24.8	18.3	20.8	4.9	2.79
218	53.8	26.5	20.0	21.4	6.2	0.99
249	38.5	25.3	9.9	15.3	2.4	2.20
250	57.8	31.8	18.5	20.6	5.5	1.79
260	55.0	21.3	30.8	28.6	7.8	2.41
2872	50.0	32.8	11.3	16.8	3.0	2.49
289	46.8	33.0	8.3	14.7	2.4	2.43
292	56.6	38.1	21.5	21.6	6.5	2.76
297	58.8	24.5	19.0	21.5	6.8	1.54
303	55.8	26.8	31.0	26.3	8.5	0.26
310	60.5	28.3	32.0	28.5	7.6	1.02
315	43.3	28.3	10.8	14.7	3.0	2.35
322	40.8	20.5	13.5	18.5	5.3	1.74
3243	46.0	32.8	10.8	15.2	2.2	2.85
3244	46.5	30.3	7.3	12.1	3.1	3.91
332	49.3	29.5	13.3	16.6	4.0	2.44
3411	52.1	29.0	8.8	13.7	3.8	2.60
3414	49.0	31.0	12.5	17.5	3.1	2.30
206	41.0	27.5	14.0	17.2	3.2	2.39
740	49.0	31.3	13.8	16.8	3.4	3.84
741	41.3	28.3	10.3	15.0	2.7	1.81
750	44.3	19.0	22.3	21.0	5.5	3.52
752	41.3	26.5	11.3	15.5	3.4	4.18
783	68.8	31.0	26.5	24.4	6.2	2.81
787	40.5	28.0	14.3	17.4	3.7	1.46
811	42.0	26.0	15.0	17.7	3.3	1.71
DK8453 (s)	47.3	27.8	13.5	18.2	4.2	
Kanjnj (r)	42.8	23.0	10.8	15.6	3.2	2.31
local 1	49.8	21.5	14.5	17.9	5.8	1.72
local 2	54.3	27.8	17.3	19.8	5.0	2.66
1992	42.8	33.5	6.8	12.9	1.8	2.88
2012	38.0	29.5	6.8	12.0	2.1	4.57
2017	45.0	28.3	7.8	13.4	3.9	1.01
2027	50.5	30.0	10.0	14.4	2.7	3.17
203	45.8	20.3	19.0	19.5	6.0	2.42
725	41.3	25.3	13.8	17.2	4.0	1.48
1983	47.0	41.5	5.0	10.6	1.0	3.16
199	45.8	27.5	13.3	17.7	3.7	2.22
569	43.8	31.8	12.3	16.1	3.1	4.09
629	50.5	31.8	18.3	20.7	5.8	2.41
637	41.8	27.5	11.0	15.9	4.0	2.58
696	41.8	21.3	21.0	22.4	4.5	2.24
736	46.8	18.8	17.8	19.2	5.5	2.45
386	41.0	26.5	8.0	11.9	3.0	1.60
403	42.8	22.8	8.0	13.7	2.7	2.49
410	40.8	29.0	13.5	16.1	4.0	1.65

Table 4.5 ………..continued

Variety	Total insect number	Insect mortality	Grain damage (%)	Grain weight loss (%)	Flour weight (g)	Yield (tons/ha)
445	45.00	36.5	8.3	14.3	2.0	3.81
539	49.75	30.0	12.5	16.6	3.2	1.35
699	26.00	26.0	8.5	13.4	2.7	2.29
226	50.25	25.3	17.5	18.7	5.1	2.06
240	59.75	31.0	18.0	20.7	6.1	1.40
243	50.75	28.5	23.8	22.6	5.2	2.61
Mean	**50.88**	**28.1**	**16.8**	**19.3**	**4.8**	
H^2	**0.87**	**0.9**	**0.9**	**0.7**	**1.0**	

4.6.2.6 Correlation between LGB resistance parameters and yield

Highly significant correlations ($p<0.001$) were observed between grain weight loss (%) and grain damage (%) (0.8828), between flour weight and grain damage (%) (0.9789), and between flour weight and grain weight loss (%) (0.8722). Correlations between yield and resistance parameters were not significant except for flour weight (0.3599) (Table 4.6).

Table 4.6: Correlation among resistance parameters for LGB and yield

Flour weight (g)	1	-					
Grain damage%	2	0.8828**	-				
Insect mortality	3	-0.1868	-0.195	-			
Total number of insects	4	0.7099**	0.6128**	0.3454*	-		
Weight loss %	5	0.8722**	0.9789**	-0.216	0.6137**	-	
Yield tons ha	6	-0.3599*	-0.2284	0.1226	-0.1858	-0.2345	-
		1	2	3	4	5	6

Note: Correlation coefficients with * were significantly correlated at $p<0.01$, ** significantly correlated at $P<0.001$

4.7 Discussion

4.7.1 Maize weevil resistance among local maize varieties

Substantial variation in resistance against maize weevil exists among local maize varieties in Malawi. The variation for resistance was confirmed by significant differences for adult mortality, total F_1 progenies, median development period, percent grain damage, percent grain weight loss and Dobie index of susceptibility among local maize varieties. These results are in agreement with reports by Giga and Mazarura (1991) who found variation for maize weevil resistance among exotic, local open pollinated varieties and maize hybrids obtained from Malawi, Zimbabwe and Mexico. Resistant varieties showed low adult mortality, low percent grain weight loss and low Dobie Index of susceptibility.

Percent grain weight loss as an indicator of susceptibility appeared to be more conservative in identifying resistant varieties than Dobie Index of susceptibility. Furthermore, percent grain weight loss and Dobie Index of Susceptibility (DIS) showed a weak but significant correlation (0.3395) at $p \leq 0.01$. This weak but significant relationship probably could be an indication that the two indicators of susceptibility have a small chance of identifying similar resistant varieties. Hence, the two indicators only identified one common resistant variety (1772) among the top most resistant varieties. In addition, DSI significantly correlated with the other resistant parameters at $P \geq 0.001$. Combining DIS, percent grain weight loss, percent grain damage, total number of F_1 progenies and adult mortality, maize varieties 1772, 1992, 3243, 3244, 148, 322, 445, 386, 218, 2012, 741, 699, 811, 1983, 249, 403 and 250, were identified as the most resistant varieties. However, using percent weight loss alone, only varieties, 148, 218, 322, 386, 445 and 1772 were identified as resistant. It is however, worth noting that distribution of variation for DIS was normal. This indicates that for DIS was a better parameter for discriminating maize varieties for weevil resistance in this study. The differences in the distribution of variation among resistance parameters signified the existing variation for weevil resistance among the maize varieties.

The use of percent grain weight loss, percent grain damage, fecundity and DIS as indicators of susceptibility or resistance has been documented (Derera et al., 2000; Kitaw et al., 2001; Abbe et al., 2009; Mwololo et al., 2012). Mwololo et al. (2012) used grain weight loss, grain damage (%) and number of insects to differentiate levels of weevil resistance among maize varieties in

Kenya. Resistant varieties showed low grain weight loss, low grain damage (%) and low number of insects as also established by Abbe et al. (2009) among varieties in Ethiopia. Derera et al. (2000) and Kitaw et al. (2001) demonstrated that resistant varieties can be identified using adult weevil mortality, grain damage (%), progeny numbers, median development period and Dobie index of susceptibility. From a breeding perspective, the grain resistance parameters showed good levels of broad-sense heritability. For example, F_1 progenies showed a broad-sense heritability of 0.89, adult mortality (0.92), weight loss (%) (0.85), MDP (0.85) and grain damage (%) (0.88).This indicates that these parameters are heritable as reported by Dhliwayo and Pixley (2003).

The resistance observed in maize varieties against maize weevil could be due to biophysical grain factors or antibiosis (Derera et al., 2000; García-Lara et al., 2004). For example, Mwololo et al. (2013) reported differences in grain hardness between resistant and susceptible varieties due to protein composition within the grain structure. Taking into consideration that many traits are involved in maize weevil resistance (Mwololo et al., 2013), a multi-trait breeding approach to maize weevil resistance breeding is crucial. For example, the use of molecular markers, exploitation of husk cover, physical grain characteristics and chemical composition (Meikle et al., 1998; Derera et al. 2000; García-Lara et al., 2004; Reif et al., 2004; Mwololo et al., 2013) can lead to a successful maize weevil resistance breeding programme. However, central to this approach is the identification of the nature of gene action controlling maize weevil resistance in maize materials (Derera et al., 2000; Dhilwayo and Pixley, 2003; Kim and Kossou, 2003; Dari et al., 2010). The nature of gene action would help in devising a strategy for enhancing resistance in the maize varieties. This is discussed in chapter 6 of the current study.

4.7.2 Larger grain borer resistance among local maize varieties

Maize varieties showed significant variation in response to LGB infestation. The variation in resistance among maize varieties were shown by highly significant differences for flour weight, insect mortality, percent grain damage and percent grain weight loss. The distribution of variation for resistance parameters among the varieties seemed to be concentrating at the centre. This indicates that most of the local varieties had moderate resistance against larger grain borer. Variation for resistance to LGB was also reported among landraces along the coastal region of Kenya (Ndiso et al., 2007). Variety differences in response to LGB are critical in the control of the pest (Rugumamu, 2006).

Exploitation of variation for flour weight, insect numbers, development periods, percent grain weight loss, percent grain damage to measure varietal resistance against LGB have been reported (Kasambala, 2009; Mwololo et al., 2010). For instance, Kasambala (2009) used insect numbers, percent grain weight loss and percent grain damage to determine the existing variation for resistance against LGB among maize varieties in Malawi. The results revealed variation for grain weight loss ranging between 7.7 and 30.3%, percent grain damage (33-66.7%) and insect numbers (41 to 99). In the study, Kanjerenjere (local variety) was identified as resistant variety. Hence, Kanjerenjere was used as a resistant check in the current study.

The current study results showed that the total number of insects ranged from 38 to 79, percent grain damage ranged from 5 to 32% and percent weight loss ranged from 10.64% to 28.61%. These ranges did not differ significantly from the results obtained by Kasambala (2009). However, some varieties outperformed the resistant check. This is an indication of high level of variation among maize varieties which should be exploited in breeding programmes. Mwololo et al. (2010) reported significant differences in grain damage, flour weights, number of dead and live insects among varieties in Kenya. Importantly, these parameters are heritable. For example, in the current study, percent grain weight loss showed a broad-sense heritability of 0.65 and percent grain damage (0.94), flour weights (0.97), adult mortality (0.85) and insect numbers (0.85).Therefore, according to the present results, these parameters can reliably discriminate varieties against larger grain borer.

Correlation analysis showed highly significant relationships between flour weights with grain damage (%), and between grain damage (%) and number of insects. This relationship is consistent with the manner LGB excavates the grain with its deflexed head and well-built mandibles (LI, 1988). Consequently, increase in number of insects resulted in the increased grain damage and high amount of flour produced. Using percent weight loss to measure susceptibility of varieties, all varieties were susceptible. However, varieties, such as 1992, 2012, 1850, 2017, 386 and 1983 had lesser percent weight loss and performed better than the resistant check (Kanjerenjere). The relatively low percent grain weight loss among the varieties was consistent with their respective percent grain damages, which were also relatively lower than the resistant check. This provides a new opportunity for new sources of resistance for use in breeding for insect resistance. It is also worth noting that varieties 1992 and 1983 showed also high level of resistance to maize weevil. This provides an opportunity to select for both maize weevil and larger grain borer resistance, since both insect pests are generally found in the same environment within the storage facilities.

The resistance observed in maize varieties against larger grain borer could be due to antibiosis (Kumar, 2002; Nhamucho et al., 2014). Of late, Mwololo et al. (2012) reported the effect of protein composition and lipids on maize resistance to LGB in tropical maize. Resistant varieties exhibited high levels of lipids and protein content. Arnason et al. (1992) reported the role of grain moisture, grain hardness, vitreous endosperm and nutritional factors in LGB development and behaviour.

4.7.3 Yield and grain resistance

Results on yield had shown that there were no significant differences for yield among maize varieties. However, some varieties showed promising yield potentials, such as 2012 (4.57 tons/ha), 1786 (4.81 tons/ha), 1795 (3.49 tons/ha), 2012 (4.57 tons/ha), 3244 (3.91 tons/ha), 445 (3.81 tons/ha), and 752 (4.18 tons/ha). Reports by Mwololo et al. (2013) indicated significant variation for grain yield among insect resistant varieties in Kenya. Combining weevil resistance and yield performance, the best local maize varieties were 1772, 1983, 1992, 3243, 3244, 750 and 752. Except for varieties, 2012, 1772 and 752 that were semi-flint, the rest of the varieties were dent. For larger grain borer, the best varieties with useful resistance levels and yields were varieties 1983 (3.16 tons/ha) and 1992 (2.88 tons/ha) and were dent.

The correlations between yield and resistance parameters for both maize weevil and larger grain borer were not significant. This means that selection for resistance can be done without significantly affecting yield. In this regard, potential varieties that have been identified as having better resistance against maize weevil and larger grain borer can be improved upon to enhance both resistance and yield.

4.8 Conclusion

Variation in resistance against maize weevil and larger grain borer exists among local maize varieties grown in Malawi. Therefore genetic diversity and grain resistance are not mutually exclusive in the maize germplasm. The results from the study have shown that resistance to larger grain borer and maize weevil can be found in a single variety as demonstrated by varieties 1992 and 1983. The identified new sources of LGB and MW resistance would be recommended for use in programmes that emphasize post-harvest insect resistance. For instance, varieties, such as 1772, 1983, 1992, 3243, 3244, 750 and 752 are good candidates for developing populations that are resistant to maize weevil. For larger grain borer resistance, 1992, 2012, and 1983 could be used in developing LGB resistant populations. However, these varieties would require recurrent selection to increase the frequency of resistant genes. Further tests on the recommended varieties should be done to ascertain their consistency in resistance levels, largely against larger grain borer to dispel pseudo-resistance among the varieties. The assessment of the top varieties should be done inclusive of other equally important agronomic attributes preferred by farmers.

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Chapter 5

Assessment of larger grain borer (*Sitophilus zeamais* Motschulsky) and maize weevil (*Sitophilus zeamais* Motschulsky) resistance and yield potential of F_1 maize hybrids in Malawi

Abstract

Maize production among smallholder farmers in Malawi is compromised by the negative effects of larger grain borer and maize weevil on maize grain in storage. Breeding for high yielding maize varieties with useful levels of resistance against these storage pests is central in improving net gain in maize production in the country. The objectives of the study were to develop insect resistant maize hybrids for use by smallholder farmers in Malawi and to evaluate the yield potential of insect resistant F_1 hybrids in target production environments in Malawi. The F_1 maize hybrids showed significant differences for grain damage (%), insect mortality, total number of insects, flour weight (g) and grain weight loss (%). Maize weevil resistant hybrids ranged from 4 to 67% across sets, while larger grain borer resistant hybrids ranged from 4 to 9% across sets. Stacking of maize weevil and larger grain borer resistance produced 67% maize weevil resistant hybrids, 14% larger grain borer resistant hybrids and 14% hybrids with resistance to both larger grain borer and maize weevil. Highly significant differences ($p<0.001$) were observed for yield among the F_1 hybrids across environments. Maize hybrids MWA06A showed a yield potential of 10 tons/ha, MWMW15106 (9.07 tons/ha) and MWMW10A (7.69 tons/ha) and good resistance to maize weevil. Maize hybrids lgMW087940 and MWlg06264 expressed high yield potential of 11.05 tons/ha and 8.16 tons/ha, respectively and good resistance to both maize weevil and larger grain borer. The stacking of maize weevil and larger grain borer resistance in single maize hybrids would provide an effective way of breeding for dual insect pest resistance. Breeding for high yielding insect resistant maize hybrids would provide a sustainable way of improving net gain in grain yield for smallholder farmers in Malawi.

Keywords: Breeding, genotype x environment, larger grain borer, maize weevil, insect resistance

5.1 Introduction

The importance of maize to Malawi cannot be overemphasized. Maize is grown by 97% of farming households and accounts for 60% of total calorie consumption (Denning et al., 2009). In 2012, the mean annual maize consumption per individual was 131.2 kg (FAOSTAT, 2014). Despite its important role as a staple food crop, the net gain in maize production in Malawi is reduced by a number of factors, one of which is post-harvest loss of maize grain in storage due to insect pests. Maize weevil (MW*) (Sitophilus zeamais* Motschulsky) and larger grain borer (LGB) (*Prostephanus truncatus* Horn) are the most important storage insect pests of maize in Malawi (Makoka, 2008; Singano et al., 2009; Kamanula et al., 2011). Therefore, breeding for high yielding maize varieties with useful levels of resistance against storage pests is an important undertaking in the country.

In Malawi, maize is grown under various environmental conditions (Ngwira, 2001) that affect maize productivity. The maize growing environments are affected by the depletion in soil nutrients, climate change, low rainfall, drought, pests and diseases among other factors (Denning et al., 2009). Nitrogen and phosphorus deficiencies are the major soil fertility constraints to maize production in Malawi (Akinnifesi et al., 2007). Furthermore, losses in yield due to drought are by far greater than any other causes (Farooq et al., 2008). For example, mild and severe water stress can reduce maize yields by 63% and 85%, respectively (Earl and Davis, 2003). Climate and environmental changes are also threatening agricultural production in the world (IFPRI, 2007) and Malawi is not an exception. The country has experienced unprecedented high temperatures, short growing seasons and unpredictable rainfall pattern (Denning et al., 2009).

The changes in environmental and climatic conditions affect the yielding potential of maize genotypes mainly due to genotype and environment interaction (GEI) (Grada and Ciulca, 2013). The GIE is the differential reaction of genotypes across environments (Yan and Kang, 2003). The GIE results from genetic differences between cultivars in their response to environmental factors such as soil nutrients, light, pests, diseases, physical injury, year of planting, and state of technology (Pereira de Oliveira et al., 2003; Yan and Kang, 2003; Banzinger et al., 2004). In addition, changes in yield and stability of genotypes in different environments are caused by significant GEI (Abera et al., 2004) which depends on sufficient differences in environments and genotypes (Yan and Hunt, 1998). Significant GEI affects heritability of traits, adaptability of genotypes, ranking and selection of superior genotypes across environments (Yan and Hunt, 1998).

The performance of genotypes across environments can only be assessed through Multiple Environment Trial (MET) through which genotypes are planted several times in one or more environments. Multi-location testing of genotypes helps to reduce the effects of GEI (Nzuve et al., 2013). The data collected on the performance of cultivars across the environments helps in the selection of superior genotypes (Setimela et al., 2007).

Different methods are available for analysis of yield data, such as Analysis of Variance (ANOVA), Linear regression analysis, GGE Biplot, Additive Main Effects and Multiplicative Interaction (AMMI) and Residual Maximum Likelihood (REML). Analysis of Variance provides main effects without GEI (Miranda et al., 2009). Finlay and Wilkinson (1963) reported the use of regression on mean model, where GEI is obtained through the variation in yielding potential of different genotypes to the change in the environment. GGE biplot and AMMI use both ANOVA and PCA to provide information about individual genotypes, environments and the degree of the interaction between genotypes and environments (Gauch, 1992; Maa'li, 2008; Miranda et al., 2009; Nzuve et al., 2013). Residual Maximum Likelihood has been reported as one of the easiest and robust methods in yield data analysis. REML analyses more than one source of error variation and it is an ideal tool for unbalanced design (Payne et al., 2009). REML manipulates both random and fixed factors as follows: Yield = mean + fixed effects + random effects (O'Neil, 2010).

Since maize grows in diverse environments and interacts with different environmental factors (Nzuve et al., 2013), the sustainability of insect resistant maize varieties in farmers' agro-environments depends on their performance in different environmental conditions. This necessitates the evaluation of insect resistant maize hybrids in different agro-environments in Malawi.

5.2 Study objectives

The objectives of the study were:

1. To develop insect resistant maize hybrids for use by smallholder farmers in Malawi.

2. To evaluate yield potential of insect resistant F_1 hybrids in target production environments in Malawi.

5.3 Materials and Methods

5.3.1 Collection of maize breeding lines

Maize breeding lines were collected from Chitedze Research Station (Malawi), CIMMYT-Kenya, and CIMMYT-Zimbabwe. Lines from CIMMYT-Kenya and CIMMYT-Zimbabwe are known to have useful resistance against larger grain borer and maize weevil, respectively, while lines from Malawi are known to have good adaptation and yield potential (Table 5.1).

Table 5.1: Breeding lines used in insect resistant F_1 hybrid development

Breeding line	**Breeding prominence**	**Source**
CKSPL10264	Larger grain borer resistance	CIMMYT-KENYA
CKSP10021	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10074	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10089	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10164	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10218	Larger grain borer resistance	CIMMYT-KENYA
CKSPL0176	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10007	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10088	Larger grain borer resistance	CIMMYT-KENYA
CKSPL10087	Larger grain borer resistance	CIMMYT-KENYA
CL106675	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106937	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106939	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106940	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106510	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106506	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106513	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106674	Maize weevil resistance	CIMMYT-ZIMBABWE
CL1012151	Maize weevil resistance	CIMMYT-ZIMBABWE
VL081446	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106511	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106508	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106690	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106674	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106512	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106676	Maize weevil resistance	CIMMYT-ZIMBABWE
CL106514	Maize weevil resistance	CIMMYT-ZIMBABWE
INBRED A	Adaptation and yield	CHITEDZE-MALAWI
CML202	Adaptation and yield	CHITEDZE-MALAWI
46C2W	Adaptation and yield	CHITEDZE-MALAWI
MAT273	Adaptation and yield	CHITEDZE-MALAWI
CML395	Adaptation and yield	CHITEDZE-MALAWI
CHIT116	Adaptation and yield	CHITEDZE-MALAWI
CML444	Adaptation and yield	CHITEDZE-MALAWI
CZ10020	Adaptation and yield	CHITEDZE-MALAWI
I(83)	Adaptation and yield	CHITEDZE-MALAWI
AR158	Adaptation and yield	CHITEDZE-MALAWI

5.3.2 Planting of maize breeding lines

Maize breeding lines were planted in pots under ambient conditions at Chitedze Research Station during the 2011/2012 growing season to generate crosses. The pots were 24 cm in diameter and 28 cm high. Loam soil mixed with organic manure was put into the pots. Two seeds were planted in each pot. Basal application of fertilizer NPK (23:21:0 +4S) and top dressing was done using Urea (46% N) at 5g/pot (50kg/ha). Weeds were removed manually from the pots every time they appeared. Insecticide ‘karate’ (lambda-cyhalothrin) was applied to the soil to control termites.

5.3.3 Generation of crosses

Five sets (**a-e**) of breeding materials (F_1s) were generated through crossing using North Carolina Design II scheme (Figure 5.1) as follows, **Set a:** Maize weevil resistant lines X locally adapted Malawi lines **Set b:** Maize weevil resistant lines X maize weevil resistant lines, **Set c:** Larger grain borer resistant lines X locally adapted Malawi lines, **Set d:** Larger grain borer resistant lines X larger grain borer resistant lines, and **Set e:** Larger grain borer resistant lines X maize weevil resistant lines.

	Females					
Males	1	2	3	4	5	6
7	1x7	2x7	3x7	4x7	5x7	6x7
8	1x8	2x8	3x8	4x8	5x8	6x8
9	1x9	2x9	3x9	4x9	5x9	6x9
10	1x10	2x10	3x10	4x10	5x10	6x10
11	1x11	2x11	3x11	4x11	5x11	6x11
12	1x12	2x12	3x12	4x12	5x12	6x12

Figure 5.1: North Carolina Design II crossing scheme

In each set, 12 lines (six females and six males) were crossed. Thirty six crosses were made per set, giving a total of 180 crosses (F_1 hybrids) for five sets (Table 5.2). At maturity, cobs were harvested and sundried in readiness for field planting during the 2012/2013 growing season.

Table 5.2 : List of F_1 hybrids in sets used in the study

Set A	**Set B**	**Set C**	**Set D**	**Set E**
MW X Adp	**MW X MW**	**LGB X Adp**	**LGB X LGB**	**LGB X MW**
MWA06A	MWMW13675	LGA264116	LGLG089264	MWLG13074
MWA06202	MWMW674675	LGA021116	LGLG021264	LGMW08706
MWA062W	MWMW151675	LGA074116	LGLG087264	LGMW16406
MWA06273	MWMW446675	LGA089116	LGLG007264	MWLG06264
MWA06395	MWMW11675	LGA164116	LGLG088264	LGMW17606
MWA6760020	MWMW1210	LGA218444	LGLGO74007	MWLG08164
MWA151A	MWMW1313-self	LGA264A	LGLG089164	MWLG13089
MWA151202	MWMW674937	LGA021A	LGLG021164	LGMW08710
MWA1512W	MWMW151937	LGA074A	LGLG087164	LGMW16410
MWA151273	MWMW446937	LGA089A	LGLG007164	LGMW26410
MWA151395	MWMW11937	LGA164A	LGLG088164	LGMW089151
MWA676202	MWMW674676	LGA0870020	LGLG007087	MWLG939074
MWA10A	MWMW13939	LGA264444	LGLG089218	LGMW021939
MWA10202	MWMW674939	LGA218I83	LGLG021218	MWLG13218
MWA102W	MWMW151939	LGA074444	LGLG087218	MWLG13074
MWA10273	MWMW446939	LGA089444	LGLG007218	MWLG10089
MWA10395	MWMW11939	LGA164444	LGLG088218	MWLG940164
MWA11312	MWMW690675	LGA264216	LGLG007218	MWLG690264
MWA11A	MWMW13940	LGA2640020	LGLG089176	LGMW08812
MWA11202	MWMW939164	LGA0210020	LGLG021176	MWLG06021
MWA112W	MWMW151940	LGA0740020	LGLG087176	LGMW16413
MWA11273	MWMW446940	LGA0890020	LGLG007176	LGMW26413
MWA11395	MWMW11940	LGA021158	LGLG088176	LGMW17613
MWA06403-3	MWMW12939	LGA176291-4	LGLG164007	MWLG151264
MWA12A	MWMW13676	LGA264I83	LGLG089074	LGMW021151
MWA12202	MWMW67410	LGA021I83	LGLG021074	MWLG11176
MWA122W	MWMW15110	LGA074I83	LGLG087074	MWLG13021
MWA08202	MWMW44610	LGA089I83	LGLG007074	LGMW087940
MWA12395	MWMW1110	LGA164I83	LGLG088074	LGMW176151
MWA080020	MWMW0611	LGA088A	LGLG007164	MWLG08007
MWA446-2W	MWMW1306	LGA264202	LGLG007264	LGMW02111
MWA15175	MWMW67406	LGA074158	LGLG007088	LGMW08711
MWA676403-3	MWMW15106	LGA088444	LGLG007176	LGMW16411
MWA100020	MWMW44606	LGA262158	LGLG089089(self)	LGMW26411
MWA446A	MWMW1106	LGA089716	LGLG089007	LGMW17611
MWA11403-3	MWMW69006	LGA087I83	LGLG218007	LGMW007939

Note: LGB = Larger grain borer resistant lines, MW = Maize weevil resistant lines, Adp = Locally adapted Malawi lines

5.3.4 Planting of insect resistant F_1 hybrids

F_1 hybrids were planted at Kandiani Irrigation Scheme during the 2012/2013 growing season under rainfed (summer), late 2013 (winter) under irrigation and at Chimoto in 2013/2014 growing season under farmers' condition. The three locations represented three different growing environments namely, rainfed, irrigation and late drought, respectively. F_1 hybrids were evaluated in 5 sets based on the type of crosses generated. The hybrids were arranged using alpha lattice design (6 blocks each with 6 or 7 entries) with 2 replications. Each plot was 6 m long. One seed was planted per planting station with 25 cm spacing between plants and 75 cm between rows. However, number of varieties planted varied among the environments due to shortage of seed. The hybrid maize variety "Kanyani" was used as guard rows. Basal application of fertilizer NPK (23:21:0 +4S) and top dressing was done using Urea (46% N) fertilizers at the rate of 100kg/ha. The fields were weeded manually thrice and insecticide karate (lambda-cyhalothrin) was applied to control termites. Full-sib mating was employed for each F_1 hybrid during the 2012/2013 growing season under rainfed (Figure 5.2).

Figure 5.2: Full Sib mating done by hand

5.3.5 Field data collection and analysis

Data collection was done from whole plot on plant height (measured from ground level to the base of the tassel after milking stage), ear placement (from ground level to the node bearing the upper most ear after milking stage), days to tasselling (number of days from sowing to when 50% of the plants had shed pollen), days to silking (number of days from sowing to when silks had emerged on 50% of the plants), husk cover, grain size, ear size, disease score, field weight and grain weight. At maturity, cobs were harvested and dried ready for yield and resistance assessments. Only maize grains harvested from 2012/2013 growing season (rainfed) were used for grain resistance testing, while yield data from all three growing environments were used for yield assessment. Data collected was analysed using GenStat Release 14 (2011). Field data was subjected to ANOVA, stability coefficients, and REML. For REML analysis, environments and genotypes were treated as having fixed effects. Data for resistant parameters was subjected to ANOVA. Broad-sense heritability was calculated based on ANOVA as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 \;\; + \sigma_\epsilon^2 / r}$$

H^2 = Broad -sense heritability

σ_g^2 = Mean sum of square for varieties

σ_ε^2 = Mean sum of square for error

r = Replication

5.3.6 Resistance screening for maize weevil and larger grain borer resistance using F_2 grain

5.3.6.1 Rearing of larger grain borer and maize weevil

The rearing of larger grain borer (LGB) and maize weevil (MW) was done at Chitedze Research Station according to the procedures outlined by CIMMYT (Tefera et al., 2010). Unsexed pests were reared in a controlled environment at 28± 1^{o}C, 65±5 RH, with a 12h: 12h light: dark regime to minimize fluctuations in temperature and relative humidity and promote insect survival (Haines, 1991). The LGB and MW were cultured on susceptible mixed maize grain in sealed but ventilated glass jars. All precautionary measures were taken to exclude other insects from contaminating the cultures. Maize varieties were evaluated for maize weevil and LGB resistance under lab conditions using four replications for sets b, c and d and three replications for sets a and e in a Complete Randomised Block Design (CRBD). About 1 kg maize grains from each variety were collected for testing. Grains were fumigated with phostoxin tablets at the rate of 1.5 g/m^3 of grain (3 tablets) for seven days to avoid carry over insects from the field. One hundred (100) grams of grain were sampled from each of the 1 kg maize grains and placed into jars. Fifty (50) unsexed adult beetles (7- 15 days old) were infested on 100 g of grain and kept inside 250 ml plastic jars for MW and in 400 ml glass jars for LGB.

5.4 Data collected

5.4.1 Maize weevil and larger grain borer resistance parameters

After 100 days, the following parameters were used for measuring insect resistance among the varieties; number of live and dead insects, total number of insects, damaged and undamaged grains based on 100 grains randomly selected from each jar. Maize hybrids were categorized into resistant groups based on percent grain damage as follows, highly resistant (0%), resistant (≤2%), moderately resistant (2.1-2.9%), moderately susceptible (3-3.9%), susceptible (4-4.9%) and highly susceptible (≥5%). Grain weight loss was calculated based on the damaged and undamaged grains using (CIMMYT protocol, Boxall, 2002) as follows: weight loss (%) = {(Wu x ND) - (Wad x Nu)/ Wu x (ND + Nu)} x 100; where Wu= weight of undamaged seed**,** NU= number of undamaged seeds, Wad = Weight of damaged seed ND = number of damaged seed. Determination of resistance based on grain weight loss was as follows: Resistant (grain weight loss ≤ 2%), moderately resistant (grain weight loss between 2.1% and 4%), moderately susceptible (grain weight loss between 4.1 and 6%), susceptible (grain weight loss of between 6.1% and 8%), highly susceptible (grain weight loss ≥8.1%). For LGB, Weight of flour produced in the jars due to insect damage was sieved and measured using an electronic weighing balance.

5.5 Results

5.5.1. Set A: Adapted Malawi lines X Maize weevil resistant lines

5.5.1.1. Grain resistance to maize weevil among F_1 hybrids

Maize hybrids showed significant differences ($p<0.05$) for percent grain damage and total number of insects, while insect mortality and percent grain weight loss did not show any significant differences (Table 5.3).

5.5.1.1.1. Grain weight loss (%)

No significant differences were observed for percent grain weight loss among the F_1 hybrids. However, maize hybrids MWA10A, MWA06A, MWA151A, MWA11273, MWA11312 and MWA12395 had the lowest grain weight losses, while maize hybrids MWA446A, MWA06403-3, MWA676403-3, MWA1512W and MWA12202 experienced high grain weight losses. Distribution of variation for percent grain weight loss showed that most of the hybrids experienced low percent weight loss (Figure 5.3).

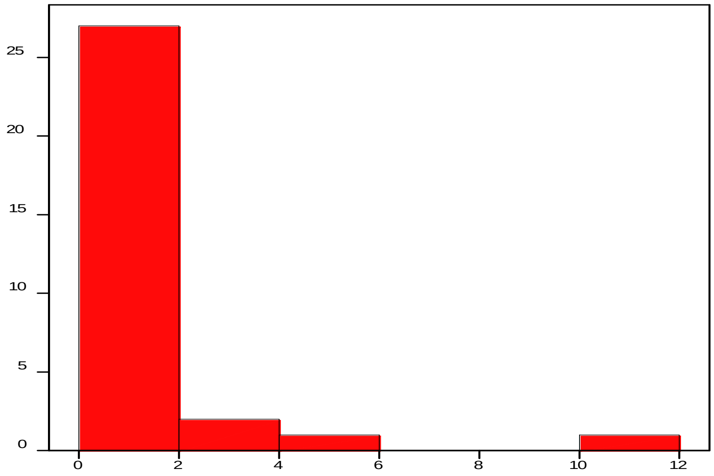

Figure 5.3: Distribution of variation for grain weight loss (%) among F_1 hybrids (Set a)

5.5.1.1.2 Total number of insects

Significant differences ($p<0.05$) were observed for total number of insects among the hybrids. The least number of insects were observed in the following hybrids, MWA112W, MWA11273, MWA151273, MWA44606, and MWA10395. The highest numbers of insects were obtained from MWA06403-3, MWA446A, MWA151175, MWA11403-3, and MWA151A (Table 5.3). The distribution of variation for total number of insects showed that most of the hybrids experienced less number of insect pests (Figure 5.4).

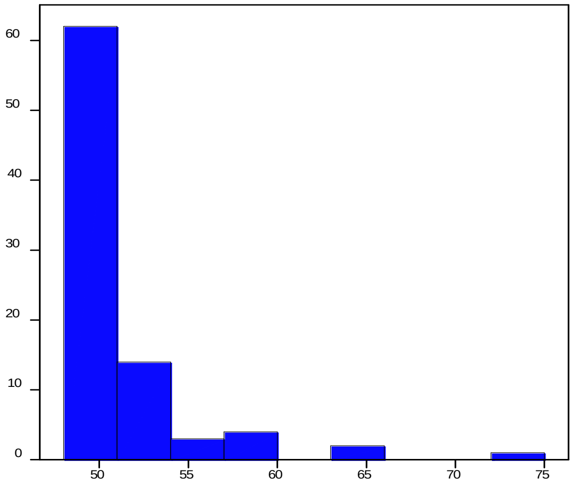

Figure 5.4: Distribution of variation for total number of insects among F_1 hybrids (set a)

6.5.1.1.3 Grain damage (%)

Significant differences ($p<0.05$) were observed for percent grain damage among the F_1 hybrids. Maize hybrids, MWA06A, MWA12395, MWA11312, MWA10A and MWA11A experienced less grain damage, while maize hybrids, MWA12202, MWA06395, MWA1512W, MWA06403-3 and MWA446A experienced the highest grain damage (Table 5.3). The distribution of variation for grain damage (%) revealed that most of the hybrids experienced less grain damage (Figure 5.5).

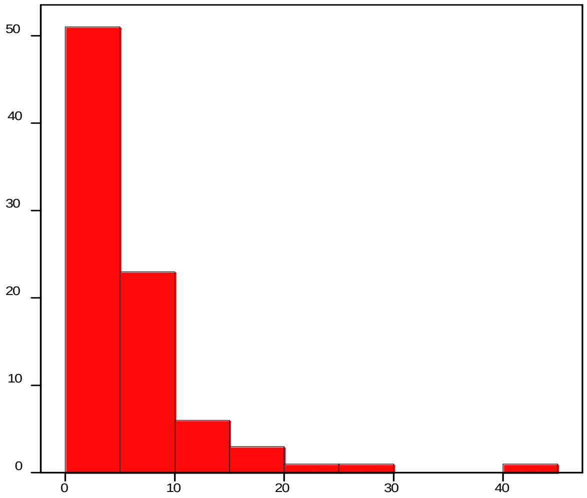

Figure 5.5: Distribution of variation for grain damage (%) among F_1 hybrids (set a)

The percent grain damage as a measure of resistance determined that 3.2% of the F_1 hybrids were highly resistant, 9.8% were resistant, 12.9% moderately resistant, 19.4% moderately susceptible, 16.1% susceptible and 38.7% highly susceptible (Figure 5.6).

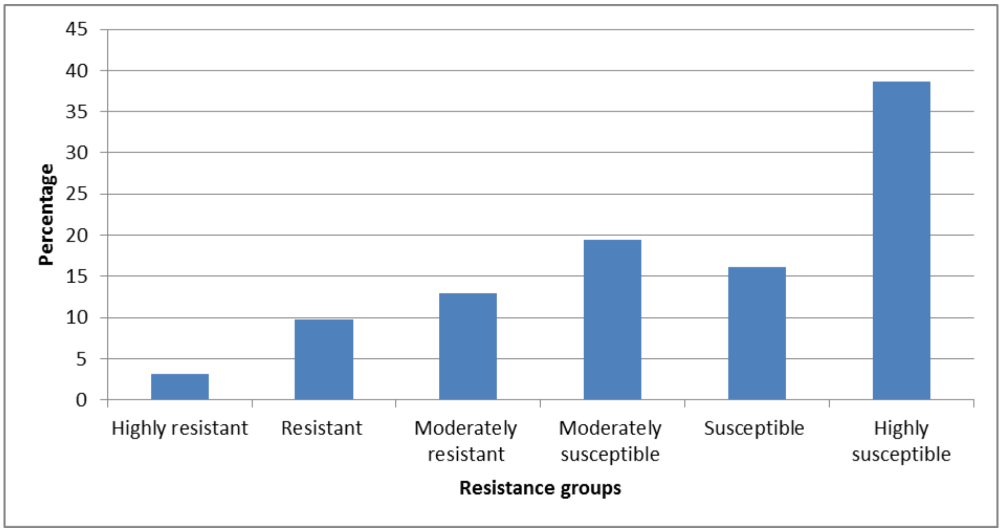

Figure 5.6: Maize weevil resistance groups based on grain damage (%) (set a)

Table 5.3: Table of means for grain resistance parameters against maize weevil (set a)

Variety	Total number of insects	Insect mortality	Weight loss (%)	Grain damage (%)	Resistance level
MWA10A	50.7	48.0	0.0	2.0	Resistant
MWA06A	50.3	47.0	0.0	0.0	Highly resistant
MWA151A	53.3	48.0	0.1	3.7	Moderately susceptible
MWA11273	50.0	46.0	0.2	2.7	Moderately resistant
MWA12395	51.3	50.3	0.2	0.4	Resistant
MWA11A	50.3	44.0	0.3	2.4	Moderately resistant
MWA151273	50.0	49.7	0.4	2.7	Moderately resistant
MWA12A	51.4	51.2	0.4	3.9	Moderately susceptible
MWA112W	49.9	46.7	0.4	4.3	Susceptible
MWA6760020	50.0	46.3	0.4	4.7	Susceptible
MWA122W	52.0	48.3	0.5	3.0	Moderately susceptible
MWA15175	54.7	50.3	0.5	4.4	Susceptible
MWA151395	50.0	44.7	0.5	8.0	Highly susceptible
MWA44606	50.0	39.3	0.6	3.4	Moderately susceptible
MWA1512W	51.0	48.3	0.6	13.4	Highly susceptible
MWA10273	50.4	44.7	0.6	3.4	Moderately susceptible
MWA062W	50.0	48.0	0.6	6.4	Highly susceptible
MWA08202	50.2	49.2	0.6	2.4	Moderately resistant
MWA4462W	52.3	48.0	0.7	5.7	Highly susceptible
MWA11312	50.3	49.3	0.7	1.7	Resistant
MWA67406	52.9	50.7	0.7	4.8	Susceptible
MWA11403-3	54.3	50.0	0.9	10.7	Highly susceptible
MWA12202	51.3	49.0	1.1	11.7	Highly susceptible
MWA10395	50.0	44.7	1.1	4.0	Susceptible
MWA06395	50.3	44.7	1.2	12.4	Highly susceptible
MWA676202	50.7	48.7	1.4	3.4	Moderately susceptible
MWA06273	53.0	52.0	1.7	10.7	Highly susceptible
MWA676403-3	50.0	44.3	2.1	11.0	Highly susceptible
MWA11202	52.4	48.7	3.8	6.4	Highly susceptible
MWA06403-3	69.4	60.7	5.8	13.8	Highly susceptible
MWA446A	58.3	47.7	11.7	15.0	Highly susceptible
P.level	sg*	nsg	nsg	sg*	
CV (%)	5.5	11.2	12.9	13.4	
lsd (0.05)	4.921	9.44	0.437	0.469	
SED	2.453	4.704	0.218	0.234	
H^2	0.77	0.76	0.74	0.83	

sg* = significant at p<0.05, nsg= not significant

5.5.1.2 Correlation between grain resistance parameters for maize weevil

Highly significant ($p<0.001$) correlations were observed between percent grain weight loss and percent grain damage (0.6965) and between percent grain damage and total number of insects (0.4653) (Appendix 5.1).

5.5.1.3 Yield potential of F_1 hybrids (set a)

Significant differences ($p<0.01$) were observed for yield potential under rainfed conditions, but no significant differences for yield potential were observed under irrigation and drought conditions. Combined yield analysis across environments showed highly significant differences ($p<0.01$) for yield potential among maize hybrids and the interaction between the hybrids and the environments. Maize varieties, MWA112W, MWA06403-3, MWA11273, MWA151A, MWA446A, MWA06273, MWA11403-3, MWA122W, MWA06A, and MWA062W had the highest mean yields across environments. The maize yields ranged from 10 to 15.64 tons/ha. The hybrids outperformed a commercial hybrid "Kanyani" which had a mean yield of (5.42 tons/ha). Maize hybrids, MWA112W, MWA11273, MWA062W, and MWA11403-3 had good general adaptation across environments (Table 5.4).

5.5.1.4 Yield potential and maize weevil resistance among F_1 hybrids

Combination of yield potential and maize weevil resistance showed that the following hybrids had good yield potential and high resistance levels against maize weevil, MWA10A (7.69 tons/ha), MWA06A (10 tons/ha), and MWA12395 (6.67 tons/ha) (Table 5.4).

Table 5.4: Yield potential, resistance and ranking of F_1 hybrids across environments (set a)

Variety	Yield (tons/ha)	Group	Rank	Superiority index	Grain damage (%)	Net yield (tons/ha)	Resistance status
MWA112W	15.64	2	5	0	4.3	14.97	Susceptible
MWA06403-3	13.60	2	15.5	0.013889	13.8	11.72	Highly susceptible
MWA11273	11.69	2	6.5	0	2.7	11.37	Moderately resistant
MWA151A	11.33	2	7	0	3.7	10.91	Moderately susceptible
MWA446A	10.98	2	8.5	0	3.4	9.32	Highly susceptible
MWA06273	10.44	2	10.75	0.000868	10.7	9.32	Highly susceptible
MWA11403-3	10.09	4	5.17	0.000868	10.7	9.01	Highly susceptible
MWA122W	10.04	4	6.67	0	3.1	9.74	Moderately susceptible
MWA06A	10.00	4	10	0.007812	0.0	9.99	Highly resistant
MWA062W	10.00	3	13.5	0.000868	6.4	9.36	Highly susceptible
MWA4462W	9.51	4	7.83	0.000868	5.7	8.97	Highly susceptible
MWA102W	8.89	2	10.75	0.000868		8.89	
MWA06395	8.71	2	17	0.007812	12.4	7.63	Highly susceptible
MWA151273	8.62	2	16	0.003472	2.7	8.39	Moderately resistant
MWA10395	8.49	4	8.83	0.000868	4.1	8.14	Susceptible
MWA08202	8.13	2	19	0	2.4	7.93	Moderately resistant
MWA1512W	7.82	2	17	0.003472	13.4	6.77	Highly susceptible
MWA080020	7.78	3	24	0.007812		7.78	
MWA11A	7.78	3	24	0.007812	2.4	7.59	Moderately resistant
MWA10A	7.69	2	21	0.013889	2.0	7.53	Resistant
MWA06202	7.56	2	21	0.003472		7.56	
MWA6760020	7.51	2	20.75	0.000868	4.7	7.16	Susceptible
MWA12A	7.47	2	14.75	0.000868	3.9	7.17	Moderately susceptible
MWA10202	7.16	2	20.5	0.007812		7.16	
MWA100020	6.80	2	21	0.007812		6.8	
MWA151395	6.76	2	21.5	0.003472	8.1	6.21	Highly susceptible
MWA12395	6.67	4	16.67	0.013889	0.4	6.64	Resistant
MWA151202	6.67	3	30	0.013889		6.67	
MWA676403-3	6.67	3	30	0.013889	11.1	5.93	Highly susceptible
MWA11395	6.49	4	12.33	0		6.49	
MWA10273	6.27	2	22	0.003472	3.4	6.05	Moderately susceptible
MWA15175	6.18	2	24	0.021701	4.4	5.91	Susceptible
Kanyani	5.42	1	13	0		5.42	
MWA08312	5.29	5	20	0		5.29	
MWA11202	4.80	2	27.5	0.007812	6.4	4.49	Highly susceptible
MWA676202	4.44	3	34	0.03125	3.4	4.29	Moderately susceptible
MWA11312	1.78	2	33.5	0.055556	1.7	1.72	Resistant
MWA12202	1.47	2	32.5	0.08	11.7	1.29	Highly susceptible
Fixed term	Wald statistic	n.d.f.	d.d.f.	F pr			
VARIETY	103.89	37	79	<0.001			
ENVIRONMENT	7.2	2	79	0.032			
VAR.ENVIR	72.67	36	79	0.005			

H^2 (yield) = 0.92

5.5.2 Set B: Maize weevil resistant lines X Maize weevil resistant lines

5.5.2.1 Grain resistance to maize weevil among F_1 hybrids

Maize hybrids showed significant differences for insect mortality, total number of insects and percent grain damage, while percent grain weight loss did not show any significant differences among the maize hybrids (Table 5.5).

5.5.2.1.1 Insect mortality

Highly significant differences ($p<0.001$) were observed for insect mortality. The highest number of insect mortalities was observed in maize hybrids, MWMW1313, MWMW1110, MWMW13675, MWMW1306, and MWMW1210. The least number of adult mortalities was observed in maize hybrids, MWMW1106, MWMW674937, MWMW13939, MWMW11675 and MWMW151937 (Table 5.5). The distribution of variation for insect mortality showed that most of the hybrids had high numbers of insect mortalities (Figure 5.7).

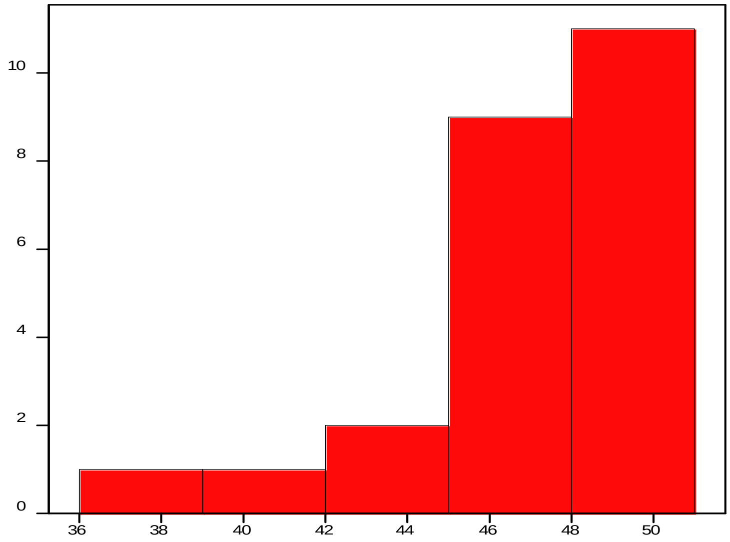

Figure 5.7: Distribution of variation for insect mortality among F1 hybrids (set b)

5.5.2.1.2 Total number of insects

Significant differences ($p<0.05$) were observed for total number of insects. The following hybrids showed the least number of insects, MWMW13939, MWMW151939, MWMW446675, MWMW151675, and MWMW11675. The highest numbers of insects were observed in maize hybrids MWMW1210, MWMW13675, MWMW690675, MWMW13676 and MWMW44606) (Table 5.5). The maize hybrids largely experienced moderate to high insect numbers (Figure 5.8).

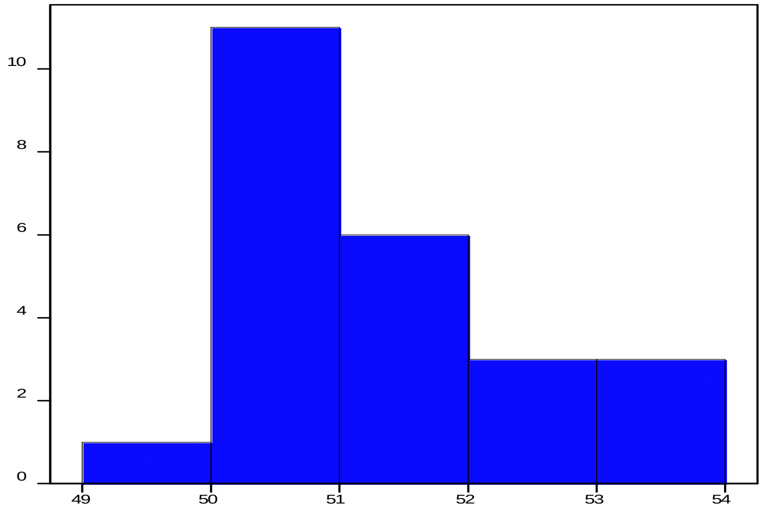

Figure 5.8: Distribution of variation for total number of insects among F1 hybrids (set b)

5.5.2.1.3 Grain weight loss (%)

No significant differences were observed for percent grain weight loss among F_1 hybrids. However, some maize hybrids experienced less weight loss, such as MWMW1313, MWMW67410, MWMW446939, MWMW151939, MWMW13939, and MWMW15106. The largest grain weight losses were observed in maize hybrids, MWMW12939, MWMW674675, MWMW13676, and MWMW151937 (Table 5.5). The majority of the hybrids experienced less grain weight loss (Figure 5.9).

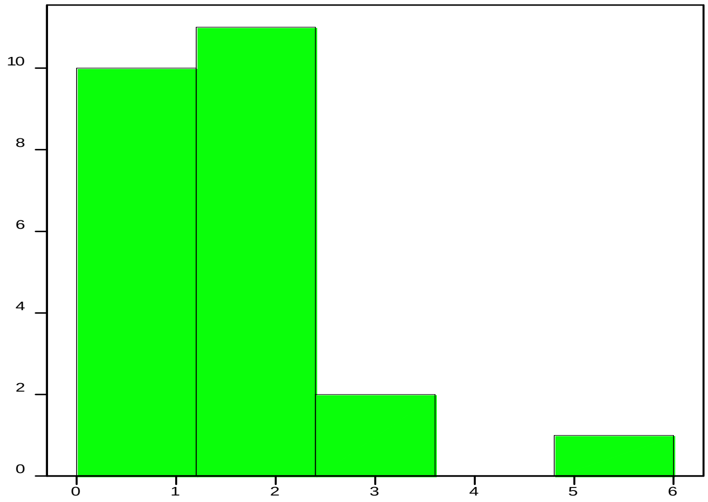

Figure 5.9: Distribution of variation for percent weight loss among F1 hybrids (set b)

5.5.2.1.4 Percent grain damage

Highly significant differences ($p<0.001$) were observed for percent grain damage. The top six hybrids with the least grain damages were, MWMW15106, MWMW446939, MWMW0611, MWMW674937, MWMW12939 and MWMW151939. Higher levels of grain damage were observed in hybrids, MWMW151937, MWMW13675, MWMW11675 MWMW446675 and MWMW674675 (Table 5.5). The majority of the hybrids experienced moderate to lower levels of grain damage (Figure 5.10).

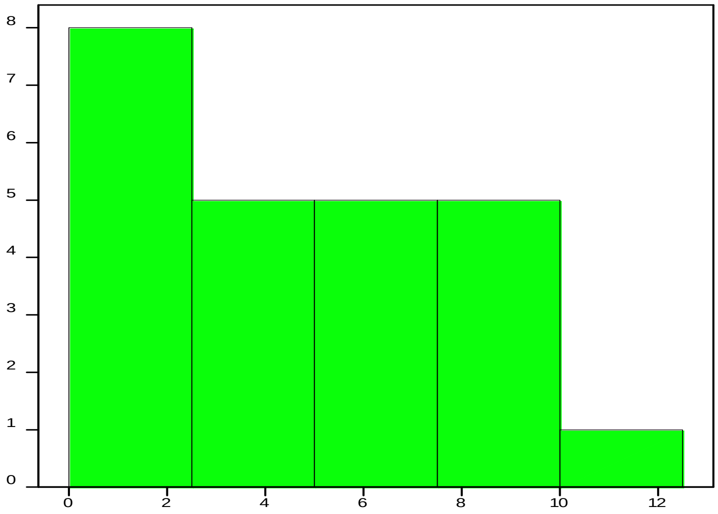

Figure 5.10: Distribution of variation for grain damage (%) among F_1 hybrids (set b)

Using grain damage (%) as a measure of resistance, the results revealed that 4.2% of the hybrids were highly resistant, 25% resistant, 8.3% moderately resistant, 8.3% moderately susceptible, 8.3% susceptible and 45.8% highly susceptible (Figure 5.11).

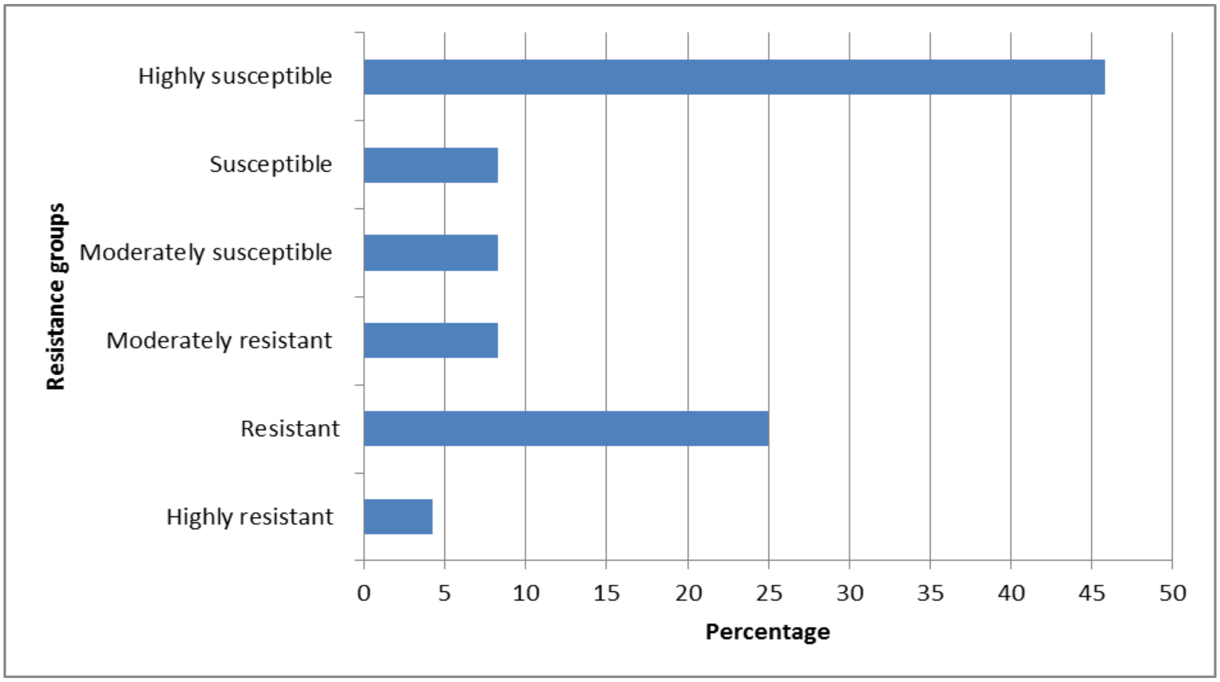

Figure 5.11: Resistance groups based on grain damage (%)

Table 5.5: Table of means for grain resistance parameters against maize weevil (set b)

Variety	Total number of inse	Insect mortalit	Weight loss (%	Grain damage (%	Resistance reaction
MWMW1313	52.12	50.61	0.00	2.0	Resistant
MWMW1110	51.87	50.36	1.86	4.0	Susceptible
MWMW13675	53.12	50.11	1.92	8.5	Highly susceptible
MWMW1306	51.37	49.86	1.86	6.5	Highly susceptible
MWMW1210	53.62	49.86	1.89	5.3	Highly susceptible
MWMW67410	50.62	49.61	0.79	2.3	Moderately resistant
MWMW446939	50.37	49.36	0.74	1.5	Resistant
MWMW674939	51.12	49.36	1.77	6.0	Highly susceptible
MWMW151939	50.12	48.36	0.56	0.8	Resistant
MWMW674675	50.87	48.11	2.55	7.5	Highly susceptible
MWMW12939	51.87	48.11	3.42	.01	Resistant
MWMW44606	53.62	47.86	1.90	7.3	Highly susceptible
MWMW690675	52.37	47.61	1.12	3.5	moderately susceptible
MWMW11937	51.12	47.61	1.32	3.8	moderately susceptible
MWMW15106	50.62	47.36	0.49	0.0	Highly resistant
MWMW13676	52.37	46.11	2.04	7.8	Highly susceptible
MWMW0611	51.12	45.86	0.71	0.5	Resistant
MWMW446675	50.12	45.86	1.76	8.0	Highly susceptible
MWMW151675	50.12	45.61	0.52	2.5	Moderately resistant
MWMW1106	50.37	45.61	1.51	4.0	Susceptible
MWMW11675	50.12	44.36	1.63	8.5	Highly susceptible
MWMW151937	50.87	43.11	5.80	12.0	Highly susceptible
MWMW13939	49.28	41.34	0.00	6.1	Highly susceptible
MWMW674937	50.12	37.36	0.71	0.5	Resistant
Mean	**51.22**	**47.06**	**1.54**	**4.57**	
P.level	Sg*	Sg**	nsg	Sg**	
CV (%)	23.6	12	16.7	19.9	
lsd (0.05)	16.45	8.5	0.402	0.586	
SED	8.28	4.3	0.202	0.295	
H^2	0.87	0.92	0.74	0.91	

sg** = significant at p<0.001, sg* = significant at p<0.05, nsg= not significant

5.5.2.2 Correlation between grain resistance parameters for maize weevil

Highly significant correlation (p<0.001) was observed between percent grain damage and percent grain weight loss (0.6364). Significant correlation (p<0.01) was also observed between total number of insects and insect mortality (0.5294) (Appendix 5.1).

5.5.2.3 Yield potential of F_1 hybrids (set b)

Significant differences ($p<0.01$) were observed for yield potential among the hybrids under irrigation. No significant differences were observed for yield potential under rainfed and drought conditions. Combined yield analysis however, showed highly significant differences ($p<0.001$) for yield potential across environments. No significant differences were observed for the interaction between maize hybrids and environments. The top five performing hybrids were MWMW13939, MWMW1106, MWMW44610, MWMW44606, and MWMW13675 with mean yields ranging from 10 to 12.76 tons/ha. These hybrids performed better than a commercial hybrid “Kanyani” which had a mean yield of 7.29 tons/ha. Superiority index showed that maize hybrids, MWMW13939, MWMW1106, MWMW13675 and MWMW44610 were generally stable across environments (Table 5.6).

5.5.2.4 Yield potential and maize weevil resistance among F_1 hybrids

Based on yield potential and resistance levels, results showed that the following hybrids had better yield potential and high resistance levels against maize weevil, MWMW15106 (9.07 tons/ha), MWMW1313 (5.84 tons/ha), and MWMW446939 (6.67 tons/ha) (Table 5.6).

Table 5.6: Yield potential, resistance and ranking of F_1 hybrids across environments (set b)

Variety	**Rank**	**Group**	**Yield (tons/ha)**	**Superiority index**	**Grain damage (%)**	**Resistance**	**Net Yield (tons/ha)**
MWMW13939	2.25	4	12.76	0.021701	6.1	Highly susceptible	11.98
MWMW1106	2	3	12.76	0.013889	4.0	susceptible	12.24
MWMW44610	4	2	11.11	0.013889			11.11
MWMW44606	2.5	3	10.98	0.021701	7.3	Highly susceptible	10.18
MWMW13675	6	2	10.00	0.007812	8.5	Highly susceptible	9.15
MWMW13676	9.5	4	9.73	0.003472	7.8	Highly susceptible	8.98
MWMW15106	15.5	4	9.07	0.000868	0.0	Highly resistant	9.07
MWMW446937	7.33	3	9.02	0.003472	0.5	Resistant	8.98
MWMW151937	10.5	4	9.02	0.013889	12.0	Highly susceptible	7.94
MWMW446940	9	3	8.27	0.003472	1.4	Resistant	8.15
MWMW15110	10.17	3	8.27	0			8.27
MWMW1210	15.75	4	8.13	0	5.3	Highly susceptible	7.71
MWMW13940	14	2	7.78	0.000868	1.5	Resistant	7.67
MWMW151675	14	2	7.78	0.000868	2.5	moderately resistant	7.58
MWMW1306	10.33	3	7.78	0.003472	6.5	Highly susceptible	7.58
MWMW1110	11.83	3	7.64	0	4.0	moderately resistant	7.45
MWMW446675	12.5	3	7.47	0	8.0	Highly susceptible	6.87
Kanyani	11	1	7.29	0			7.29
MWMW11937	17	4	6.93	0.000868	3.8	Moderately susceptible	6.67
MWMW674675	15.67	3	6.76	0.000868	7.5	Highly susceptible	6.25
MWMW446939	19.5	2	6.67	0	1.5	Resistant	6.57
MWMW67406	13.67	3	6.53	0.000868			6.53
MWMW67410	15.67	3	6.18	0.003472	2.3	moderately resistant	6.04
MWMW69010	13	1	6.09	0			6.09
MWMW690675	18	3	6.04	0.000868	3.5	Moderately susceptible	5.83
MWMW1313	16.33	3	5.96	0.000868	2.0	moderately resistant	5.84
MWMW11675	18.33	3	5.69	0.000868	8.5	Highly susceptible	5.21
MWMW11939	26	2	5.56	0.000868			5.56
MWMW12939	26.5	4	4.62	0.000868	1.0	Resistant	4.58
MWMW151940	23.17	3	4.53	0.003472	9.11	Highly susceptible	4.12
MWMW0611	31.5	2	4.44	0.003472	0.5	Resistant	4.42
MWMW674937	31.5	2	4.44	0.003472	0.5	Resistant	4.42
MWMW674676	27.25	4	4.44	0.003472			4.44
MWMW674939	21	3	4.27	0.000868	6.0	Highly susceptible	4.01
MWMW151939	34	2	3.56	0.006806	0.8	Resistant	3.53
MWMW69006	35	2	3.33	0.007812	8.3	Highly susceptible	3.06
MWMW11940	30.5	4	2.84	0.013889	1.1	Resistant	2.81

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
VARIETY	95.13	37	2.57	<0.001
ENVIRONMENT	43.06	2	21.53	<0.001
VAR.ENVIRON	40.93	41	1	0.474

H^2 (yield) = 0.95

5.5.3. SET C: Adapted Malawi lines X LGB resistant lines

5.5.3.1. Grain resistance of maize hybrids to larger grain borer

Maize hybrids showed highly significant differences ($p<0.001$) for adult mortality, total number of insects, grain damage (%), flour weight and grain weight loss (%) (Table 5.7).

5.5.3.1.1 Total number of insects

Highly significant differences ($p<0.001$) were observed for total number of insects. The least number of insects were noted in the following hybrids, LGA089I83, LGA089118, LGA087I83, and LGA264158. The highest numbers of insects were found in LGA164444, LGA264A, LGA264216, and LGA218I83 (Table 5.7). The distribution of variation for total number of insects showed that most of the hybrids had moderate to low numbers of insect pests (Figure 5.12)

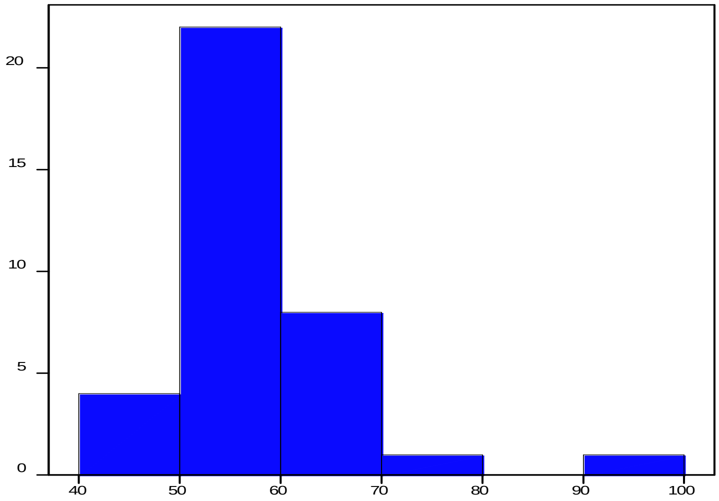

Figure 5.12: Distribution of variation for total number of insects among F_1 hybrids (set c)

5.5.3.1.2 Insect mortality

Significant differences ($p<0.05$) were observed for insect mortality among the F_1 hybrids. The following hybrids had the highest number of adult mortalities; LGA089I83, LGA074158, LGA074116, LGA264116, and LGA264202, while the least number of insect mortalities were observed in LGA264158, LGA264A, LGA089716, LGA021158 and LGA218444 (Table 5.7). The distribution of variation for insect mortality indicated that most hybrids had large numbers of dead insects (Figure 5.13).

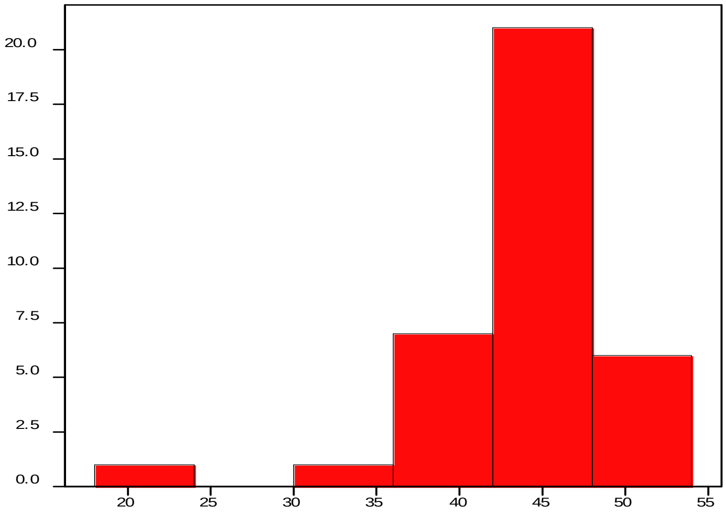

Figure 5.13: Distribution of variation for insect mortality among F1 hybrids (set c)

5.5.3.1.3 Flour weight (g)

Highly significant differences ($p<0.001$) were observed for flour weight among maize hybrids. The least amount of flour was obtained from hybrids, LGA089118, LGA264158, LGA088A, LGA0740020, and LGA264202, while highest amount of flour were observed in the following hybrids; LGA264A, LGA164444, LGA218I83, LGA264216 and LGA264444 (Table 5.7). The majority of the hybrids produced moderate to low amount of flour (Figure 5.14).

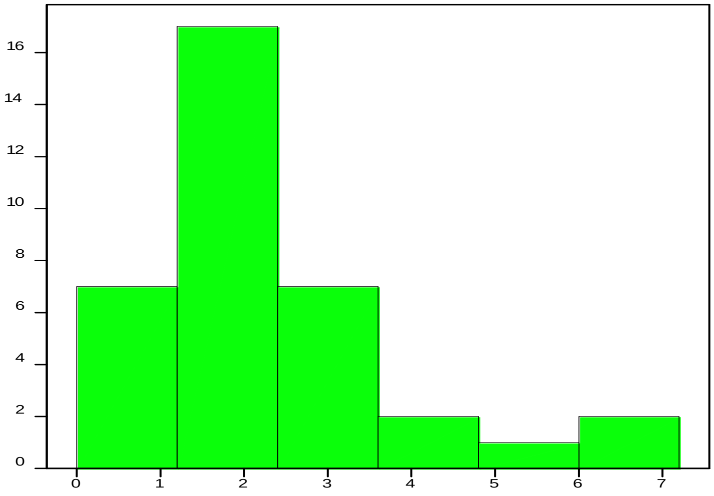

Figure 5.14: Distribution of variation for flour weight among F_1 hybrids (set c)

5.5.3.1.4 Grain weight loss (%)

Highly significant differences (p>0.001) were observed for grain weight loss (%) among F_1 hybrids. The following hybrids experienced less grain weight loss, LGA089116, LGA087I83, and LGA088A. The highest grain weight loss was observed in hybrids, LGA264A, LGA164444 and LGA218I83 (Table 5.7). The distribution of variation for grain weight loss showed that the majority of F_1 hybrids experienced moderate to low levels of grain weight losses (Figure 5.15).

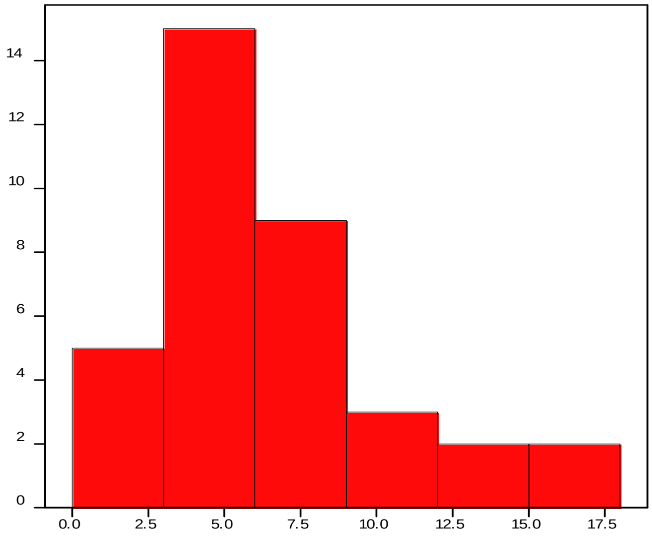

Figure 5.15: Distribution of variation for grain weight loss (%) among F1 hybrids (set c)

5.5.3.1.5 Grain damage (%)

Highly significant differences (p<0.001) were obtained for percent grain damage among the hybrids. The least grain damage was observed in the following hybrids, LGA089116, LGA087I83, and LGA088A. Highest grain damage was observed in hybrids, LGA264A, LGA164444, LGA264216, LGA218I83 and LGA021A (Table 5.7). The distribution of variation for grain damage revealed that most of the hybrids experienced moderate to low grain damage (Figure 5.16).

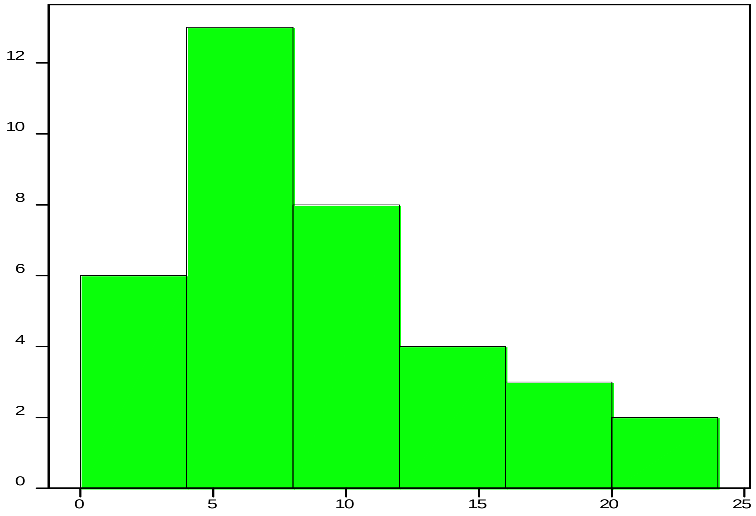

Figure 5.16: Distribution of variation for grain damage (%) among F1 hybrids (set c)

Based on percent grain damage, 5.5% of the hybrids were resistant, 2.8% moderately resistant, 8.3% moderately susceptible, 5.5% susceptible and 77.8% highly susceptible (Figure 5.17).

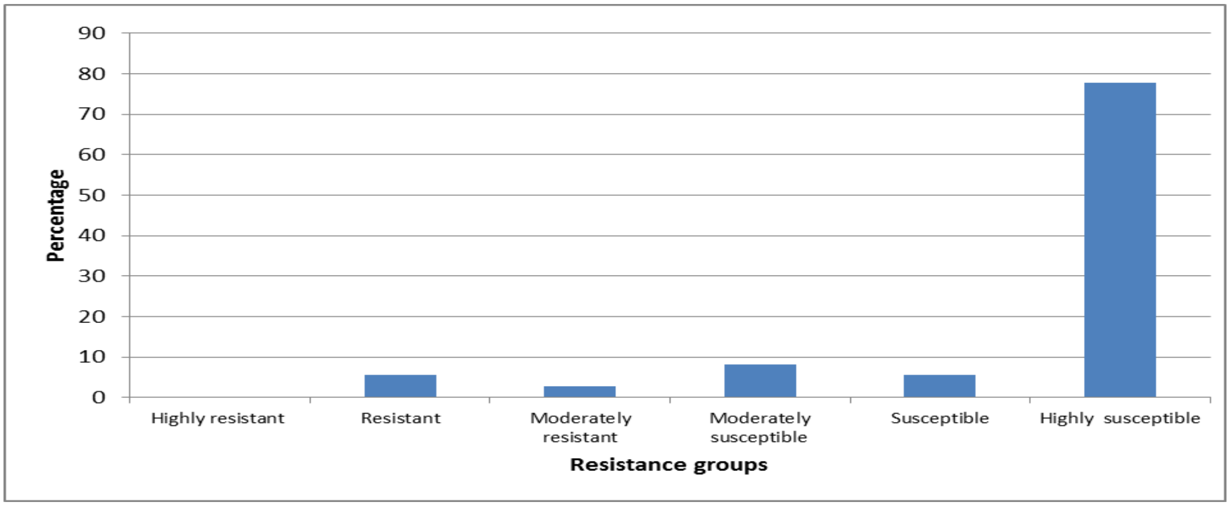

Figure 5.17: Discrimination of F_1 hybrids into resistance groups using grain damage (%)

Table 5.7: Table of means for grain resistance parameters against larger grain borer (set c)

Variety	**Total number of insects**	**Insect mortality**	**Weight loss (%)**	**Grain damage (%)**	**Flour (g)**	**Resistance status**
LGA089116	51.57	48.07	0.0	1.5	1.43	Resistant
LGA087I83	46.93	47.95	0.9	1.9	1.28	Resistant
LGA088A	50.33	46.21	1.5	2.8	0.44	Moderately resistant
LGA0870020	56.08	47.96	1.9	3.6	0.99	Moderately susceptible
LGA0740020	50.08	41.46	2.4	3.3	0.67	Moderately susceptible
LGA264202	53.33	48.46	3.0	4.1	0.79	Susceptible
LGA089I83	46.08	50.84	3.0	4.9	1.62	Susceptible
LGA264158	46.93	23.00	3.2	7.9	0.20	Highly susceptible
LGA176291-4	53.58	47.96	3.5	5.3	1.29	Highly susceptible
LGA089118	46.93	46.00	3.6	3.9	0.03	Moderately susceptible
LGA021I83	53.08	45.71	3.8	5.8	1.14	Highly susceptible
LGA021158	50.08	37.21	3.9	5.8	1.57	Highly susceptible
LGA074444	53.58	46.21	4.0	5.6	1.47	Highly susceptible
LGA164116	54.83	47.71	4.0	5.6	1.42	Highly susceptible
LGA0890020	56.58	43.21	4.1	6.1	1.84	Highly susceptible
LGA264116	57.58	49.71	4.9	6.6	1.57	Highly susceptible
LGA164I83	54.08	42.21	5.2	8.6	2.44	Highly susceptible
LGA074158	58.58	50.71	5.4	7.8	1.44	Highly susceptible
LGA074I83	52.08	44.21	5.7	7.6	1.34	Highly susceptible
LGA264444	61.08	42.21	5.9	9.3	3.62	Highly susceptible
LGA089A	51.33	43.46	6.2	7.6	1.24	Highly susceptible
LGA089716	50.58	36.96	6.3	9.3	1.79	Highly susceptible
LGA074A	51.58	45.46	6.5	9.6	1.37	Highly susceptible
LGA218444	63.83	38.96	6.6	10.1	3.07	Highly susceptible
LGA089444	65.35	46.51	7.2	11.9	3.32	Highly susceptible
LGA2640020	54.91	45.07	7.9	12.3	2.14	Highly susceptible
LGA164A	62.33	39.71	8.1	11.6	3.09	Highly susceptible
LGA264I83	59.33	44.46	8.2	10.8	1.84	Highly susceptible
LGA088444	57.58	43.46	8.7	15.1	3.04	Highly susceptible
LGA074116	62.57	49.73	9.8	12.8	2.16	Highly susceptible
LGA0210020	61.33	39.71	10.8	15.8	3.32	Highly susceptible
LGA021A	58.08	40.96	10.9	16.6	3.52	Highly susceptible
LGA264216	69.33	46.21	13.6	19.1	4.22	Highly susceptible
LGA218I83	68.83	43.21	13.8	17.8	5.14	Highly susceptible
LGA164444	91.08	43.71	16.3	23.1	6.29	Highly susceptible
LGA264A	71.08	34.96	16.7	23.8	6.49	Highly susceptible
Mean	**56.52**	**43.91**	**6.3**	**9.26**	**2.16**	
P.level	Sg**	Sg*	Sg**	Sg**	Sg**	
CV (%)	20.41	13.3	19.5	21.8	27.05	
lsd (0.05)	18.1	9.12	0.645	0.758	0.4412	
SED	9.12	4.59	0.325	0.382	0.2223	
H^2	0.9	0.89	0.93	0.94	0.97	

sg* = Significant at $p<0.05$, sg** = Significant at $p<0.001$

5.5.3.2 Correlation between grain resistance parameters

Highly significant correlations ($p<0.001$) were obtained among all parameters except for insect mortality. Percent grain damage and percent grain weight loss had a correlation coefficient of 0.9847, percent grain damage and total number of insects (0.8187), percent grain weight loss and total number of insects (0.8287) (Appendix 5.3).

5.5.3.3 Yield potential of F_1 hybrids (set c)

Analysis of variance for yield potential within each of the three environments (rainfed, irrigated, drought) showed no significant differences among the hybrids. However, combined yield analysis across environments showed significant differences ($p<0.01$) for yield potential and environmental effects. There was no evidence of significant interaction between the environments and varieties. The following were the best yielding maize hybrids, LGA089444, LGA0890020, LGA218I83, LGA164A, LGA087I83, LGA0870020, LGA0210020, LGA164444, and LGA021A. These hybrids had the highest yield across the three environments ranging from 7.96 to 14.44 tons/ha. Except for LGA021A, the rest of the hybrids out performed “Kanyani” the commercial hybrid that showed a yield potential of 8.42 tons/ha. LGA089444, LGA0890020 and LGA164A had better general adaptation across the environments (Table 5.8).

5.5.3.4 Yield potential and larger grain borer resistance among F_1 hybrids

Using levels of resistance and yield potential across the environments as criteria for selection of hybrids, only two hybrids, LGA087I83 and LGA089116 showed high yield potential (8.89 tons/ha) and (6.6 tons/ha) respectively and high levels of resistance to larger grain borer (Table 5.8).

Table 5.8: Yield potential and resistance levels of maize hybrids to LGB (set c)

Variety	Yield (tons/ha)	Group	Mean rank	Superiority Index	Grain damage (%)	Net yield (tons/ha)	Resistance status
LGA089444	14.44	2	1	0	11.9	12.73	Highly susceptible
LGA0890020	10.55	3	1.33	0.01451	6.1	9.91	Highly susceptible
LGA218I83	10.00	2	4	0.01389	17.8	8.22	Highly susceptible
LGA164A	9.58	4	8	0.0236	11.6	8.47	Highly susceptible
LGA087I83	8.89	2	7	0.0217	1.9	8.72	Resistant
LGA0870020	8.89	2	7	0.0217	3.6	8.57	Moderately susceptible
LGA0210020	8.52	3	6.67	0.01748	15.8	7.18	Highly susceptible
LGA164444	8.48	3	7.5	0.03643	23.1	6.52	Highly susceptible
Kanyani	8.42	1	9.5	0.0039			
LGA021A	7.96	3	10.17	0.04581	16.6	6.65	Highly susceptible
LGA021I83	7.78	2	11.5	0.03125	5.8	7.33	Highly susceptible
LGA074158	7.78	2	11.5	0.03125	7.8	7.17	Highly susceptible
LGA089716	7.78	2	11.5	0.03125	9.3	7.05	Highly susceptible
LGA264I83	7.59	4	14.75	0.05368	10.8	6.77	Highly susceptible
LGA264444	7.40	3	9.5	0.03125	9.3	6.71	Highly susceptible
LGA218444	7.24	3	9	0.06112	10.1	6.51	Highly susceptible
LGA164116	7.13	3	11.17	0.05627	5.6	6.73	Highly susceptible
LGA089I83	6.93	3	11.83	0.05626	4.9	6.59	Susceptible
LGA264A	6.90	3	14	0.07123	23.8	5.26	Highly susceptible
LGA074A	6.81	4	23	0.08536	9.6	6.16	Highly susceptible
LGA088A	6.75	4	22.25	0.08903	2.8	6.56	Moderately resistant
LGA264158	6.74	3	13.17	0.05737	7.9	6.21	Highly susceptible
LGA074116	6.67	2	16.5	0.04253	12.8	5.81	Highly susceptible
LGA088444	6.67	2	16.5	0.04253	15.1	5.66	Highly susceptible
LGA264202	6.67	4	16.5	0.04253	4.1	6.40	Susceptible
LGA089116	6.60	4	22.5	0.07948	1.5	6.51	Resistant
LGA0740020	5.96	3	17.33	0.0733	3.3	5.76	Moderately susceptible
LGA074I83	5.56	2	21.5	0.05556	7.6	5.14	Highly susceptible
LGA2640020	5.55	3	16.17	0.07499	12.3	4.87	Highly susceptible
LGA021158	5.16	3	18.17	0.07465	5.8	4.86	Highly susceptible
LGA089A	5.13	4	21.25	0.11162	7.6	4.74	Highly susceptible
LGA164I83	4.94	3	18.67	0.10864	8.6	4.52	Highly susceptible
LGA176291-4	4.67	2	25.5	0.06722	5.3	4.42	Highly susceptible
LGA021116	4.44	2	30	0.07031	7.9	4.09	Highly susceptible
LGA074444	4.44	2	30	0.07031	5.6	4.20	Highly susceptible
LGA264216	3.33	2	34.5	0.08681	19.1	2.70	Highly susceptible
LGA264116	2.44	2	36	0.10125	6.6	2.28	Highly susceptible

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
VARIETY	57.1	36	1.59	0.014
ENVIRONMENT	79.53	2	39.77	<0.001
VAR.ENVIRO	29.87	33	0.91	0.624

H^2 (yield) = 0.91

5.5.4 Set D: LGB resistant lines X LGB resistant lines

5.5.4.1 Resistance of F_1 hybrids to larger grain borer

Ssignificant differences were observed for percent grain damage, flour weight, and total number of insects. No significant differences were observed for insect mortality and percent grain weight loss (Table 5.9).

5.5.4.1.1 Total number of insects

Significant differences (p<0.01) were observed for total number of insects among maize hybrids. Maize hybrids, LGLG087218, LGLG089089, LGLG089218, LGLG021074, and LGLG088218 had the least number of insects. On the other hand, maize hybrids, LGLG007074, LGLG087074, LGLG007088, LGLG021264 and LGLG088164 had the highest number of insects (Table 5.9). The majority of the hybrids experienced moderate to high numbers of insects (Figure 5.18).

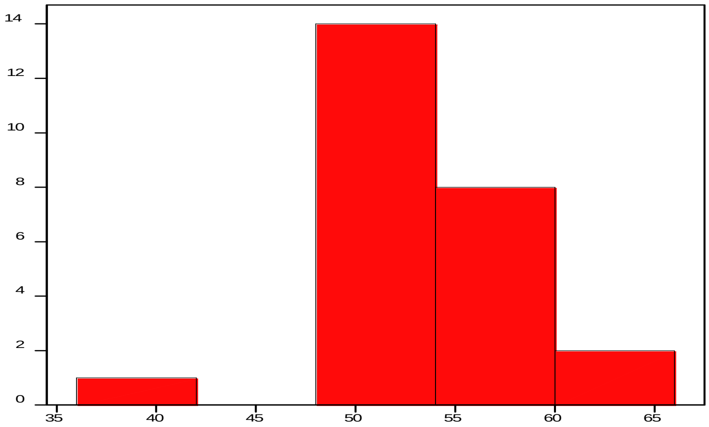

Figure 5.18: Distribution of variation for total number of insects among F1 hybrids (set d)

5.5.4.1.2 Flour weight (g)

Highly significant differences (p<0.001) were observed for flour weight among the hybrids. The following hybrids produced the least amount of flour, LGLG021074, LGLG089089, LGLG088218, LGLG074007 and LGLG089218. Maize hybrids, LGLG007088, LGLG007074, LGLG088176, LGLG021264 and LGLG089074 produced the highest amount of flour (Table

5.8). The distribution for variation for flour weight among the hybrids indicated that most of the hybrids produced moderate to little amount of flour (Figure 5.19).

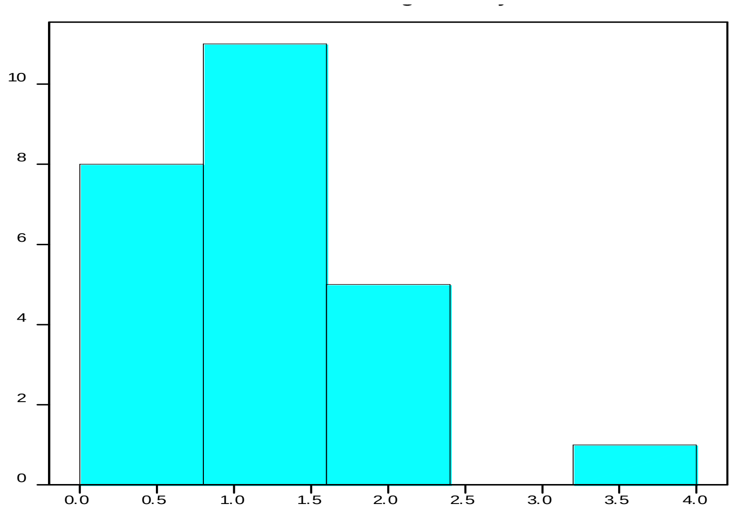

Figure 5.19: Distribution of variation for flour weight (g) among F1 hybrids (set d)

5.5.4.1.3 Percent grain weight loss

No significant differences were observed for percent grain weight loss among the F_1 hybrids (Table 5.9). However, maize hybrids, LGLG089218, LGLG088218, LGLG021074, LGLG021161, LGLG007164 and LGLG087264 showed less grain weight loss, while maize hybrids, LGLG007088, LGLG021264, LGLG164007 and LGLG089074 experienced high grain weight losses. The distribution of variation for grain weight loss showed that most of the hybrids experiencing moderate grain weight losses (Figure 5.20).

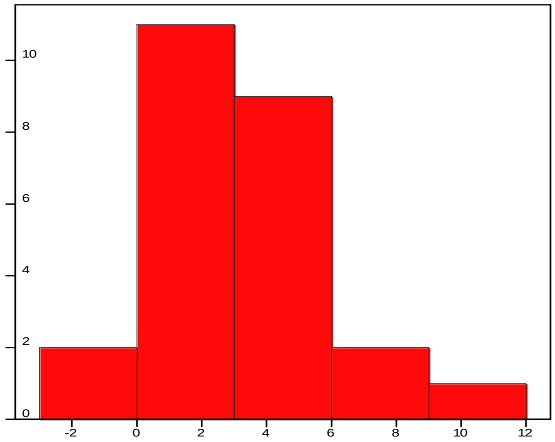

Figure 5.20: Distribution of variation for grain weight loss (%) among F1 hybrids (set d)

5.5.4.1.4 Grain damage (%)

Highly significant differences ($p<0.001$) were observed for percent grain damage among the hybrids. The following hybrids experienced the least grain damage, LGLG089218, LGLG021074, LGLG007164, LGLG087264, and LGLG088218, while maize hybrids, LGLG007088, LGLG021264, LGLG021164 and LGLG007074 experienced substantial grain damage (Table 5.9). Distribution of variation for percent grain damage showed that most hybrids experienced moderate to low grain damage (Figure 5.21).

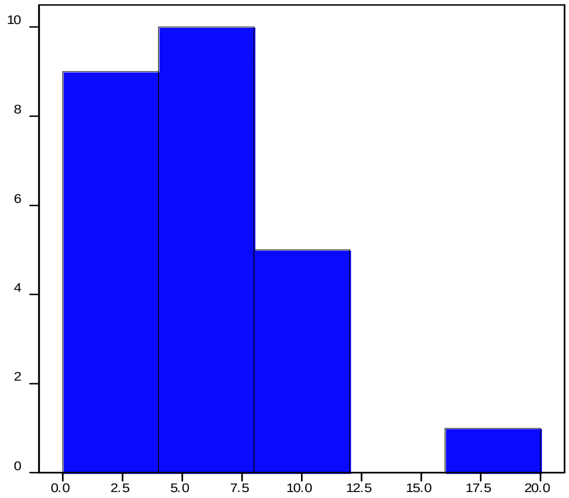

Figure 5.21: Distribution of variation for percent grain damage among F_1 hybrids (set d)

Grain damage as a measure of resistance determined that 4% of the hybrids were resistant, 24% moderately resistant, 8% moderately susceptible, 8% susceptible and 56% highly susceptible (Figure 5.22).

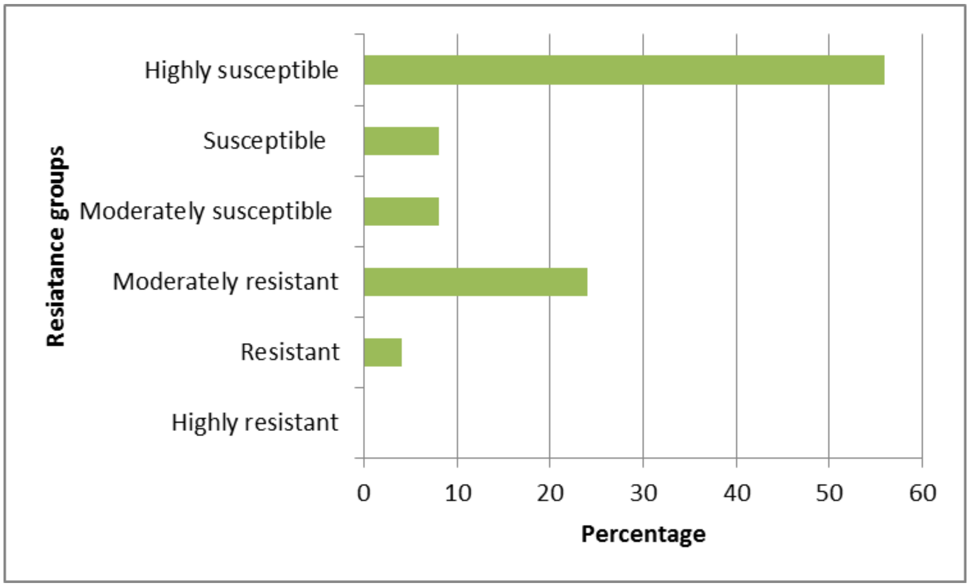

Figure 5.22: Grouping of F_1 hybrids into resistant groups based on grain damage (%)

Table 5.9: Table of means for grain resistance parameters against larger grain borer (set d)

Variety	**Total number of insects**	**Insect mortality**	**Weight loss (%)**	**Grain damage (%)**	**Flour weight (g)**	**Resistance status**
LGLG089218	36.4	53.1	2.1	1.9	0.22	Resistant
LGLG088218	50.0	45.2	0.0	3.0	0.43	Moderately resistant
LGLG021074	50.2	46.5	0.9	2.1	0.36	Moderately resistant
LGLG089089	50.4	45.8	3.2	5.5	0.35	Highly susceptible
LGLG087218	51.0	47.7	2.2	3.0	0.51	Moderately resistant
LGLG081218	51.0	41.2	4.7	8.2	0.66	Highly susceptible
LGLG074007	51.5	49.2	2.5	3.5	0.50	Moderately susceptible
LGLG087264	51.5	46.1	0.7	2.7	1.13	Moderately resistant
LGLG088264	51.7	47.0	3.8	5.0	0.86	Susceptible
LGLG007264	52.0	44.5	5.3	7.2	0.86	Highly susceptible
LGLG088176	52.2	43.5	2.3	4.0	1.86	Moderately susceptible
LGLG089176	52.2	48.2	4.6	6.0	0.96	Highly susceptible
LGLG164007	52.5	42.2	5.8	8.0	1.03	Highly susceptible
LGLG089074	52.7	43.7	5.8	8.2	1.76	Highly susceptible
LGLG007218	52.8	46.1	2.4	4.8	1.38	Susceptible
LGLG21164	54.2	49.0	0.0	10.2	1.46	Highly susceptible
LGLG089007	54.7	46.0	2.0	7.3	1.11	Highly susceptible
LGLG007164	55.5	51.2	0.3	2.6	0.81	Moderately resistant
LGLG021176	56.0	46.7	4.9	7.0	1.43	Highly susceptible
LGLG007176	57.0	50.2	2.2	3.0	0.61	Moderately resistant
LGLG088164	58.0	49.0	0.6	7.2	1.76	Highly susceptible
LGLG021264	58.5	44.5	6.4	10.5	1.83	Highly susceptible
LGLG007088	58.5	40.2	11.0	18.2	3.33	Highly susceptible
LGLG087074	60.7	39.7	4.3	6.7	0.86	Highly susceptible
LGLG007074	61.5	45.7	6.0	9.0	2.23	Highly susceptible
Mean	**52.1**	**45.2**	**3.4**	**6.4**	**1.16**	
P.level	Sg*	nsg	nsg	Sg**	Sg**	
CV (%)	18.7	15.1	25.4	15.1	7.76	
Isd(0.05)	14.45	10.43	0.6679	0.5215	0.1935	
Sed	7.246	5.23	0.3343	0.2613	0.097	
H^2	0.89	0.85	0.83	0.93	0.94	

Sg* = Significant at p<0.01, sg** = Significant at p<0.001, nsg= not significant

5.5.4.2 Correlations between grain resistance parameters

Highly significant correlation ($p<0.001$) was observed between percent grain damage and percent weight loss (0.7658), significant correlation ($p<0.01$) was observed between percent grain damage and total number of insects (0.5174) (Appendix 5.4).

5.5.4.3 Yield potential of F_1 hybrids (set d)

Under drought conditions maize hybrids showed significant differences ($p<0.01$) for yield potential. No significant differences were observed among the hybrids for yield potential under irrigation and rainfed conditions. Highly significant differences ($p<0.001$) were observed for yield potential and environmental effects across environments. Significant differences ($p<0.01$) were also observed for the interaction between environments and the hybrids. The following hybrids were the top five high yielding varieties, LGLG218089, LGLG088264, LGLG088176, LGLG021007, LGLG087218, and LGLG007264 with yields ranging from 6.22 to 8.80 tons/ha. However, only varieties LGLG218089 (8.80 tons/ha) and LGLG088264 (7.16 tons/ha) outperformed the commercial hybrid (Kanyani) (7.11 tons/ha). The superiority index revealed that maize varieties, LGLG218089, LGLG088264, and LGLG088176 were generally adapted to the three environments (Table 5.10).

5.5.4.4 Yield potential and maize weevil resistance of F_1 hybrids

Combining yield potential and resistance, only three maize hybrids, LGLG087218 (6.36 tons/ha), LGLG088218 (6.00 tons/ha) and LGLG021074 (4.13 tons/ha) met the criteria for selection. However, the hybrids were moderately resistant against larger grain borer (Table 5.10).

Table 5.10: Mean yield and resistance levels for F_1 hybrids (set d)

Variety	Mean rank	Group	Superiority index	Yield (tons/ha)	Grain damage (%)	Resistance status	Net yield (tons/ha)
LGLG218089	8	4	0.00437	8.80			
LGLG088264	8.5	2	0.02506	7.16	4.96	Susceptible	6.80
Kanyani	7.5	1	0.00981	7.11			
LGLG088176	7.33	3	0.01968	6.67	3.96	Moderately susceptible	6.40
LGLG021007	20	4	0.01674	6.53			
LGLG087218	11.67	3	0.02053	6.36	2.96	Moderately resistant	6.17
LGLG007264	7.67	3	0.01534	6.22	7.21	Highly susceptible	5.77
LGLG007218	6.67	3	0.0069	6.18	4.75	Susceptible	5.88
LGLG087074	9	3	0.04647	6.04	6.71	Highly susceptible	5.64
LGLG088218	10.83	3	0.0194	6.00	2.96	Moderately resistant	5.82
LGLG087176	15	2	0.01602	5.82	4.15	Susceptible	5.58
LGLG007074	17	2	0.03403	5.51	8.96	Highly susceptible	5.02
LGLG089264	12.83	3	0.02575	5.33			
LGLG088074	12.5	5	0.03516	5.07			
LGLG164007	14.83	3	0	5.07	7.96	Highly susceptible	4.66
LGLG164089	26	4	0.02971	5.02			5.02
LGLG007088	20	2	0.04949	4.76	18.21	Highly susceptible	3.89
LGLG021218	15	3	0.01407	4.71	8.21	Highly susceptible	4.32
LGLG089007	13.17	3	0.00393	4.62	7.25	Highly susceptible	4.29
LGLG089176	13.5	3	0.00392	4.53	5.96	Highly susceptible	4.26
LGLG087264	14	6	0.00391	4.44	2.74	Moderately resistant	4.32
LGLG089089	14	6	0.00391	4.44	5.51	Highly susceptible	4.20
LGLG021164	16.67	3	0.00721	4.44	10.21	Highly susceptible	3.99
LGLG176089	29	4	0.03682	4.31			
LGLG021074	20.67	3	0.04829	4.13	2.07	Moderately resistant	4.05
LGLG088164	11.67	3	0.00447	4.09	7.21	Highly susceptible	3.79
LGLG007087	24	2	0.03224	4.09			
LGLG218007	18.5	3	0.02356	3.91			
LGLG089164	19.5	3	0.01235	3.82			
LGLG007176	19.83	3	0.01883	3.82	2.96	Moderately resistant	3.71
LGLG089074	19.33	3	0.0389	3.56	8.21	Highly susceptible	3.26
LGLG021176	21.33	3	0.01663	3.51	6.96	Highly susceptible	3.27
LGLG007164	17.83	3	0.00941	3.47	2.55	Moderately resistant	3.38
LGLG087164	19.33	3	0.00833	3.16			
LGLG089218	16.5	5	0.00098	2.76	1.87	Resistant	2.70
LGLG021264	23	5	0	1.96	10.46	Highly susceptible	1.75
LGLGO74007	30.25	2	0.0871	1.69	3.46	Moderately susceptible	1.63

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
VARIETY	86.89	36	2.41	<0.001
ENVIRONMENT	130.04	2	65.02	<0.001
VARIETY.ENVIRON	80.68	50	1.61	0.004

H^2 (yield) = 0.91

5.5.5 SET E: Larger gain borer resistant lines X Maize weevil resistant lines

5.5.5.1 Grain resistance of F_1 hybrids to maize weevil

Highly significant differences (p<0.001) were observed among the hybrids for adult mortality, total number of insects, and percent grain damage. No significant differences were observed for percent grain weight loss (Table 5.11).

5.5.5.1.1 Insect mortality

Highly significant differences (p<0.001) were obtained for insect mortality among maize hybrids. Maize hybrids, MWIg939164, MWIg151264, MWIg13089, IgMW26411, and IgMW08812 showed the highest number of insect mortalities. On the other hand, maize hybrids MWIg08164, IgMW08711, MWIg06264, IgMW26410 and IgMW02111 had the least number of insect mortalities (Table 5.11). Distribution of variation for insect mortalities indicated that most hybrids experienced low levels of insect mortalities (Figure 5.23).

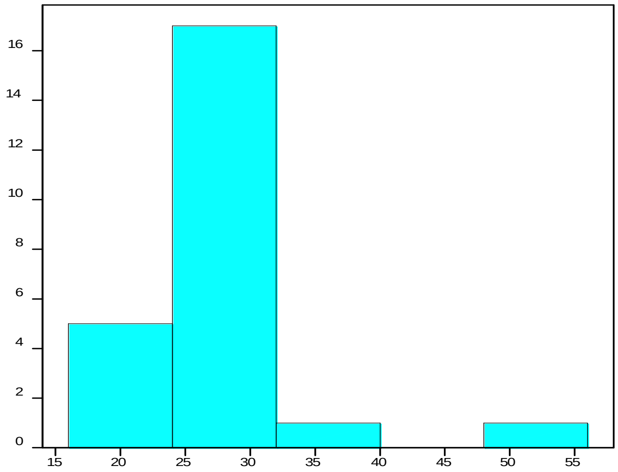

Figure 5.23: Distribution of variation for insect mortality among F1 hybrids (set e)

5.5.5.1.2 Total number of insects

Highly significant differences (p<0.001) were observed for total number of insects among the hybrids. Maize hybrids MWIg06264, MWIg11176, IgMW16413, IgMW087940, and IgMW02111 had the lowest number of insects, while maize hybrids, MWIg939164, MWIg151264, MWIg13089, IgMW08812, and MWIg13218 had the largest total number of insects (5.11). The majority of the hybrids experienced lower numbers of insects (Figure 5.24).

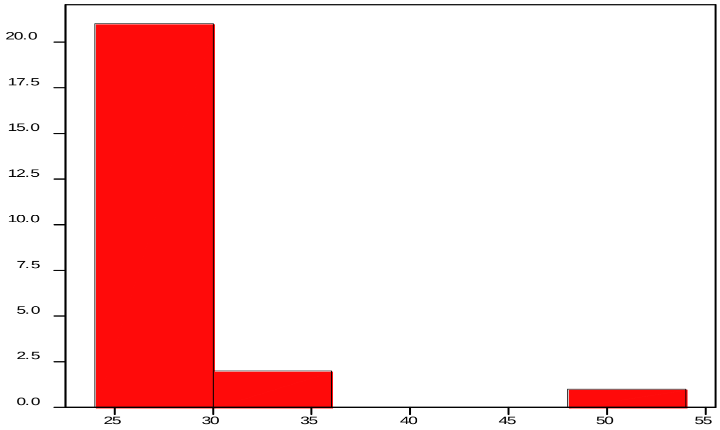

Figure 5.24: Distribution of variation for total number of insects among F1 hybrids (set e)

5.5.5.1.3 Grain weight loss (%)

There were no significant differences for weight loss among the F_1 hybrids. However, maize hybrids IgMW007940, IgMW089151, IgMW16410, IgMW087940, MWIg06264 and MWIg08164 experienced lower grain weight loss, while maize hybrids IgMW26410, IgMW087711, MWIg939164 and MWIg13089 showed large grain weight loss (Table 5.11). Distribution of variation for grain weight loss (%) revealed that most of the F_1 hybrids experienced minimal grain weight loss (Figure 5.25).

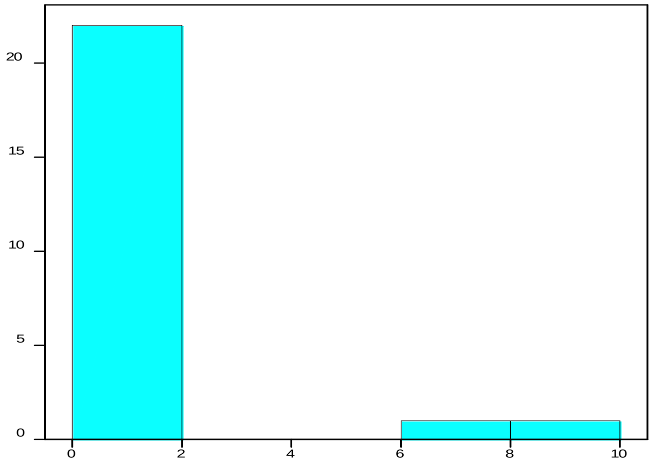

Figure 5.25: Distribution of variation for grain weight losses (%) among F1 hybrids (set e)

5.5.5.1.4 Grain damage (%)

Highly significant differences (p<0.001) were observed for percent grain damage among maize hybrids. The least number of damaged grains were observed in the following maize hybrids, IgMW007940, IgMW087940, MWIg06264, IgMW089151, MWIg08007, MWIg06021, IgMW08710 and MWIg08164, while maize hybrids, IgMW26410, IgMW08711, IgMW16411, IgMW16410 and MWIg13089 experienced the highest grain damage (Table 5.11). Distribution of variation for grain damage (%) revealed that most of the hybrids experienced less grain damage (Figure 5.26).

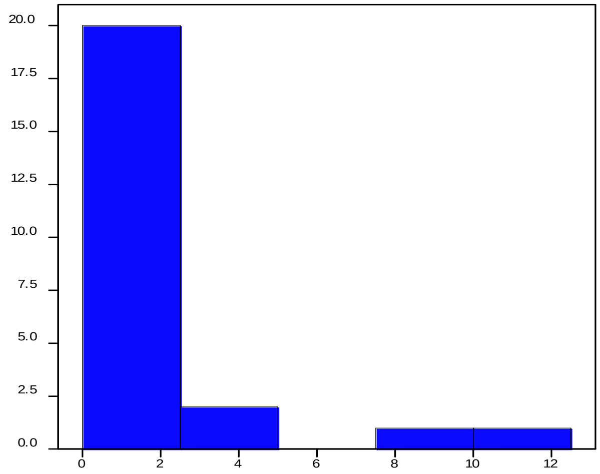

Figure 5.26: Distribution of variation for grain damage (%) among F1 hybrids (set e)

Grain damage (%) as a measure of resistance determined that 16.7% of the F_1 hybrids were highly resistant, 50% were resistant, 21% moderately resistant, 4.2% moderately susceptible and 8.3% highly susceptible (Figure 5.27).

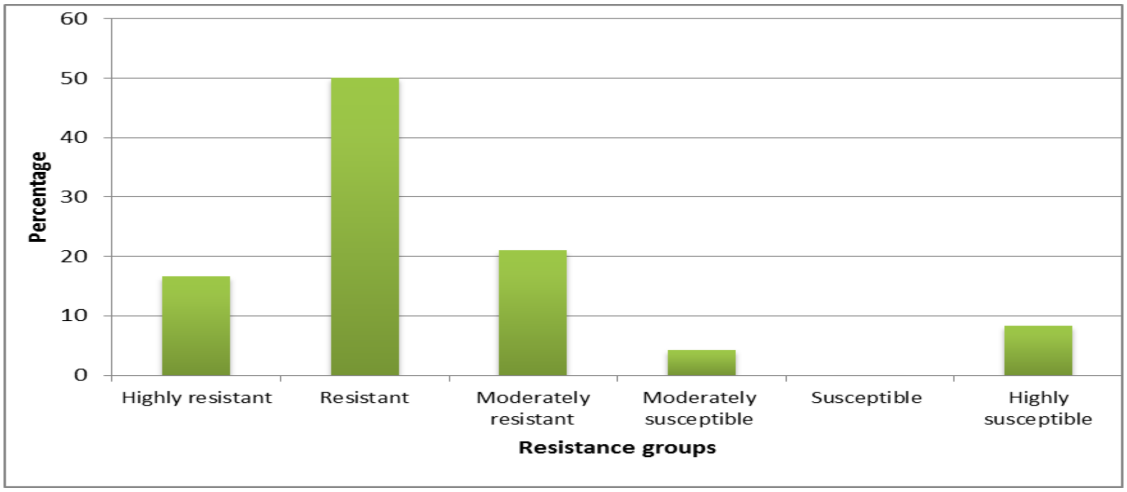

Figure 5.27: Grouping of F_1 hybrids into resistance groups using grain damage (%)

Table 5.11: Table of means for grain resistance parameters against maize weevil (set e)

Variety	**Total number of insects**	**Adult mortality**	**Weight loss (%)**	**Grain damage (%)**	**Resistance reaction**
IgMW007940	25.35	24.7	0.0	0.0	Highly resistant
IgMW021151	26.02	25.4	0.0	0.3	Resistant
IgMW089151	25.35	25.0	0.0	0.0	Highly resistant
IgMW16410	25.35	24.7	0.0	2.7	Moderately resistant
MWIg06021	25.68	25.7	0.0	1.0	Resistant
MWIg08007	25.35	24.7	0.0	0.0	Highly resistant
MWIg08164	25.44	22.4	0.0	0.0	Highly resistant
IgMW26411	27.68	27.4	0.0	2.3	Moderately resistant
MWIg06264	25.02	23.4	0.1	1.3	Resistant
IgMW08812	29.02	28.7	0.1	0.7	Resistant
MWIg13218	29.02	26.7	0.1	2.0	Resistant
MWIg151264	34.02	33.7	0.1	0.3	Resistant
MWIg11176	25.02	24.4	0.2	1.0	Resistant
IgMW02111	25.02	23.7	0.2	2.3	Moderately resistant
IgMW16413	25.02	24.7	0.2	0.3	Resistant
IgMW08710	25.68	25.0	0.2	1.3	Resistant
IgMW087940	25.02	24.4	0.3	0.7	Resistant
IgMW17606	26.68	26.7	0.6	2.3	Moderately resistant
IgMW16411	26.35	24.7	0.7	3.7	Moderately susceptible
MWIg13074	26.35	25.7	0.9	1.7	Resistant
MWIg13089	31.35	31.0	1.0	2.3	Moderately resistant
MWIg939164	52.68	52.7	1.7	1.0	Resistant
IgMW08711	25.68	23.0	6.0	10.0	Highly susceptible
IgMW26410	25.02	23.4	8.4	11.0	Highly susceptible
Mean	**27.63**	**26.7**	**0.9**	**2.0**	
P.level	Sg*	Sg*	nsg	Sg*	
CV (%)	3.9	4.8	21.8	16.8	
lsd (0.05)	0.218	0.263	0.66	0.468	
SED	0.108	0.131	0.328	0.233	
H^2	0.92	0.91	0.82	0.89	

sg*= significant at p<0.001, nsg= not significant

5.5.5.1.5 Correlation between resistance parameters for maize weevil

Highly significant correlations ($p<0.001$) were observed between percent grain damage and percent grain weight loss (0.9324) and between insect mortality and total number of insects (Appendix 5.5).

5.5.5.2 Grain resistance of F_1 hybrids to larger grain borer

Significant differences were observed for percent grain damage, flour weight, and percent grain weight loss. Insect mortality and total number of insects did not show any significant differences (Table 5.12).

5.5.5.2.1 Flour weight (g)

Significant differences ($p<0.05$) were observed for flour weight among the hybrids. Maize hybrids, IgMW089151, IgMW08710, MWIg06021, IgMW021151, and MWIg06264, produced the least amount of flour. The highest amount of flour was observed on maize hybrids, MWIg11176, IgMW26411, MWIg13074, IgMW08711 and IgMW16411 (Table 5.12). Most hybrids produced moderate to small amount of flour (Figure 5.28).

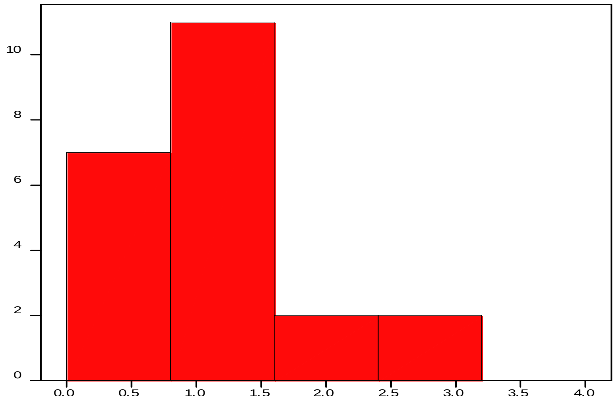

Figure 5.28: Distribution of variation for flour (g) among F1 hybrids (set e)

5.5.5.2.2 Grain weight loss (%)

Highly significant differences ($p<0.001$) were observed for grain weight loss among F_1 hybrids. Maize hybrids IgMW087940, MWIg06264, IgMW08710, IgMW089151 experienced less grain weight loss, while maize hybrids MWIg11176, IgMW16411, MWIg13074 and IgMW26411 showed high grain weight loss (Table 5.12). Distribution of variation for grain weight loss revealed that maize hybrids experienced low to high levels of grain weight loss (Figure 5.29).

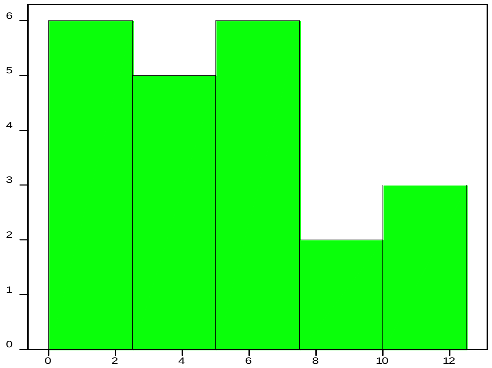

Figure 5.29: Distribution of variation for percent grain weight loss among F1 hybrids (set e)

5.5.5.2.3 Percent grain damage (%)

Highly significant differences ($p<0.001$) were observed for percent grain damage among maize hybrids. Maize hybrids MWIg06264, IgMW087940, IgMW089151, IgMW08710, and IgMW08812 sustained minimal grain damage, while maize hybrids IgMW26411, MWIg11176, IgMW16411, MWIg13074 and IgMW17606 experienced the largest number of damaged grains (Table 5.12). Distribution of variation for grain damage (%) showed that the majority of the hybrids experienced low to high grain damage (%) (Figure 5.30).

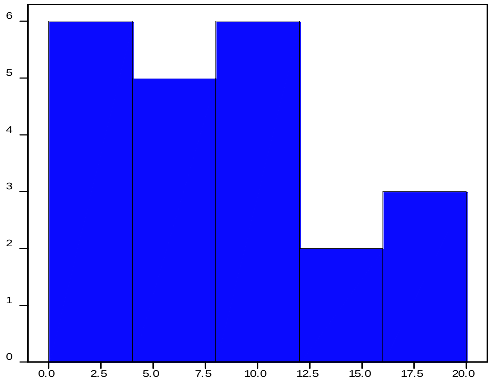

Figure 5.30: Distribution of variation for grain damage among F1 hybrids (set e)

The percent grain damage as an indicator of resistance revealed that 9% of the hybrids were resistant, 4.5% moderately resistant, 13.6% moderately susceptible, 4.5% susceptible and 68% highly susceptible. (Figure 5.31).

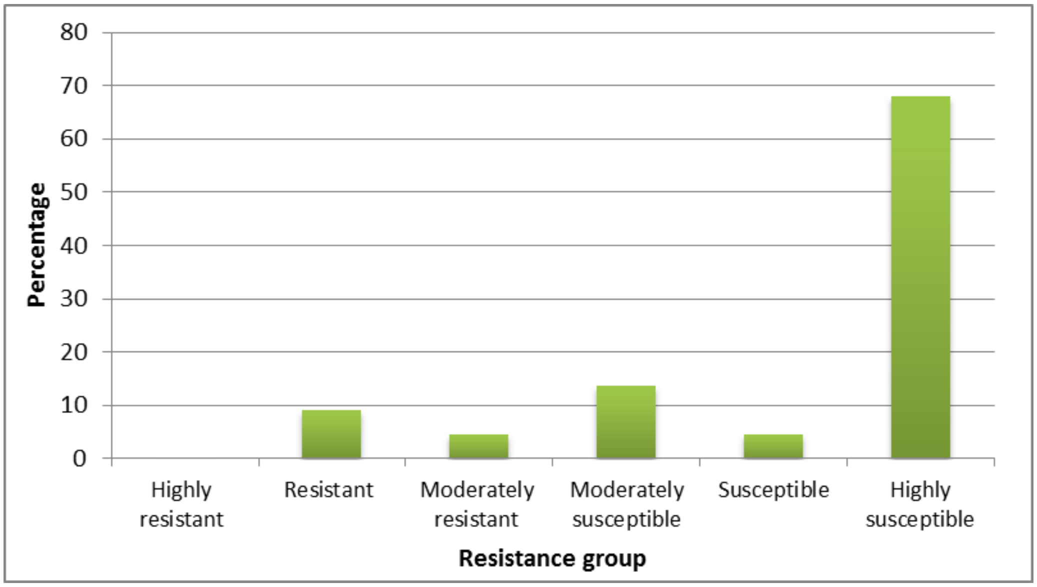

Figure 5.31: Grouping of F_1 hybrids based on grain damage (%) among F1 hybrids (set e)

Table 5.12: Table of means for grain resistance parameters against larger grain borer (set e)

Variety	Total number of insects	Insect mortality	Weight loss (%)	Grain damage (%)	Flour weight (g)	Resistance reaction
IgMW087940	25.03	23.0	0.0	1.7	0.90	Resistant
MWIg06021	25.03	20.7	2.8	4.7	0.30	Susceptible
IgMW08710	25.36	21.3	1.7	3.0	0.24	Moderately susceptible
IgMW089151	26.36	24.7	1.7	2.7	0.10	Moderately resistant
IgMW021151	27.03	23.0	2.4	3.7	0.36	Moderately susceptible
MWIg13218	27.03	20.0	4.1	7.0	0.90	Highly susceptible
IgMW08711	28.36	22.7	4.8	8.7	1.40	Highly susceptible
IgMW26410	29.03	24.3	6.1	9.3	1.28	Highly susceptible
IgMW02111	29.36	24.7	5.5	8.7	1.04	Highly susceptible
IgMW007939	29.36	23.0	6.0	11.7	1.07	Highly susceptible
IgMW08812	30.03	22.3	2.4	3.3	0.40	Moderately susceptible
MWIg151264	30.69	26.0	5.8	8.7	1.20	Highly susceptible
MWIg13089	31.03	25.7	5.3	6.0	0.67	Highly susceptible
IgMW17606	31.62	26.3	7.8	12.8	1.28	Highly susceptible
IgMW16413	33.03	21.0	6.2	8.7	1.00	Highly susceptible
MWIg06264	33.36	28.0	0.8	1.3	0.40	Resistant
IgMW26411	34.03	21.0	11.9	19.7	2.37	Highly susceptible
IgMW16410	35.69	31.7	3.4	5.3	0.90	Highly susceptible
MWIg13074	36.36	26.0	8.0	15.0	1.60	Highly susceptible
MWIg08164	37.36	29.0	4.9	6.3	1.07	Highly susceptible
IgMW16411	40.36	23.0	11.2	16.7	2.43	Highly susceptible
MWIg11176	47.03	20.3	10.1	19.0	3.10	Highly susceptible
Mean	**31.48**	**24.0**	**5.0**	**8.4**	**1.09**	
P.level	nsg	nsg	Sg**	Sg**	Sg*	
CV	24.9	21.8	22.1	15.6	22.3	
lsd(0.05)	13.05	8.712	0.6927	0.6165	0.4179	
Sed	6.461	4.314	0.3425	0.3053	0.2064	
H^2	0.81	0.75	0.9	0.9	0.85	

* Significant at $p<0.05$, **significant at $p<0.001$, nsg=not significant

5.5.5.3 Correlation between resistance parameters for larger grain borer

Highly significant correlations ($p<0.001$) were observed between percent grain damage and percent grain weight loss (0.9726), between percent grain damage and total number of insects (0.6685), and between percent grain weight loss and total number of insects (0.6771) (Appendix 5.6).

5.5.5.4 Yield potential of F_1 hybrids (set e)

Analysis of individual environments revealed significant differences ($p<0.01$) for yield potential among the hybrids under rainfed and no significant differences for yield potential were observed among the hybrids under irrigation and drought. Combined yield analysis revealed highly significant differences ($p<0.001$) for yield potential and significant differences ($p< 0.01$) for the genotype and environment interaction among the hybrids. Maize hybrids, lgMW26413, MWlg08264, lgMW087940, lgMW26411, lgMW08710, lgMW16411, lgMW13218, lgMW02111, MWlg939074 and MWlg08089 showed highest yield potential, with mean yields ranging from 8.92 to 14.23 tons/ha. These hybrids out yielded the check “Kanyani” which had a mean yield of 6.34 tons/ha. The following hybrids exhibited general adaptability, lgMW26413, lgMW26411, lgMW087940, lgMW08710, lgMW26411 and lgMW16411 (Table 5.13).

Table 5.13: Mean yield potential and mean rank of F_1 hybrids across environments (set e)

Variety	Yield (tons/ha)	Group	Mean rank	Superiority Index
LGMMW26413	14.23	2	4.75	0.05769
MWLG08264	11.08	5	10	0.00002
LGMW087940	11.05	2	10.25	0.0146
LGMW26411	10.30	3	5.83	0.02632
LGMW08710	10.16	2	8.75	0.0078
LGMW16411	10.00	6	2.5	0.01351
MWLG13218	9.93	2	12.25	0.00435
LGMW02111	9.37	3	7.5	0.01059
MWLG939074	8.98	2	13.25	0.01925
MWLG08089	8.92	4	9	0.01959
LGMW17611	8.89	6	8.5	0.0076
MWLG13021	8.86	3	7.17	0.02121
MWLG13089	8.81	2	17.75	0.00152
MWLG06087	8.61	5	20	0.00345
MWLG06264	8.16	2	17.25	0.02736
MWLG08007	8.16	2	18.25	0.00187
MWLG939264	7.98	4	14.25	0.01282
LGMW16413	7.78	6	13.5	0.00338
LGMW08706	7.67	2	19.75	0.00084
LGMW26410	7.60	3	13.5	0.01511
MWLG151089	7.37	5	27	0.00823
LGMW021151	7.09	2	22.25	0.00847
MWLG13074	6.68	3	15.33	0.00493
LGMW16410	6.67	6	18.5	0.00084
MWLG690264	6.64	3	15.17	0.01301
MWLG13218	6.56	3	10.5	0.01958
Kanyani	6.34	1	2	0.00067
MWLG10089	6.22	2	25.5	0.0064
LGMW08711	6.22	3	14.5	0.02829
MWLG06089	6.13	4	17.5	0.01582
MWLG940164	6.02	3	18.67	0.01039
MWLG08164	5.80	3	18.83	0.0424
LGMW08812	5.59	3	18.83	0.00795
MWLG11176	5.56	6	24	0
MWLG06021	5.40	7	16.25	0.00891
MWLG089151	5.24	3	20.83	0.01475
MWLG13164	5.17	5	33	0.02177
lgMW26413	4.93	1	5	0.00044
LGMW17613	4.82	3	22.33	0.01798
LGMW089151	4.68	2	31	0.02452
LGMW13089	4.65	5	35	0.02592
LGMW007939	4.17	3	28.17	0.04069
LGMW176151	3.80	3	25.67	0.03135
LGMW021939	3.72	2	32.75	0.01926
LGMW17606	3.54	3	27.67	0.0448
LGMW16406	2.37	2	36.25	0.05276
MWLG151264	1.98	7	27.75	0.02362

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
VARIETY	**132.31**	**49**	**2.7**	**<0.001**
ENVIRONMENT	112.19	2	56.1	<0.001
VARIETY.ENVIRONMENT	77.58	49	1.58	0.006

H^2 (yield) = 0.93

5.5.5.5 Yield potential and resistance levels of F_1 hybrids

Combining yield performance and resistance levels as criteria for selecting hybrids, maize hybrids lgMW087940 (11.05 tons/ha), MWlg06264 (8.16 tons/ha) and lgMW08710 (10.16 tons/ha), MWlg13218 (9.93 tons/ha) and lgMW089151 (5.24 tons/ha) were resistant to maize weevil and revealed high yield potential. Maize hybrids, lgMW08710, MWlg06264 and lgMW089151 were resistant to larger grain borer (Table 5.14).

Table 5.14: Yield potential, yield ranking and dual resistance among F_1 hybrids from set e

	Larger grain borer resistance		**Maize weevil resistance**			
Variety	**Grain damage (%)**	**Resistance reaction**	**Grain damage (%)**	**Resistance reaction**	**Yield (tons/ha)**	**Rank**
lgMW087940	1.7	Resistant	0.7	Resistant	11.05	1
MWlg06021	4.7	Susceptible	1.0	Resistant	5.40	19
lgMW08710	3.0	Moderately susceptible	1.3	Resistant	10.16	3
lgMW089151	2.7	Moderately resistant	0.0	Highly resistant	5.24	20
lgMW021151	3.7	Moderately susceptible	0.3	Resistant	7.09	12
MWlg13218	7.0	Highly susceptible	2.0	Resistant	9.93	6
lgMW08711	8.7	Highly susceptible	10	Highly Susceptible	6.22	15
lgMW26410	9.3	Highly susceptible	11	Highly susceptible	7.60	11
lgMW02111	8.7	Highly susceptible	2.3	Moderately resistant	9.37	7
lgMW08812	3.3	Moderate susceptible	0.7	Resistant	5.59	17
MWlg151264	8.7	Highly susceptible	0.3	Resistant	1.98	22
MWlg13089	6.1	Highly susceptible	2.3	Moderately resistant	8.81	8
lgMW17606	12.8	Highly susceptible	2.3	Moderately resistant	3.54	21
lgMW16413	8.7	Highly susceptible	0.3	Resistant	7.78	10
MWlg06264	1.3	Resistant	1.3	Resistant	8.16	9
lgMW26411	19.7	Highly susceptible	2.3	Moderately resistant	10.30	2
lgMW16410	5.3	Highly susceptible	2.7	Moderately resistant	6.67	14
MWlg13074	15.0	Highly susceptible	1.7	Resistant	6.68	13
MWlg08164	6.3	Highly susceptible	0.0	Highly resistant	5.80	16
lgMW16411	16.7	Highly susceptible	3.7	Moderately susceptible	10.00	4
MWlg11176	19.0	Highly susceptible	1.0	Resistant	5.56	18

5.5.6 Analysis for yield and resistance across sets (A-E)

5.5.6.1. Response of maize hybrids to maize weevil across three sets (A, B & E)

Highly significant differences ($p<0.001$) were observed for grain damage across three sets (Table 5.15).

Table 5.15: Analysis of Variance for percent grain damage across three sets

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Set	2	7.9595	3.9797	27.78	<.001
Variety	84	37.1165	0.4419	3.08	<.001
Residual	190	27.2165	0.1432		
Total	276	72.2925	0.261		

CV = 19.27, SE= 0.3785, LSD= 0.9394, P<0.001

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
set	22.56	2	11.28	<0.001
Variety. Set	253.06	83	3.05	<0.001

5.5.6.2 Top 20 maize weevil resistant F_1 hybrids

Using percent grain damage to compare maize hybrids for grain resistance against maize weevil across sets, the results showed that among the top 20 most maize weevil resistant hybrids, 18 hybrids came from set a “crosses between adapted Malawi lines and maize weevil resistant lines” and 2 hybrids from set b “crosses between maize weevil resistant lines”. The top five most ranked maize weevil resistant hybrids were MWA06A, MWA12395, MWA08202, MWA11312 and MWA10A belonged to set a (Appendix 5.7).

5.5.6.3 Response of F_1 hybrids to larger grain borer across sets (C, D & E)

Highly significant differences ($p<0.001$) were observed for percent grain damage across three sets (Table 5.16).

Table 5.16: Analysis of variance for percent grain damage across three sets

Analysis of variance	d.f.	s.s.	m.s.	v.r.	F pr.
Set	2	4.019	2.0095	8.7	<.001
Variety	83	65.2517	0.7862	3.4	<.001
Residual	211	48.7343	0.231		
Total	296	118.0049	0.3987		

CV= 22.48, se= 0.4806, lsd =0.9241, p<0.001

5.5.6.3.1 Top 20 larger grain borer resistant hybrids

Using percent grain damage to determine overall top 20 resistant hybrids against larger grain borer, the results revealed that ten hybrids came from set c “crosses between adapted Malawi lines and larger grain borer resistant lines”, seven hybrids came from set d “crosses between larger grain borer resistant lines” and three hybrids came from set e “crosses between maize weevil resistant lines and larger grain borer resistant lines” (Table 5.18). However, top three resistant hybrids came from set c, namely, LGA089116, LGA087I83, and LGA088A (Appendix 5.8).

5.5.6.4 Combined yield analysis of F_1 hybrids across environments

Highly significant differences (p<0.001) were observed for yield potential, environmental effects and the interaction between varieties and environments (Table 5.17)

Table 5.17: Analysis of variance for combined yield across environments

Fixed term	Wald statistic	d.f.	Wald/d.f.	chi pr
VARIETY	674.62	195	3.46	<0.001
ENVIRONMENT	263.05	2	131.52	<0.001
VARIETY.ENVIRONMENT	324.32	214	1.52	<0.001

Among the top hybrids with high yielding potential, set a contributed seven hybrids, five hybrids came from set b, two hybrids from set c, six hybrids from set e and none from set d (Figure 5.32).

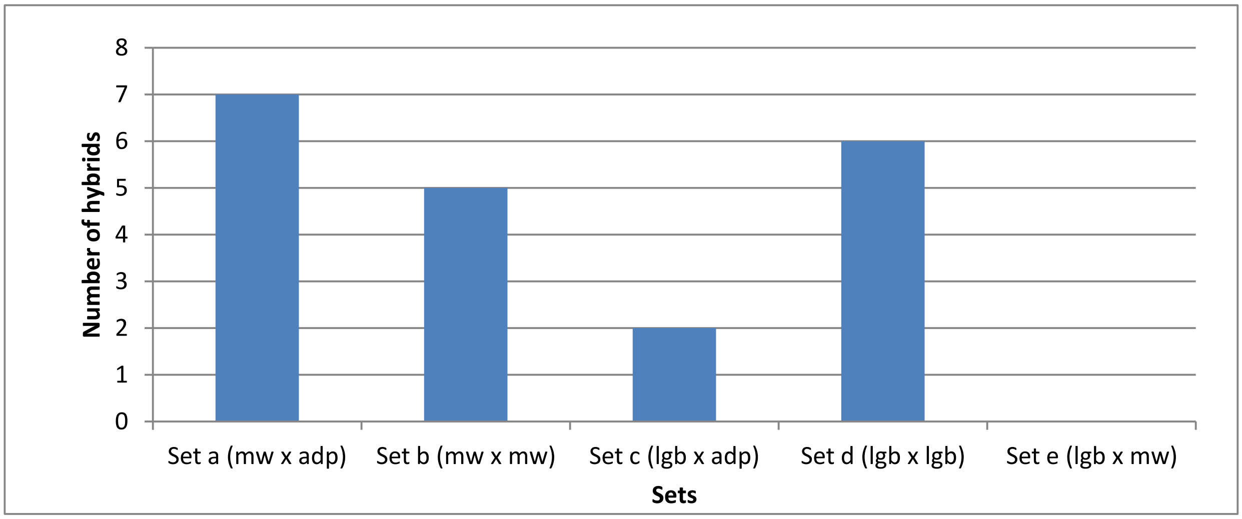

Figure 5.32: Contribution of each set to top 20 high yielding hybrids

Note: MW = maize weevil resistant lines, LGB = LGB resistant lines, Adp = adapted Malawi lines

The F_1 hybrids from crosses between locally adapted Malawi lines and maize weevil resistant lines produced the highest mean yields. These were followed by hybrids from crosses between adapted Malawi lines and larger grain borer resistant lines (Figure 5.33).

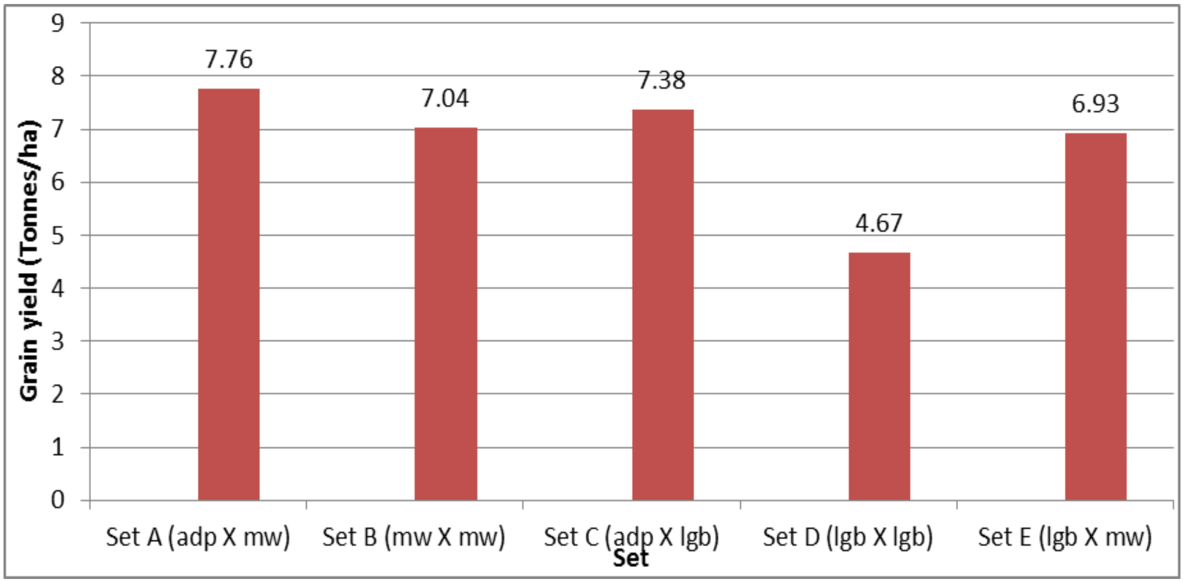

Figure 5.33: Mean maize yields across sets

5.5.6.5 Insect resistance among top 20 high yielding F_1 hybrids

Maize hybrids MWA11273, lgMW087940, lgMW08710 and MWA06A were the highest yielding maize weevil resistant hybrids across sets. Maize hybrid, lgMW087940 was the best larger grain borer and maize weevil resistant hybrid with high yield potential (Table 5.18).

Table 5.18: Resistance among top most high yielding F_1 hybrids

		Resistance levels	
VARIETY	**Yield (tons/ha)**	**Maize weevil resistance**	**Larger grain borer resistance**
MWA112W	15.64	Susceptible	
LGA089444	14.14		Highly susceptible
MWA06403-3	13.60	Highly susceptible	
MWMW13939	12.76	Highly susceptible	
MWMW1106	12.76	Susceptible	
MWA151A	11.33	Moderately susceptible	
MWA11273	11.69	Moderately resistant	
MWLG08264	11.08		
LGMW087940	11.05	Resistant	Resistant
MWMW44606	10.98	Highly susceptible	
LGMW26411	10.30	Moderately resistant	Highly susceptible
MWA06273	10.44	Highly susceptible	
LGA0890020	10.55		Highly susceptible
LGMW08710	10.16	Resistant	Moderately susceptible
MWA446A	10.98	Highly susceptible	
MWMW44610	11.11		
LGMW16411	10	Moderately susceptible	Highly susceptible
MWA06A	10	Highly resistant	
MWMW13675	10	Highly susceptible	

5.6 Discussion

5.6.1 Response of maize varieties to maize weevil infestation

Significant variation in levels of resistance against maize weevil was observed among the F_1 hybrids. The variation was revealed through significant differences obtained among the hybrids for the number of insect mortalities, total number of insects, and percent grain damage. Resistant varieties showed high insect mortality numbers, experienced less grain damage, and had less total number of insects (Abebe et al., 2009; Tefera et al., 2011; Mwololo et al., 2012). From each set, maize hybrids with useful amount of resistance were identified using grain resistant parameters. For instance, using percent grain damage to cluster maize materials into resistant groups and the distribution of variation for the resistant parameters was so variable among the hybrids. This was indicative of varietal differences in their response to weevil attack.

Hence, genetic variation for resistance against maize weevil existed among the maize hybrids (Kim and Kossou, 2003).

Correlation analysis among the resistant parameters showed significant and non-significant relationships. In general, percent grain weight loss and percent grain damage had consistently showed highly significant positive correlations. Correlations between number of insects with both percent grain weight loss and percent grain damage were not consistent. For example in set a, total number of insects showed highly significant positive correlation with percent grain damage, in set b, the relationship between the two parameters was positive and non-significant, and in set e the relationship was negative and non-significant. The only apparent difference between the sets was the availability of LGB resistant genes in set e. Hence, use of insect number as an indicator of resistant to maize weevil was unreliable. In view of this development, percent grain weight loss and percent grain damage were better indicators of resistance among maize hybrids. In addition, these indicators were largely heritable. For example, percent grain damage had broad- sense heritability ranging from 0.83 to 0.91 across sets, while percent grain weight loss had broad-sense heritability ranging from 0.74 to 0.82 across sets.

Using both percent grain damage and percent weight loss to identify the top maize weevil resistant hybrids across sets, in set a, both percent grain damage and percent grain weight loss identified MWA10A, MWA06A, MWA12395 and MWA11312 as resistant hybrids. In set b, both indicators identified maize hybrids MWMW15106, MWMW151939, MWMW0611, MWMW674937, MWMW1313 and MWMW446939. While for set e, the parameters identified lgMW007940, lgMW089151, MWlg06021, MWlg08007, and MWlg08164 as being resistant to maize weevil. Kitaw et al. (2001) and Abebe et al. (2009) found strong positive correlation between percent grain damage, weight loss and insect numbers. The increase in number of insect pests led to increase grain damage subsequently increase in weight loss.

Mechanical and biochemical factors have been attributed to the observed variation in resistance among maize varieties. For example, phenolic compounds were reported to be responsible for providing both mechanical resistance and antibiosis in maize grain (Arnason et al., 1994; Derera et al., 2000; García-Lara et al., 2004). Ferulic and P-coumaric acids (feruloyl and P-coumaroyl arabinoxylans) were reported to be responsible for mechanical resistance against maize weevil, while phenolic amides such as diferuloyl and dicoumaroyl putrescine were responsible for antibiosis against maize weevil (Arnason et al., 1994). Phenolics play a pivotal role in strengthening the cell wall structures in cereals, (Garcia-Lara et al., 2004). For instance, simple phenolic acids, diferulates strengthen pericarp cell wall and dehydrodiferulates join polymers in plant walls, there by conferring resistance to maize weevil (Garcia-Lara, et al., 2004).

5.6.2 Response of maize varieties to larger grain borer infestation

Variation in varietal response to larger grain borer was observed among the hybrids. The distribution of variation for resistant parameters was also variable. Resistant varieties exhibited high insect mortality rates, low grain damage (%), less amount of flour produced and less grain weight loss (%). The use of grain weight loss (%), grain damage (%), flour weight and number of insects as indicators of susceptibility among maize varieties have been reported (Ndiso et al., 2007; Kasambala, 2009; Mwololo et al., 2010; Tefera et al., 2011). Tefera et al. (2011) reported significant variation in amount of dust, grain weight loss and grain damage in a three way cross maize hybrids. Ndiso et al. (2007) used amount of flour and weight loss to isolate resistant varieties among landraces in Kenya. Mwololo et al. (2010) reported variation in varietal resistance to LGB among hybrids and OPVs in Kenya. The varieties showed significant differences in flour weight, grain damage and number of insects. Report from Malawi by Kasambala (2009) indicated significant variation was observed for grain weight loss, grain damage and number of insects among commercial maize hybrids in Malawi.

Highly significant correlations were observed among the resistance parameters, especially between grain weight loss (%), grain damage (%) and insect numbers. This implied that the three resistance parameters, which are also heritable, could be used to distinguish resistant maize varieties from the susceptible varieties when exposed to LGB infestation. For instance, the heritability of percent grain damage ranged from 0.90-0.94, percent grain weight loss (0.83-0.93) and total number of insects (0.81-0.89). Tefera et al. (2011) reported significant correlations between insect numbers, amount of dust and weight loss. Maize varieties with large number of insects produced the highest amount of flour and had the largest grain weight losses.

Using both percent grain weight loss and percent grain damage to identify resistant hybrids, in set c, both parameters identified varieties LGA087I83, LGA088A, and LGA089116 as top LGB resistant hybrids. In set d, the two parameters identified LGLG088218, LGLG021074, LGLG087264, and LGLG007164, and set e, maize hybrids lgMW087940, lgMW089151, lgMW08710, and MWlg06264 were resistant. The resistance observed in these varieties could be attributed to structural (physical) factors and chemical compound, such as amylose, lipids, protein and phenolic compounds that are found within the grain (Arnason et al., 1994; Garcia-Lara et al., 2004; Nhamucho et al., 2014). In contrast, Meikle et al. (1998) reported that husk cover extension had greater ability in providing resistance against LGB than grain characteristics.

5.6.3 Stacking of larger grain borer and weevil resistance in F_1 maize hybrids

The stacking of larger grain borer and weevil resistance in F_1 maize hybrids has proven to be effective in breeding for both maize weevil and larger grain borer resistant hybrids. For instance, the stacking of maize weevil and larger grain borer resistance produced 67% maize weevil resistant hybrids and 14% larger grain borer resistant maize hybrids and 14% hybrids with resistance to both larger grain borer and maize weevil. Of interest were maize hybrids, lgMW087940, lgMW089151, and MWlg06264 that were resistant to both larger grain borer and maize weevil. This could suggest that the hybrids contained a combination of genes (similar genes or complementally genes) conferring resistance to both maize weevil and larger grain borer. This was in agreement with an observation by Tefera et al. (2011) who reported the existence of maize hybrids in Kenya that were able to confer resistance to both LGB and maize weevil. The identification of such resistant hybrids means that selection for both maize weevil and larger grain borer among maize germplasm is a practical and feasible option to reduce the damage caused by the insect pests (Tefera et al., 2011). This dual resistance found in the hybrids could be exploited for breeding maize varieties that are resistant to both insect pests in Malawi. However, screening for dual resistance should involve the use of larger grain borer insect pests as also recommended by Tefera et al. (2011). The results in this study have shown that hybrids that were resistant to LGB were also found to be resistant to maize weevil, while most of the hybrids resistant to maize weevil were found to be susceptible to larger grain borer. In addition, the development of dual resistant maize hybrids should use LGB resistant lines as female parents. Results have shown that most of the hybrids that were resistant to both maize weevil and larger grain borer, the female parents were largey LGB resistant. As observed in maize weevil resistance by Derera et al. (2000) and Kim and Kossou, (2003), it appears, though not conclusive that female effects could play a significant role in development of dual resistant maize hybrids.

5.6.4 Combined analysis of maize weevil and larger grain borer resistance across sets

Combined analysis for maize weevil resistance across sets revealed that among top 20 weevil resistant hybrids, 18 hybrids came from set a “adapted maize lines x maize weevil resistant lines”, only 2 hybrids came from set b “maize weevil resistant lines x maize weevil resistant lines”. The top most maize weevil resistant hybrids were MWA06A, MWA12395, MWA10A and MWA11312 that came from set a. The presence of larger number of resistant hybrids from set a meant that maize germplasm in Malawi have genes for weevil resistance but have never been fully explored and exploited in breeding programmes (Gilbert and Jones, 2012).

Combined analysis of larger grain borer resistance across sets showed that among the top 20 larger grain borer resistant hybrids, ten hybrids came from set c “adapted Malawi lines x larger grain borer resistant lines”, Seven (7) hybrids came from set d “larger grain borer resistant lines x larger grain borer” and set e “maize weevil resistant lines x larger grain borer resistant lines” produced three hybrids. Maize hybrids, LGA089116, LGA087I83, and LGA088A were the top most resistant hybrids. The large presence of resistant varieties originating from set c was also indicative of the availability of genes for larger grain borer resistant among the locally adapted lines. Although Kasambara (2009) reported the susceptibility of all commercial hybrids to larger grain borer in Malawi, current results have demonstrated that improvement of LGB resistance among productive maize germplasm in Malawi is possible.

5.6.5 Yield potential of insect resistant F_1 hybrids across environments

Yield potential among the hybrids within environments did not differ significantly except for sets d under drought, set b under irrigation, and sets a and e under rainfed. But combined yield analysis across environments showed significant differences for yield potential, environmental effects and the genotype and environment interaction. This was in agreement with Kanyamasoro et al. (2010) who also reported significant variation for yield potential especially among weevil resistant varieties. Variation in environmental conditions can result in significant genotype and environment interaction which affects maize productivity (Sibiya et al., 2011; Grada and Ciulca, 2013). Significant interaction between genotype and environment affects selection of genotypes (Kanyamasoro et al., 2010; Mendes et al., 2012). The assessment of the top 20 high yielding varieties across the sets and across environments revealed that most of the high yielding varieties came from crosses between locally adapted Malawi lines with LGB or MW resistant lines. Crosses from all LGB or MW performed poorly possibly due to poor adaptation to the testing environments. Stability and adaptability of genotypes are important factors when selecting cultivars for planting (Scapim et al., 2000). Using mean ranking or superiority index to measure general adaptation of varieties across environments in each set, the following maize hybrids were identified as having good general adaptability; LGA089444, MWA112W, MWA11273, LGLG218089, MWMW13939, MWMW1106, lgMW26413, MWA06403-3, LGA151A, MWMW44606, MWlg08264 and lgMW087940. The adaptable varieties had the highest mean yields across environments, as such the combination of mean yield and superiority index can help in the selection and isolation of superior varieties (Scapim, 2000). Isolating hybrids that are stable and adaptable to the environments minimizes the effect of genotype and environmental interaction (Eberhart and Russell, 1966).

5.6.6 Breeding of high yielding insect resistant hybrids in Malawi

Breeding for high yielding maize varieties with high levels of resistance to maize weevil should involve the use crosses between weevil resistant lines and crosses between adapted Malawi lines and weevil resistant lines. This has been demonstrated by the development of maize hybrids, such as MWA06A (10 tons/ha), MWMW15106 (9.07 tons/ha), MWA10A (7.69 tons/ha), and MWMW446939 (6.67 tons/ha) and MWMW12939 (6.67 tons/ha) that have good resistance to maize weevil and high yield potential across environments and some of the hybrids out performed a commercial hybrid. Better performances of weevil resistant varieties against commercial hybrids were also reported by Tefera et al. (2012) among improved hybrids with different resistance levels in Kenya. For larger grain borer resistance, crosses between larger grain borer resistant lines and maize weevil resistant lines, and crosses between locally adapted maize lines and larger grain borer resistant lines should be used to develop maize hybrids with high yield potential and high levels of resistance to LGB. For instance, lgMW087940 (11.05 tons/ha), LGA087I83 (8.89 tons/ha), MWlg06264 (8.16 tons/ha) and lgMW089116 (6.6 tons/ha) have shown to have good resistance to larger grain borer and better yield potential across environments.

5.7 Conclusion

The study has demonstrated that development of insect resistant hybrids is possible. The developed F_1 hybrids exhibited significant variation in yield potential and resistance to both maize weevil and larger grain borer. The study has also demonstrated that stacking of maize weevil and larger grain borer resistance can lead to increase in number of maize weevil resistant hybrids and the development of dual resistant maize hybrids. Insect resistance can form part of an integrated pest management strategy for storage pests in Malawi. However, productive hybrids would be obtained largely by crossing local adapted lines with maize weevil and larger grain borer resistance sources, and partly from crosses between maize weevil and larger grain borer resistance sources. Therefore, study results demonstrated that insect resistant hybrids would provide a sustainable way of reducing post-harvest grain losses in storage and increase net grain yield among smallholder farmers in the country. The developed LGB and MW resistant hybrids would have acceptable productivity in the field and stability in the target environment resulting in superior net yield on farm.

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Chapter 6

Combining ability for grain yield and resistance among maize weevil and larger grain borer resistant maize lines

Abstract

Identification of maize lines with good combining ability for yield and resistance is central in the development of acceptable insect resistant maize varieties by farmers in Malawi. Determination of the nature of gene action would help in devising breeding strategy for the development of maize varieties with yield superiority and useful levels of resistance. Single cross F_1 hybrids were developed from maize weevil and larger grain borer resistant lines using North Carolina Design II. The objectives of the study were to estimate general combining ability (GCA) and specific combining ability (SCA) between maize lines and their hybrids for grain yield and resistance to maize weevil and larger grain borer. Significant GCA ($p<0.01$) and highly significant SCA ($P<0.001$) were obtained for weevil resistance. Additive and non-additive gene actions were responsible for determining weevil resistance in the maize hybrids. Parental lines CL106940 and CL106674 showed good combining ability for resistance as male and female, respectively. A cross between CL106675 and CL1012151 showed good specific combining ability for resistance. Significant GCA ($p<0.05$) was observed for grain yield, indicative of additive gene action being responsible for grain yield in the maize hybrids. Maize line CL106940 had good general combining for both yield and resistance. For larger grain borer, GCA was highly significant ($p<0.001$) for both resistance and grain yield suggesting that additive gene actions were responsible for both resistance and grain yield in the maize hybrids. Maize lines CKSPL10218 and CKSPL10007 showed good combining ability for resistance as male and female parents, respectively. Maize lines CKSPL10074 and CKSPL10088 showed good GCA for yield as male and female parents, respectively. The preponderance of additive gene effects over dominance gene effects in the maize hybrids gives a practical option for selecting for both resistance and grain yield.

Key words: GCA, grain yield, insect resistance, larger grain borer, maize weevil, design II mating scheme, SCA

6.1 Introduction

Maize cultivation in Malawi is faced by a number of constraints that adversely affect sustainability of high levels of maize production in the country (Denning et al., 2009). Like most of the Eastern and Southern African countries, drought, low soil fertility and climate change are the most important stress factors affecting maize production in Malawi (Zambezi, 1993; Bänziger and Diallo, 2001; FAOSTAT, 2008). Apart from these stress factors, maize yields in the country are further reduced by post-harvest losses due to maize weevil and larger grain borer in storage facilities (Makoka, 2008; Singano et al., 2009; Kamanula et al., 2011). Development of insect resistant maize varieties is crucial in reducing posts-harvest losses of maize grain in storage. Selection of maize lines with good combining ability for yield and resistance is central in the development of insect resistant maize varieties with yield superiority that can easily be accepted by farmers. For instance, the success in breeding for higher yielding maize varieties depends on the ability to select maize lines with good combining ability for yield, resulting in the development of superior varieties (Sleeper and Poehlman, 2006; Brown and Caligari, 2008; Balestre et al., 2009).

The type of combining ability (general/specific) identified in potential crosses indicates the nature of gene action. General combining ability (GCA) is an average performance of a line in all its crosses expressed as a deviation from the mean of all crosses. GCA points to additive gene effects (Falconer and Mackay, 1996). Specific combining ability (SCA) implies any deviation from the sum of the general combining ability of two parental lines. SCA provides non-additive gene effects (Falconer and Mackay, 1996). However, significant SCA in some cases points to the presence of additive and dominance gene actions, in such cases, the ratio of GCA to SCA greater than 1 is indicative of preponderance of additive gene action over non-additive gene action (Falconer and Mackay, 1996; Muraya et al., 2006).

The success in selecting for combining abilities depends on availability of variation within the breeding germplasm. From variation and statistical perspective, the variance of GCA is equivalent to additive variance, while the variance for SCA specifies non-additive variance (Falconer and Mackay, 1996). Estimation of genetic variances is possible through the use of mating designs. Mating designs provide information for the determination of general and specific combining ability, consequently the nature of gene actions (Bridgewater, 1992; Durel, 1998).

Hierarchical, diallel and factorial (North Carolina II) designs are some of the commonly used mating designs in plant breeding (Pepper, 1983). For example, North Carolina design II (NC Design II) gives two independent estimates of GCA with expected mean squares for males and females giving GCA and the interaction between females and males providing SCA (Hallauer et al., 2010).

Studies have been conducted to determine combining abilities of different traits in maize. Malik et al. (2004) determined combining abilities for days to pollen shade, plant height, ear size, leaf area, ear weight, kernel rows and grain yield in maize inbred lines within temperate, subtropical and tropical environments. Combining ability and nature of gene action for maize weevil resistance have been explored and reported. Derera et al. (2000) reported significant maternal effects, significant GCA and SCA in determining susceptibility index, weevil emergency and grain weight loss in maize. Additive gene action, dominance gene action and maternal effects were important in maize weevil resistance. Kim and Kossou (2003) evaluated maize cultivars and crosses between inbred lines, the results showed significant general combining ability and specific combining ability. Both additive and non-additive gene actions contributed significantly to maize weevil resistance. Significant general and specific combining abilities were also reported among lines and hybrids for grain weight loss and emerged F_1 weevils in maize (Dari et al., 2010). Derera et al. (2000) successfully estimated GCA and SCA for weevil resistance using NC design II.

Importantly, not much is documented about the nature of gene action for resistance against larger grain borer. Very little has been done in breeding programmes to exploit genetic variation in maize weevil germplasm for development of maize weevil resistant materials (Dhliwayo and Pixley, 2003). Identification of maize lines with good combining ability for yield and resistance is crucial in the development of insect resistant maize varieties in Malawi. The nature of gene action would help in devising breeding strategy for the advancement of maize varieties with yield superiority and high levels of resistance to maize weevil and larger grain borer.

6.2 Study objectives

The objectives of the study were:

1. To estimate general combining ability (GCA) and specific combining ability (SCA) between maize inbred lines for resistance to maize weevil and larger grain borer.

2. To estimate general combining ability (GCA) and specific combining ability (SCA) between maize lines for grain yield.

6.3 Materials and Methods

6.3.1 Collection of materials

A total of 20 maize breeding lines were collected from CIMMYT-Kenya and CIMMYT-Zimbabwe. Maize lines from Kenya and Zimbabwe were known to have useful resistance against larger grain borer and maize weevil, respectively (Table 6.1). Set B (MW x MW) was comprised of crosses between maize weevil resistant lines while Set D (LGB X LGB) was made up of crosses between LGB resistant lines.

Table 6.1: Breeding lines for combining ability analysis for yield and insect resistance

Set B (MW x MW)			**Set D (LGB x LGB)**		
Maize weevil resistant lines			**Larger grain borer resistant lines**		
Maize line	**Source**	**Role**	**Maize line**	**Source**	**Role**
CL106513	CIMMYT-Zimbabwe	Female	CKSPL10088	CIMMYT-Kenya	Female
CL106674	CIMMYT-Zimbabwe	Female	CKSPL10087	CIMMYT-Kenya	Female
CL1012151	CIMMYT-Zimbabwe	Female	CKSPL10021	CIMMYT-Kenya	Female
VL081446	CIMMYT-Zimbabwe	Female	CKSPL10089	CIMMYT-Kenya	Female
CL106511	CIMMYT-Zimbabwe	Female	CKSPL10007	CIMMYT-Kenya	Female
CL106675	CIMMYT-Zimbabwe	Male	CKSPL10264	CIMMYT-Kenya	Male
CL106937	CIMMYT-Zimbabwe	Male	CKSPL10164	CIMMYT-Kenya	Male
CL106939	CIMMYT-Zimbabwe	Male	CKSPL10218	CIMMYT-Kenya	Male
CL106940	CIMMYT-Zimbabwe	Male	CKSPL10176	CIMMYT-Kenya	Male
CL106506	CIMMYT-Zimbabwe	Male	CKSPL10074	CIMMYT-Kenya	Male

6.3.2 Planting of breeding lines

Maize lines were planted in pots filled with loam soil mixed with organic manure at Chitedze Research Station during the 2011/2012 growing season. The pots were 24 cm in diameter and 28 cm high. Two seeds were planted in each pot. Basal application of fertilizer was done using

NPK (23:21:0 +4S) and top dressing was done using Urea (46% N) at the rate of 5g/pot. Weeds were removed from the pots each time they appear. Insecticide ‘karate’ (lambda-cyhalothrin) was applied to the soil to control termites.

6.3.3 Generation of crosses

Crosses were generated in each set of breeding lines (maize weevil lines and larger grain borer lines) using North Carolina Design II crossing scheme (Figure 6.1).

	Females				
Males	1	2	3	4	5
7	1x7	2x7	3x7	4x7	5x7
8	1x8	2x8	3x8	4x8	5x8
9	1x9	2x9	3x9	4x9	5x9
10	1x10	2x10	3x10	4x10	5x10
11	1x11	2x11	3x11	4x11	5x11

Figure 6.1: North Carolina Design II crossing scheme

For maize weevil, 16 crosses were generated for combining ability analysis for yield and 25 crosses for resistance. For larger grain borer, 16 crosses were generated for combining ability analysis for resistance and 25 crosses for yield. At maturity, cobs were harvested and sundried in readiness for field planting during the 2012/2013 growing season.

6.3.4 Planting of crosses

F_1 hybrids were planted at Kandiani Irrigation Scheme during the 2012/2013 growing season. Hybrids were arranged using alpha lattice design (6 blocks each with 6 or 7 entries) in 2 replications. Each plot was 6 m long. One seed was planted per planting station with 25 cm spacing between plants and 75 cm between rows. A commercial maize hybrid “Kanyani” was used as guard rows. Full-sib mating was employed for each cross. Basal application of fertilizer NPK (23:21:0 +4S) and top dressing using Urea (46% N) was done at the rate of 100kg/ha. The field was weeded thrice and insecticides “karate” (lambda-cyhalothrin) was applied to control termites. At maturity, cobs were harvested and dried ready for yield assessment and resistance screening at Chitedze Research Station (crop storage laboratory).

6.3.5 Resistance screening for maize weevil and LGB resistance using F_2 grain

6.3.5.1 Rearing of larger grain borer and maize weevil

The rearing of LGB and maize was done at Chitedze Research Station (crop storage facilities) according to the procedures outlined by CIMMYT (Tefera et al., 2010). Unsexed pests were reared in a controlled environment at 28± 1^{o}C, 65±5% RH, with a 12h: 12h light: dark regime to minimize fluctuations in temperature and relative humidity and promote insect survival (Haines, 1991). The LGB and maize weevils were cultured on susceptible mixed maize grains in sealed but ventilated glass jars. All precautionary measures were taken to exclude other insects from contaminating the cultures. Maize varieties were evaluated for maize weevil and LGB resistance under lab conditions using four replications in a Complete Randomised Block Design (CRBD). About 1 kg maize grains from each variety were collected for testing. Grains were fumigated with phostoxin (Aluminum Phosphide) tablets for seven days to avoid carry over insects from the field at the rate of 1.5g of phostoxin (3 tablets) per 1 m^3 of maize grain. One hundred (100) grams of grain were sampled from each of the 1 kg maize grains and placed into jars. 50 unsexed adult beetles (7- 15 days old) were infested on 100 g of grain and kept inside 250 ml plastic jars for maize weevil and in 400 ml glass jars for LGB. Percent grain damage was used as indicator susceptibility in the study. Percent grain damage as a parameter for resistance has consistently shown strong correlations with other resistance parameters such as percent grain weight loss. In addition, grain damage is an indicator that farmers use to determine susceptibility of maize varieties to storage pests.

6.3.5.2 Data collected

After 100 days, data on a number of resistance parameters were collected such as number of live and dead insects, total number of insects, damaged and undamaged grains based on 100 grains randomly selected from each jar.

Yield data (grain weights) collection was based on whole plot. Data on grain weights and percent grain damage was analysed for combining ability using SAS (2001). The following statistical model was used to determine general and specific combining ability for both yield and resistance in one environment (Comstock and Robinson, 1948) as follows:

$Y_{ijk} = \mu + r_k + gi + g_j + h_{ij} + e_{ijk}$

Y_{ijk} = Observed performance of the ith female parent, the jth male parent, in kth replication

μ= grand mean

r_k = replication effect

gi = the GCA effect of the ith female parent

g_j = the GCA effect of the jth male parent

h_{ij} = the SCA effect specific to a cross between ith female parent and jth male parent

e_{ijk}= random error for Y_{ijk} observation

Broad sense heritability was calculated based on Analysis of Variance as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + +\sigma_{\varepsilon/r}^2}$$

σ_g^2 = Genotype (crosses) mean sum of square

σ_ε^2 = Error mean sum of square

r = Replication

6.4 Results

6.4.1 SET B: Combining ability analysis for resistance and grain yield among maize weevil resistant lines

6.4.1.1 Analysis of Variance for grain damage (%)

Significant differences for effects on grain damage (%) were observed for crosses, males, and interaction between males and females, while female effects did not significantly affect grain damage (%) (Table 6.2).

Table 6.2: Analysis of variance for grain damage (%) among maize weevil lines

Source of variation	df	ss	ms	Pr>f
Replications	3	27.76	9.253	0.6047
Crosses	24	1137.06	47.378**	0.001
Males	4	267.06	66.765*	0.0028
Females	4	34.96	8.74	0.6745
Males x Females	16	835.04	52.19**	0.001
Residual	72	1075.74	14.941	

*Significant at p<0.01, **significant at p<0.001

H^2 = 0.93

The best GCA estimate for resistance among males was from parent CL106940, while the best GCA estimate among females was from parent CL106674. The contribution of male effects on grain resistance was at 23.49%, female effects (3.10%) and effects from the interaction between males and females were at 73.44% (Table 6.3)

Table 6.3: Estimates for GCA for maize weevil resistance among maize weevil lines

Maize line	Parental role	GCA
CL106513	Female	0.42
CL106674	**Female**	**-1.08**
CL1012151	Female	0.62
VL081446	Female	-0.08
CL106511	Female	0.12
CL106675	Male	3.12
CL106937	Male	-0.23
CL106939	Male	-0.98
CL106940	**Male**	**-1.58**
CL106506	Male	-0.33
Male Effects	23.49%	
Female effects	3.10%	
Males x Females effects	73.44%	
Additive genetic variance (F=1)	-0.18	
Dominance genetic variance (F=1)	9.312	

The best estimate for specific combining ability for resistance was from a cross between parent CL106675 and parent CL1012151 (-5.12) (Table 6.4).

Table 6.4: Estimates for SCA for maize weevil resistance

	Females				
Males	CL106513	CL106674	CL1012151	VL081446	CL106511
CL106675	1.08	1.58	**-5.12**	1.08	1.38
CL106937	-3.07	-2.07	7.73	-2.57	-0.02
CL106939	0.68	4.18	-2.77	-1.32	-0.77
CL106940	-1.22		4.33	-0.97	-0.92
CL106506	2.53			3.78	0.33

6.4.1.2 Analysis of Variance for grain yield

Significant differences ($p<0.05$) were observed for male effects on yield among maize lines. No significant effects on grain yield were observed for crosses, females, and the interaction between males and females (Table 6.5).

Table 6.5: Analysis of variance for grain yield among maize weevil lines

Source of variation	**df**	**SS**	**MS**	**Pr>F**
Replications	1	0.911	0.911	0.1042
Crosses	15	9.319	0.621	0.0895
Males	3	3.134	1.045*	0.0444
Females	3	1.696	0.565	0.1804
Males x Females	9	4.489	0.499	0.1914
Residual	15	4.569	0.305	

*Significant at $p<0.05$

$H^2 = 0.80$

The best estimate for general combining ability for grain yield among males was from parent CL106940. Though effects of females on yield were not significant, female parent VL081446 had a better GCA estimate for yield. Males contributed 33.63% of the effects, females (18.20%) and contribution from the interaction between males and females was 48.17% (Table 6.6).

Table 6.6: Estimates for GCA for grain yield among maize weevil lines

Maize line	Parental role	GCA
CL106674	Female	-0.3313
CL1012151	Female	-0.0688
VL081446	**Female**	**0.2938**
CL106511	Female	0.1063
CL106675	Male	-0.2063
CL106937	Male	0.1688
CL106939	Male	-0.3813
CL106940	**Male**	**0.4188**
Male Effects	33.63%	
Female effects	18.20%	
Males x Females effects	48.17%	
Additive genetic variance (F=1)	0.013	
Dominance genetic variance (F=1)	0.097	

Although not significantly different in the overall analysis, estimates for specific combining ability showed that the interaction between CL106937 and CL1012151 had a better SCA for grain yield (Table 6.7).

Table 6.7: Estimates for SCA for grain yield among maize weevil lines

	Females			
Males	CL106674	CL1012151	VL081446	CL106511
CL106675	0.14375	0.38125	-0.23125	-0.29375
CL106937	-0.48125	**0.75625**	-0.10625	-0.16875
CL106939	0.31875	-0.39375	-0.05625	0.13125
CL106940	0.01875	-0.74375	0.39375	0.33125

6.4.1.3 Comparison of GCAs for resistance and grain yield among maize lines

Focusing on both GCAs for resistance and grain yield, the results showed that male parent CL106940 had good combining ability for both grain yield and resistance (Table 6.8).

Table 6.8: GCAs for both yield and resistance among maize weevil lines

		Weevil resistance	Grain yield
Line	**Parental role**	**GCA**	**GCA**
CL106513	Female	0.42	
CL106674	Female	-1.08	-0.331
CL1012151	Female	0.62	-0.069
VL081446	Female	-0.08	0.2938
CL106511	Female	0.12	0.1063
CL106675	Male	3.12	-0.206
CL106937	Male	-0.23	0.1688
CL106939	Male	-0.98	-0.381
CL106940	Male	**-1.58**	**0.419**
CL106506	Male	-0.33	

6.4.2 SET D: Combining ability analysis for resistance and grain yield among larger grain borer resistant lines

6.4.2.1 Grain resistance

Highly significant differences (p<0.001) were observed for effects of crosses and females on grain damage (%) among the maize lines. Male effects and the interaction between males and females had no significant effects on grain damage (%) (Table 6.9).

Table 6.9: Analysis of Variance for grain damage (%) among LGB lines

Source of variation	df	SS	MS	Pr>F
Replications	3	25.63	8.54	0.382
Crosses	15	490.5	32.7*	0.0002
Males	3	60.38	20.13	0.0747
Females	3	334.75	111.58*	0.0001
Males X Females	9	95.38	10.6	0.2656
Residual	45	367.89	8.18	

*significant at p<0.001

H^2 = 0.94

The best estimates for GCA were observed in male parent CKSPL10218 and female parent CKSPL10087. The results further revealed that males contributed 12.31% effects on resistance, females (68.25%), and the interaction between males and females contribution on effects was at 19.44% (Table 6.10).

Table 6.10: Estimates for GCA for LGB resistance

Maize line	Parental role	GCA
CKSPL10088	Female	-0.4375
CKSPL10087	**Female**	**-2.1875**
CKSPL10021	Female	3.8125
CKSPL10007	Female	-1.1875
CKSPL10264	Male	1.375
CKSPL10164	Male	0.4375
CKSPL10218	**Male**	**-1.0625**
CKSPL10176	Male	-0.75
Male effects	12.31%	
Female effects	68.25%	
Male x Female effects	19.44%	
Additive genetic variance (F=1)	1.151	
Dominance genetic variance (F =1)	0.606	

Though the interaction between males and females were not significant, in the overall analysis, the cross between CKSPL10007 and CKSPL10164 produced a better estimate for specific combining ability for LGB resistance (Table 6.11).

Table 6.11: Estimates for SCA for LGB resistance

	Males			
Females	CKSPL10264	CKSPL10164	CKSPL10218	CKSPL10176
CKSPL10088	-1.1875	2	-0.75	-0.0625
CKSPL10087	-1.6875	-1	1	1.6875
CKSPL10021	1.0625	0.75	-0.5	-1.3125
CKSPL10007	1.8125	**-1.75**	0.25	-0.3125

6.4.2.2 Grain yield

Significant differences for effects on grain yield were observed for replications, treatments, crosses, male and female parents. However, effects from the interaction between males and females were not significant (Table 6.12)

Table 6.12: Analysis of variance for grain yield among LGB maize lines

Source of variation	**df**	**ss**	**ms**	**Pr>F**
Replications	1	1.095	1.095*	0.0063
Crosses	24	8.979	0.374*	0.0041
Males	4	3.795	0.949**	0.0004
Females	4	2.725	0.681*	0.0026
Males x Females	16	2.459	0.154	0.2986
Residual	24	2.935	0.122	

*significant at $p<0.01$, **significant at $p<0.001$

H^2 = 0.86

Estimates of GCA for grain yield showed that male parent CKSPL10074 had a good GCA for grain yield, while female parent CKSPL10088 had good GCA for grain yield. Male effects on grain yield were 42.26%, female effects (30.35%) and the interaction between males and females contributed 27.39% of the effects in the hybrid (Table 6.13).

Table 6.13: Estimates for GCA for grain yield among LGB maize lines

Line	Parental role	GCA
CKSPL10088	**Female**	**0.368**
CKSPL10087	Female	0.068
CKSPL10021	Female	-0.272
CKSPL10089	Female	-0.232
CKSPL10007	Female	0.068
CKSPL10264	Male	0.168
CKSPL10164	Male	-0.382
CKSPL10218	Male	-0.032
CKSPL10176	Male	-0.172
CKSPL10074	**Male**	**0.418**
Male effects	42.26	
Female effects	30.35	
Male x Female effects	27.39	
Additive genetic variance (F=1)	0.017	
Dominance genetic variance (F =1)	0.016	

Despite the non-significance of the interaction between males and females, the best estimate for specific combining ability for grain yield was between parental lines CKSPL10264 and CKSPL10007 (Table 6.14).

Table 6.14: Estimates for SCA for grain yield among LGB maize lines

	Females				
Males	CKSPL10088	CKSPL10087	CKSPL10021	CKSPL10089	CKSPL10007
CKSPL10264	-0.068	-0.268	-0.428	0.2802	**0.482**
CKSPL10164	-0.268	-0.218	0.372	0.082	0.032
CKSPL10218	-0.118	0.182	0.022	-0.018	-0.068
CKSPL10176	0.272	0.072	-0.038	-0.128	-0.178
CKSPL10074	0.182	0.232	0.072	-0.218	-0.268

6.4.2.3 Comparison of GCAs for resistance and grain yield among LGB maize lines

No line had good general combining abilities for both resistance to larger grain borer and grain yield (Table 6.15).

Table 6.15: Comparison of GCAs for grain yield and resistance among LGB lines

Line	**Parental role**	**Grain yield GCA**	**Resistance GCA**
CKSPL10088	Female	0.368	-0.4375
CKSPL10087	Female	0.068	-2.1875
CKSPL10021	Female	-0.272	3.8125
CKSPL10089	Female	-0.232	
CKSPL10007	Female	0.068	-1.1875
CKSPL10264	Male	0.168	1.375
CKSPL10164	Male	-0.382	0.4375
CKSPL10218	Male	-0.032	-1.0625
CKSPL10176	Male	-0.172	-0.75
CKSPL10074	Male	0.418	

6.5 Discussion

6.5.1 Gene action controlling grain resistance and grain yield in maize weevil resistant maize hybrids

General combining ability (GCA) and Specific combining ability (SCA) effects were significant for maize weevil resistance. The significance of both GCA and SCA demonstrated that both additive gene action and non-additive gene actions were responsible for determining weevil resistance in maize hybrids (Falconer and Mackay, 1996; Muraya et al., 2006). The mean sum of square for males was found to be significant, while the mean sum of square for females was not significant. In addition, males contributed more effects (23.49%) on resistance than females (3.07%). Hence, male effects were more dominant than female effects in determining resistance among the maize hybrids. The results further showed that grain damage as a parameter for maize weevil resistance was highly heritable (0.93). As such it can be used in selection of materials to improve weevil resistance. The results from a study by Kim and Kossou (2003) on maize cultivars and crosses between inbred lines showed that both additive and non-additive gene actions contributed significantly to maize weevil resistance among the genotypes. Dari et al. (2010) also reported the significance of both general combining ability, and specific combining ability among lines and hybrids for grain resistance using weight loss and emerged F_1 weevils. However, Dhliwayo and Pixley (2003) reported the significance of only additive gene action in determining weevil resistance. Derera et al. (2000) demonstrated that additive gene action, dominance gene action and maternal effects were responsible for weevil resistance in maize germplasm. Dhliwayo et al. (2005) studying 14 Southern African maize inbred lines reported that additive gene action only was responsible for weevil resistance. Nonetheless, the present study had shown that additive gene effects, non–additive gene effects, and male effects affected maize weevil resistance among maize hybrids

The significance of general combining ability in determining grain yield among maize hybrids was an indication that additive gene action significantly determined grain yield in maize weevil resistant hybrids. Males contributed more effects (33.63%) than females (18.20%). This was also confirmed by the significance of mean sum of square for males and non-significance of mean sum of square for females. In this regard, males were predominant in their effects on grain yield than females. The heritability value was for grain yield was at 0.80. The heritability value was slightly lower can partly due to environmental effects (Chapter 5). This means that yield can be improved upon among maize weevil varieties through selection. Of interest was male parent CL106940 which had good combining ability for both grain yield and resistance. This parent would be an ideal candidate for developing maize weevil resistant hybrids with high yield potential. Kanyamasoro et al. (2010) reported the significance of additive and non-additive effects in determine grain yield in maize inbred lines. The nature of gene action controlling grain yield in general has been reported. Singh (2010) reported the significance of SCA in determining grain yield in short duration maize. Santos et al. (2007) reported that both SCA and GCA were responsible for grain yield in maize populations. Derera et al. (2007) and Musila et al. (2010) reported that only additive gene action was responsible for grain yield.

6.5.2 Gene action controlling grain resistance and grain yield in larger grain borer resistant maize hybrids

The significance of GCA for grain resistance demonstrated that additive gene action was responsible for determining grain resistance to larger grain borer among the maize hybrids. Females contributed more effects to resistance (68.25%) than males (12.31%). Furthermore, females mean sum of square was significant, while mean sum of square for males was not significant. It is therefore conclusive that female effects played a significant role in determining larger grain borer resistance. At the moment, there are no published reports on gene action responsible for larger grain borer resistance. However, in the previous study (Chapter 6) and reports by Tefera et al. (2011) revealed the existence of maize hybrids conferring resistance to both LGB and MW. It was construed that same genes confer resistance to both maize weevil and larger grain borer (Tefera et al., 2011). Based on the premise that genes conferring resistance to maize weevil are the same genes providing resistance to larger grain borer and on the evidence from the current study that additive gene actions are responsible for maize weevil resistance. It can therefore be concluded that additive gene action is largely responsible for determining larger grain borer resistance. The higher heritability value of 0.94 for percent grain damage means that the resistance parameter is highly heritable. Therefore selection can easily be applied in developing larger grain borer resistant maize germplasm.

Additive gene effects were responsible in determining grain yield. Both male effects (42.26%) and female effects (30.35%) significantly contributed to grain yield. In addition, maize hybrids showed high broad sense heritability (0.86) for grain yield. At present there are no published reports on gene action responsible for grain yield, specifically for larger grain borer resistant maize hybrids. However, studies on gene effects on grain yield suggest that mainly GCA and partly SCA are responsible for determining grain yield (Derera et al., 2007; Santos et al., 2007; Musila et al., 2010; Singh, 2010)

6.6 Conclusion

Both additive and non-additive gene action were responsible for determining weevil resistance in the maize hybrids, while only additive gene action was responsible for determining grain yield. For larger grain borer, both resistance and grain yield were determined by additive gene action. Maize lines with significant GCA, especially for resistance should be crossed with adapted Malawi lines to develop varieties with both yield superiority and insect resistance. The preponderance of additive gene effects over non-additive effects suggests that selection is possible for both resistance and grain yield among maize germplasm. The male parent CL106940 showed good combining ability for both grain yield and weevil resistance. This parent would be useful as a male parent in breeding maize hybrids for both maize weevil resistance and grain yield. It is therefore concluded that LGB and MW resistant maize lines have good combining ability for resistance and grain yield which can be exploited in developing hybrids and synthetic populations. Additive gene effects are responsible for controlling resistance to LGB and MW in maize germplasm, suggesting that selection can be used to enhance the resistance.

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Chapter 7

General overview

7.1 Introduction

This chapter provides a synopsis of the study on "Diversity analysis and breeding for maize weevil and larger grain borer resistance in productive germplasm in Malawi." The synopsis details the main objectives of the study, major findings, challenges, breeding implications and recommendations for future maize breeding activities for larger grain borer and maize weevil resistance in Malawi.

7.2 Main objectives

The study was conducted to achieve 5 objectives as outlined in chapter 1, section 9.

7.3. Major findings

7.3.1. Farmers' perceptions on yield, maize production constraints and storability of local maize varieties

a. Farmers continue to grow both hybrids and local varieties on their farms. Hybrids are mainly grown because of their high yield potential and early maturity than landraces, while local varieties are grown due to good tolerance to pests and diseases, large grain size, good yields under low fertility, white colour, superior poundability, drought tolerance and high storability than hybrids.

b. Major maize production constraints were lack of fertilizer, low soil fertility, pests, lack of high quality seeds, and drought.

c. Yield is the single most important factor for selecting varieties for planting, however, if highly susceptible to storage pests, farmers may opt for another variety.

d. Larger grain borer and maize weevil were the most important and common storage pests of maize among the farmers.

e. Farmers are aware of differences in variety resistance to larger grain borer and maize weevil.

f. Grain hardness, grain size, grain colour, poundability and grain texture were the main characteristics farmers use to identify maize varieties that are tolerant to maize weevil and larger grain borer.
g. Farmers practice integrated pest management to control storage pests, as such host resistance can easily be integrated in the farmers' IPM strategies.
h. Farmers use many traits when selecting varieties for planting. Therefore, breeding for new hybrids and local varieties should include as many traits as practically possible to meet the needs of farmers.

7.3.2 Genetic marker diversity of the potential breeding sources for use in introgressing larger grain borer and maize weevil resistance genes in farmer-preferred local varieties

a. Based on phenotypic data, local maize varieties in Malawi are highly diverse.
b. Phenological differences among the varieties were mainly due to differences in plant height, ear placement, kernel weight, days to tasselling and days to silking.
c. Phenotypic data produced 8 local maize clusters.
d. Gene diversity exists among local maize varieties. The SSR markers exhibited 97.56% polymorphism, gene diversity of 0.5115, and heterozygosity of 0.5233.
e. A total of 165 alleles were obtained, ranging from 2-9 alleles and an average of 4 alleles per locus.
f. Ten clusters were observed using molecular data. The largest genetic distance (0.9001) was between varieties 206 (cluster 9) and local 2 (cluster 1), while the shortest genetic distance was 0.2189 between varieties 203 (cluster 6) and 811 (cluster 3).
g. Gene migration between populations per generation was at 0.33. This was <1Nm, which suggest that gene flow among the varieties was low. Hence, unique germplasm can be identified within and between the maize populations.

7.3.3 Variation for resistance to larger grain borer and maize weevil among local maize varieties

a. Variation for resistance to maize weevil and larger grain borer exists among local varieties in Malawi.
b. For maize weevil, 14.5% of the local varieties studied were resistant, 21.7% moderately resistant, 24.6% moderately susceptible, 23.25% susceptible and 16% highly

susceptible. For larger grain borer all varieties were susceptible except for varieties 2012, 1992, 386 and 1983 that showed moderate resistance.

c. Local varieties 2012, 386, 1992 and 1983 were resistant to maize weevil and moderately resistant to larger grain borer.

7.3.4 Levels of insect resistance among F_1 hybrids

a. Grain weight loss (%) and grain damage (%) were the most consistent grain resistant parameters for discriminating resistant from susceptible hybrids. The two parameters were highly correlated to each other and generally produced similar groups of resistant hybrids.

b. Larger grain borer resistant hybrids were also resistant to maize weevil, while not many of maize weevil resistant hybrids were resistant to larger grain borer.

c. The percentage of maize weevil resistant hybrids developed ranged from 4 to 67% across sets, while larger grain borer resistant hybrids ranged from 4 to 9% across sets.

d. Stacking of maize weevil and larger grain borer resistance produced 67% maize weevil resistant hybrids, 14% larger grain borer resistant hybrids, 14% maize hybrids with resistance to both larger grain borer and maize weevil.

e. Maize hybrids, MWMW151939, MWMW446929, MWA06A, MWA12395, lgMW087940, MWlg06264, MWA11312 and MWA10A were resistant to maize weevil. Maize hybrids, LGLG088218, LGLG021074, lgMW087940, LGA089116, LGA087I83, LGA088A, and MWlg06264 were resistant to larger grain borer.

f. For maize weevil, the majority of resistant hybrids came from crosses between locally adapted maize lines and maize weevil resistant lines. For larger grain borer, most of the resistant hybrids came from crosses between locally adapted maize lines and larger grain borer resistant lines. This means therefore, that maize genes conferring resistance to weevil and larger grain borer exists among locally available maize germplasm. The identified germplasm can be used to develop insect resistant hybrids. But have generally been unnoticed or ignored in the country.

g. Maize hybrids lgMW087940, lgMW089151 and MWlg06264 were resistant to both maize weevil and larger grain borer, implying that same gens could be conferring resistance to both maize weevil and larger grain borer.

h. The majority of maize hybrids with resistance to both maize weevil and larger grain borer, the female parent came from the larger grain borer resistant lines. It appears that female effects were important in dual insect resistant maize hybrids.

7.3.5. Value for cultivation of larger grain borer and maize weevil resistant hybrids, as reflected by combination of high productivity and stability

a. F_1 hybrids showed differences in yield potential across environments, therefore genetic differences for yield exist among the hybrids.

b. Genotype x Environment interaction was significant and that affected the selection of hybrids across environments.

c. Environmental effects were significantly different, as such the test environments ably represented some of the target production environments for farmers in Malawi.

d. Crosses among maize weevil resistant lines and larger grain borer resistant lines performed poorly across environments possibly due to poor adaptation to the testing environments. Therefore, resistance to larger grain borer and maize weevil should be bred into locally adapted maize lines.

e. Maize hybrids MWA06A (10 tons/ha), MWMW15106 (9.07 tons/ha), MWA10A (7.69 tons/ha), MWMW446939 (6.67 tons/ha), and MWMW12939 (6.67 tons/ha) showed high yield potential and high levels of resistance to maize weevil.

f. Maize hybrids lgMW087940 (11.05 tons/ha), LGA087I83 (8.89 tons/ha), LGA089116 (6.6 tons/ha) and MWlg06264 (8.16 tons/ha) revealed high resistance levels to larger grain borer and high yield potential.

g. Maize hybrids lgMW087940 (11.05 tons/ha) and MWlg06264 (8.16 tons/ha) showed high levels of resistance to both larger grain borer and maize weevil and high yield potential.

7.3.6. General combining ability (GCA) and specific combining ability (SCA) among maize lines for resistance to larger grain borer, maize weevil and grain yield

a. Both additive (GCA) and non-additive gene actions (SCA) determined weevil resistance in maize weevil resistant hybrids. The interaction between males and females contributed significant effects to grain resistance (73.44%).

b. Only additive gene action (GCA) influenced grain yield in weevil resistant maize hybrids. Males and the interaction between males and females contributed more effects to grain yield, 33.63% and 48.17%, respectively.

c. Male line CL106940 showed good combining ability for both weevil resistance and grain yield. This line is a good candidate for crossing with locally adapted germplasm for the development of high yielding weevil resistant hybrids.

d. Additive gene effects (GCA) determined both grain yield and resistance in larger grain borer resistant maize hybrids. Female effects contributed significantly to larger grain borer resistance (68.25%), while both male effects (42.26%) and female effects (30.35) significantly contributed to grain yield.

7.4 Challenges to breeding for insect resistant varieties

a. Maize breeding programme in Malawi does not have a well developed (structured) breeding programme for insect resistant as such setting up a workable structure could drag the process of kick-starting insect resistance breeding programme.

b. Rearing of insects requires specialized equipment which could drain already limited resources for such breeding programmes.

d. Farmers and other potential users of the insect resistant varieties may look for a quick fix in breeding for insect resistant varieties. This could compromise the programme through fast tracking the release of resistant hybrids before adequate evaluation and testing is done. This may lead to early breakdown in resistance.

e. Agro-dealers and multinational companies in Malawi that promote chemical control of storage insect pests may perceive breeding for insect resistance as a threat to their business. This view may affect progress in developing insect resistant maize varieties.

f. Intellectual property Rights may prohibit or hinder the release of insect resistant maize hybrids due to the use of lines from other countries or institutions.

7.5 Opportunities

a. Insect pest damage in maize storage facilities has been recognized as a national problem that threatens food security at household level in Malawi.

b. Malawi has on-going maize breeding programmes that can easily integrate insect resistance breeding in its programmes.

c. The current high costs of chemical products and environmental concerns would work in favor of breeding for insect resistant maize.

d. Chitedze Research Station has a functional laboratory and trained personnel for insect handling and grain resistance evaluation, critical for kick starting the insect resistant breeding programme.

7.6 Breeding implications

Farmers use a wide range of traits when choosing varieties for planting. Selection of a large breeding population with diverse traits is a prerequisite when developing maize varieties for small holder farmers. Breeding for insect pest resistant maize varieties should focus on yield and other biophysical characteristics such as grain hardness, grain size, grain color, poundability and grain texture. This would make sure that varieties are easily accepted by farmers. Breeding programmes should also consider selection of other important traits such as drought tolerance, diseases resistance, grain size and cob size that were perceived as critical by famers. Since farmers tend to keep their own local varieties, apart from developing hybrids, breeding initiatives should also focus on developing or improving the existing open pollinated varieties kept by farmers.

Local maize varieties in Malawi expressed good levels of genetic diversity. The expressed diversity would offer a good source of genes for the breeding programmes in Malawi. The availability of local maize varieties with resistance to both larger grain borer and maize weevil offer new opportunities for Malawi to kick start storage insect resistant breeding programmes. The same maize materials would be used for both larger grain borer and maize weevil resistant screening. This would make the breeding process more efficient and cost effective. In addition, lines can be developed from dual resistant varieties for introgressing genes into susceptible but high yielding maize varieties. Varieties with desirable characteristics for farmers but with moderate resistance could be improved upon through recurrent selection that exploits the additive and non-additive variances to increase the frequency of resistant genes.

For maize hybrids, many of the adapted Malawi lines displayed a good combination of yield potential and resistance with larger grain borer and maize weevil lines. These could be a good source of breeding materials for developing lines for grain yield and insect resistance. The strong presence of additive gene effects for both maize weevil and larger grain borer resistant hybrids suggest that recurrent selection can be applied to increase the frequency of desirable genes. Where dual resistance (weevil and LGB) screening is pursued, breeders should focus on initially using larger grain borer for screening. Results have shown that all maize hybrids with resistance to larger grain borer were also resistant to maize weevil, while most of the maize hybrids resistant to maize weevil were susceptible to larger grain borer. By making initial selection with larger grain borer, the selected germplasm will likely be resistant to maize weevil. Ultimately productive hybrids will be formed by crossing the adapted lines by the insect resistant maize inbred lines.

7.7 Recommendations

a. Varieties with superior characteristics for yield performance and resistance must further be evaluated in more diverse environments and seasons to confirm performance before recommendation to farmers.

b. Analysis of levels of phenolic compounds among the selected varieties must be carried out to link levels of resistance and amount of phenolic compounds as a screening tool. This could not be done in the current study due to prohibitive costs.

c. Malawi should develop a well-structured programme for insect resistance breeding focusing more on maize weevil and larger grain borer. The government of Malawi should set aside extra funding for maize research for the integration of insect resistance breeding in conventional maize programmes so that insect resistance is incorporated in productive varieties to enhance net grain yield.

d. QTL analysis should be conducted to validate the existence of the same genes conferring resistance to both maize weevil and larger grain borer among maize varieties. Results have shown that quantitative genes were largely responsible for resistance for both larger grain borer and maize weevil hybrids.

Appendices

Appendix 2.1: Survey questionaire on farmers' perception on yield performance and storageability of landraces and locally adapted improved varieties

Name of Intervewee……………………………………………………..…..

Sex…………………………………………………………….………..……..

Region………………………………………………….……….…..………...

District…………………………………………………………………………

EPA……………………………………………………………………………

Village…………………………………………………………………………

A. Interviewee details

1.	Household head	1. Yes		2. No
2.	Gender of interviewee	1. Female		2. Male
3.	Marital status	1. Married 4. Divorce	2. Single 5. Separated	3. Widowed
4	Age (years):	1. 15-20 4. 46-55	2. 21-35 5. Above 55	3. 36-45
5.	Educational level:	1. None 4. Tertially	2. Primary 5. Other	3. Secondary

B. Sources of income

1. Farming
2. Business
3. Employment
4. None

C. Type of Farming

1 Field crop production

2 Animal production

3 Poultry production

4 Horticulture productions

6 Fishing

D. If 1 (what is the size of the farm)
1. less than 2ha
2. Beween 2-5ha,
3. Between 5-10 ha
4. More than 10 ha

E. Type of crops grown

1. Maize 2.Cassava 3. Sweet potato 4. Sorghum 5.Millets
6. Groundnuts 7. Beans. 8. Irish potato 9. Cotton 10.tobacco
11. Others

F*. (On maize)* Does you produce enough for the whole year?
1. Yes with surplus- a. Yes b. No
2. No

G. How many 50 kg bags of maize do you realise per year
1. 1 – 5 bags
2. 5 – 10 bags
3. 11- 15 bags
4. 16 – 20 bags
5. More than 20 bags

H. Constrants to maize production
1 Lack of seeds
2 lack of resources to buy in puts
3. Low yields varieties
5 lack of Fertiliser not available
7 Diseases
8.Pests
9 Drought
10.Post harvest losses
11.Poor poundability
12.Shortage of labour
13. Lack of market
14. Low soil fertility
15. Other (specify)

I. How much maize do you lose in storage due to pests?
1. all the harvest
2. Half of the harvest
3. Three quarter of te harvest
4. None
5. Not sure

J. Common storage pests
1. Rodents
2. Larger grain borer

3. Maize weevil
4. Moisture
5. Moulds
6. Theft by people

K. Type of seed used or preferred (maize only)

1. Land races and Locally adapted cultivars/varieties
2. Hybrids
3. Both

L. Why do the farmers prefer that type of seeds?

1. High yelding
2. Readily available
3. Locally found
4. Other reasons

M Source of seeds

1. Keep on seed
2. Friends
3. Admarc
4. Seed companies

N. Important attributes for selecting land races and locally adapted maize varieties for planting

1. Grain size
2. Cob size
3. Yield
4. Poundability
5. Colour
6. Taste
7. Storability
8. Resistance to pests and diseases
9. Drought torelant
10. Other (specify)

O. Type of storage facilities for maize grain

1. Traditional structures
2. Bags
3. Modern facilities
4. Others

P. Farmers' level of knowledge on storage problems

1. Excellent
2. Very good
3. Good
4. Fair
5. Poor
6. Very poor
7. Not sure

Q. Farmers interaction with extension workers on problems of storage pests

1. Excellent
2. good
3. Fair
4. Fairly

5. None

R. Control of storage pests

1. Use of natural pesticides/ indeginous plants
2. Chemicals
3. Grain processing (pounding)
4. Use of resistant varieties
5. General sanitation
6. None
7. Other (specify)

S. Can you use grain storage resistant varieties with slightly lower yields than the current susceptible high yielding varieties?

1. Yes
2. No
3. Not sure

T. What is an ideal crop (important attrebutes) for the farmer (ideotype)?

Appendix 3.1: Mean trait values for each local maize variety

Variety	yield (tons/ha)	PH (cm)	KW(g)	ED	EPL(cm)	HC	KS	EP	TB	DT	KC	KRA	KT	KR	DS
139	2.32	200.2	332	0.245	106.5	6.82	2.235	1.061	16.56	76.9	1.031	1.366	2.132	9.9	80.42
145	3.43	210.4	363	0.135	96.5	6.969	2.42	1.351	17.29	73.91	1.104	1.327	2.042	11.02	78.98
148	1.51	218.9	380	0.181	113.1	6.933	2.414	1.125	16.49	74.33	1.151	1.555	2.436	9.92	79.3
154	2.77	228.1	391	0.27	111.2	6.862	2.471	1.449	18.68	78.87	1.15	1.59	2.577	10.04	77.53
163	4.15	216.4	291	0.354	108.6	6.825	2.029	1.26	18.46	75.02	1.082	1.383	2.614	11.54	78.22
164	2.19	233.9	379	0.126	126.8	6.894	2.154	1.237	14.99	76.46	1.004	1.31	2.225	10.58	76.39
172	1.8	200.3	319	0.151	115.7	7.102	2.047	1.063	17.17	74.65	1.039	1.407	2.37	11.64	74.8
1772	3.76	194.7	323	0.314	93.5	6.29	2.165	1.07	16.74	73.93	1.172	1.258	2.362	10.12	81.12
1786	4.81	198.2	381	0.209	99.2	6.954	2.346	1.249	16.83	73.98	1.023	1.565	2.347	10.92	75.36
1795	3.49	195.9	296	0.306	104.3	6.985	2.025	1.114	14.73	79.51	1.711	1.353	2.621	11.24	79.69
1845	1.93	221.6	412	0.281	100.2	6.924	2.204	1.063	15.94	74.36	1.268	1.622	2.631	8.63	76.82
1850	1.7	217.2	293	0.259	122.9	6.255	2.075	1.201	29.84	75.02	1.227	1.502	2.534	11.17	78.97
1857	1.29	208.4	438	0.286	100.3	6.889	2.436	1.031	17.73	72.82	1.184	1.411	2.645	9.36	76.84
1892	2.8	205.4	391	0.246	106	6.904	2.485	1.278	14.23	74.99	1.03	1.305	2.258	9.41	74.5
1915	2.28	242.8	398	0.189	126.3	6.991	2.348	1.22	16	78.17	1.688	1.457	2.536	9.71	76.99
193	2.79	172.9	362	0.164	102.3	6.766	2.234	0.992	17.56	76.66	1.028	1.311	2.299	10.68	79.3
1983	3.16	178.6	356	0.278	105.4	6.976	2.064	1.013	17.4	72.13	1.075	1.19	2.541	9.75	72.5
199	2.22	207.6	348	0.162	103.4	6.359	2.212	1.06	15.5	74.98	1.216	1.466	2.552	9.82	79.03
1992	2.88	197.3	325	0.325	95.1	6.817	2.083	1.223	15.32	75.53	1.055	1.185	2.756	10.31	76.22
2012	4.57	213.2	373	0.152	110.4	6.919	2.259	1.159	15.8	72.39	1.477	1.391	2.446	11.71	77.57
2017	1.01	223.1	385	0.199	114.5	7.081	2.396	1.179	17.03	73.37	1.074	1.32	2.488	9.88	75.55
2027	3.17	206.9	322	0.448	108.1	6.732	1.98	1.017	15.66	72.98	1.083	1.618	2.629	10.67	78.98
203	2.42	230.4	318	0.225	126.4	6.807	2.319	1.165	17.84	73.27	1.254	1.536	2.293	9.23	77.35
206	2.39	242.3	404	0.224	128.6	6.825	2.363	1.315	17.11	73.26	1.163	1.286	2.455	12.03	79.75
218	0.99	229.6	390	0.277	119.5	7.181	2.346	0.994	14.73	78.55	0.981	1.336	2.09	9.93	75.94
226	2.06	212.2	361	0.269	91.5	7.275	2.266	1.043	16.93	74.22	1.055	1.467	2.014	9.98	75.69
240	1.4	230.6	413	0.13	116.5	6.964	2.556	1.41	17.8	76.66	1.083	1.408	2.302	9.99	80.63
243	2.61	204.8	331	0.135	102.6	7.011	2.27	1.053	17.68	76.57	1.063	1.381	2.388	8.34	75.78
249	2.2	206.3	349	0.178	104.5	6.733	2.029	1.042	15.84	76.87	1.462	1.436	2.55	10.52	75.72
250	1.79	192.3	381	0.33	99.9	7.026	2.257	1.06	15.68	77.94	1.203	1.518	2.51	9.48	77.6
260	2.41	222	395	0.296	119.4	7.46	2.386	1.107	14.7	72.56	1.138	1.41	1.688	10.03	76.96
2862		107	140	0.664	23.4	6.89	1.055	1.05	8.47	75.6	1.1	1.411	2.996	8.64	62.46

Appendix 3.1….continued

Variety	Yield (tons/ha)	PH (cm)	KW (g)	ED	EPL (cm)	HC	KS	EP	TB	DT	KC	KRA	KT	KR	DS
2872	2.49	174.3	356	0.574	67.1	6.249	2.246	1.177	13.87	72.14	1.215	1.239	2.276	12.23	73.77
289	2.43	210.7	323	0.134	107.7	6.849	2.081	1.177	15.4	72.81	1.169	1.426	2.34	10.46	75.77
292	2.76	224	339	0.137	2119	6.919	2.233	1.252	16.1	76.11	1.29	1.318	2.116	9.99	78.92
297	1.54	260.5	381	0.153	138.3	6.856	2.291	1.159	18.62	73.61	1.042	1.493	2.276	10.56	79.86
303	0.26	234.8	397	0.2	122.7	6.104	2.444	1.149	16.6	74.04	1.175	1.181	2.031	10.27	74.62
310	1.02	222.7	380	0.2	119.1	7.022	2.386	0.995	16.1	74.78	1.026	1.341	2.511	9.26	77.39
315	2.35	208.3	313	0.492	107.3	6.562	1.974	1.109	16.67	73.31	1.061	1.599	2.296	11.26	76.51
322	1.74	218.8	351	0.39	116	7.009	2.311	1.17	16.21	75.89	1.071	1.436	2.013	9.62	77.72
3243	2.85	161.7	292	0.298	62.8	7.124	1.606	1.299	14.69	72.2	2.693	1.544	2.856	10.8	72.35
3244	3.91	190	303	0.139	92.4	7.044	1.734	1.075	16.46	72	1.291	1.422	2.788	11.59	73.68
332	2.44	204.2	324	0.197	100.6	7.083	2.188	1.046	16.25	78.27	1.506	1.549	2.538	9.72	75.17
3411	2.6	212.2	374	0.228	100.8	7.086	2.167	0.942	15.91	71.46	1.137	1.454	2.473	9.95	76.55
3414	2.3	182.2	328	0.231	87.7	7.016	2.222	1.255	16.11	77.86	1.282	1.369	2.299	10.6	75.48
386	1.6	225.5	334	0.239	118.6	6.917	2.23	1.238	15.25	73.06	1.086	1.262	2.393	10.48	76.39
403	2.49	210.7	258	0.13	110.4	7.055	1.88	1.218	17.43	73.88	1.484	1.514	2.228	10.58	78.89
410	1.65	208.2	258	0.271	123.2	6.917	1.949	1.199	13.43	80.54	1.248	1.466	2.845	8.48	79.28
445	3.81	203.7	263	0.151	102.7	6.958	1.717	1.397	15	71.7	1.192	1.586	2.084	11.01	72.82
539	1.35	224.4	337	0.163	113.9	7.012	2.059	1.181	15.47	71.78	1.209	1.546	2.479	11.06	73.63
569	4.09	159.6	213	0.293	90.4	6.264	1.503	1.014	16.23	74.68	1.021	1.31	2.557	11.34	74.04
584	2.13	213.4	369	0.467	179.6	6.847	2.15	1.128	16.6	71.79	1.018	1.331	2.174	8.8	74.7
629	2.41	180.6	327	0.292	101.2	6.921	2.011	1.067	14.71	71.68	1.121	1.288	2.041	8.31	78.64
637	2.58	197.8	308	0.321	97.6	6.56	2.068	1.183	15.88	72.59	1.298	1.615	2.435	11.1	74.65
696	2.24	179.8	305	0.145	83.5	6.357	1.969	1.045	14.26	75.32	1.057	1.456	2.527	9.49	74.87
699	2.29	218.9	348	0.234	107.8	7.045	2.191	1.404	15.53	74.54	1.11	1.382	2.236	9.68	78.12
725	1.48	231.4	380	0.163	124.2	6.974	2.362	1.078	16.3	72.99	1.306	1.336	2.485	10.06	78.5
736	2.45	182.9	331	0.266	84.6	6.185	2.199	1.061	15.1	72.45	1.317	1.251	2.431	9.59	74.05
740	3.84	199.8	337	0.183	101.4	6.275	2.134	1.037	15.81	75.25	1.104	1.293	2.383	10.44	82.05
741	1.81	219.5	334	0.255	115	6.92	2.268	1.096	14.68	72.93	1.124	1.289	2.189	10.84	74.65
750	3.52	233.8	340	0.313	124.3	6.974	2.047	1.149	16.07	76.7	1.388	1.331	2.624	8.88	79.29
752	4.18	196.7	277	0.179	92.3	6.357	1.909	1.045	14.93	75.32	1.22	1.403	2.274	10.95	76.2
783	2.81	218.2	323	0.225	106.1	6.894	1.959	1.061	14.2	72.12	1.131	1.421	2.441	9.34	75.7
787	1.46	191.1	320	0.253	98.7	7.021	1.964	1.198	15.94	74.16	1.152	1.305	2.277	10.14	75.51
811	1.71	224.6	331	0.258	115.2	6.933	1.943	1.143	15.56	71.47	1.049	1.297	2.631	10.31	78.02
Knjnj	2.31	203.9	318	0.355	98.3	6.9	2.069	1.077	15.1	71.37	1.156	1.418	2.326	9.02	75.25
Local 1	1.72	218.9	465	0.035	100.1	6.92	2.64	1.5	16.06	74.8	1.069	1.643	2.912	7.16	77.63
Local 2	2.66	199.9	335	0.383	107.8	6.15	2.222	1.089	15.84	69.9	1.223	1.327	2.207	9.76	72.73

Days_to_silking (DS), Ear damage (ED), Ear placements (EPL), Husk cover (HC), Kernel size (KS), Number_of_ears_plant (EP), Number_of_tassel_branches (TB), Plant_hieght (PH), days_to_tassel (DT), kernel colour (KC), number_of_kernels_row (KR), kernel type (KT), kernel weight (KW), kernel_role_arrangement (KRA)

Appendix 3.2: Genetic diatances between local maize varieties

811																					
1850	0.2741																				
Local 1	0.2385	0.3362																			
303	0.4578	0.3175	0.4649																		
199	0.3454	0.2875	0.3927	0.39																	
386	0.4374	0.4134	0.4011	0.4136	0.359																
250	0.4496	0.2887	0.3484	0.4006	0.4359	0.5195															
740	0.3515	0.4263	0.4011	0.4418	0.3467	0.5021	0.3654														
445	0.4324	0.3828	0.4095	0.3958	0.3918	0.4009	0.3738	0.4009													
206	0.446	0.4733	0.4481	0.4757	0.4699	0.6112	0.439	0.643	0.4756												
1786	0.3889	0.3769	0.3885	0.4818	0.4096	0.418	0.4041	0.4578	0.4133	0.5762											
1857	0.4545	0.4438	0.4053	0.3774	0.4139	0.5227	0.4376	0.4233	0.4054	0.4986	0.4763										
2872	0.3621	0.5	0.4094	0.4337	0.3214	0.4394	0.4526	0.3881	0.3096	0.5413	0.4137	0.5272									
569	0.4213	0.422	0.3015	0.4475	0.3929	0.5074	0.4804	0.5654	0.5038	0.6296	0.439	0.4572	0.3732								
172	0.3311	0.3649	0.3046	0.3852	0.3724	0.331	0.3654	0.3674	0.2442	0.452	0.418	0.3222	0.3298	0.4263							
584	0.4653	0.3814	0.5339	0.4252	0.4884	0.5114	0.5407	0.5972	0.2954	0.492	0.5371	0.411	0.4538	0.505	0.3277						
539	0.3818	0.3702	0.4366	0.3684	0.3918	0.3116	0.4718	0.4988	0.4378	0.4756	0.362	0.3788	0.4348	0.3578	0.3745	0.4665					
240	0.3818	0.2749	0.3203	0.382	0.3048	0.4009	0.3475	0.4417	0.3185	0.3955	0.3873	0.34	0.3702	0.3096	0.2896	0.4402	0.2697				
1332	0.3633	0.2928	0.3504	0.3208	0.436	0.3542	0.3397	0.5021	0.3489	0.4934	0.3192	0.3453	0.4263	0.3513	0.2732	0.291	0.2524	0.3239			
1857	0.3172	0.3732	0.4221	0.3548	0.2987	0.4263	0.3364	0.3881	0.2636	0.4096	0.3531	0.2839	0.2932	0.3614	0.274	0.336	0.3455	0.2863	0.2483		
203	0.2189	0.3015	0.2538	0.3815	0.2513	0.4408	0.4004	0.3629	0.3082	0.4221	0.3885	0.3052	0.2902	0.3721	0.2819	0.3917	0.3326	0.2844	0.3382	0.2355	
750	0.3574	0.2636	0.3576	0.3549	0.4181	0.3616	0.3093	0.4143	0.331	0.4896	0.3251	0.3788	0.3955	0.4482	0.3251	0.3552	0.2697	0.383	0.2185	0.2749	0.3082
699	0.4721	0.36	0.5195	0.3683	0.4581	0.4408	0.3234	0.5108	0.4095	0.435	0.5079	0.4594	0.4613	0.4883	0.4267	0.4038	0.3842	0.4095	0.3141	0.4221	0.4495
696	0.446	0.3973	0.4613	0.3804	0.4053	0.3881	0.5384	0.5216	0.4215	0.6609	0.4783	0.5869	0.3497	0.4473	0.4783	0.5317	0.4482	0.4084	0.3634	0.3732	0.4613
193	0.32	0.3071	0.3397	0.4255	0.2252	0.331	0.3781	0.4051	0.4002	0.439	0.4055	0.4355	0.3298	0.3278	0.3216	0.5371	0.3373	0.3373	0.331	0.2849	0.3279
249	0.4374	0.4894	0.3523	0.4918	0.4994	0.3319	0.4965	0.5815	0.4917	0.5435	0.5214	0.5289	0.4134	0.4763	0.4174	0.5292	0.3739	0.4246	0.3436	0.4507	0.4382
2027	0.553	0.362	0.4909	0.411	0.4867	0.5082	0.4552	0.6645	0.4644	0.454	0.5605	0.526	0.5553	0.442	0.4578	0.4273	0.5036	0.4266	0.3503	0.373	0.3703
741	0.4863	0.5576	0.5663	0.5648	0.4306	0.4749	0.6591	0.5927	0.5058	0.7007	0.6006	0.6773	0.5744	0.493	0.5832	0.5565	0.457	0.5226	0.5576	0.4324	0.5158
292	0.324	0.2518	0.3788	0.4403	0.3211	0.3839	0.3569	0.3979	0.4531	0.4898	0.3042	0.447	0.4318	0.3904	0.4235	0.5067	0.3661	0.312	0.3839	0.3904	0.3926
403	0.4496	0.4946	0.559	0.3728	0.4779	0.4325	0.448	0.4607	0.4146	0.5236	0.4581	0.5572	0.4123	0.5236	0.4173	0.5407	0.3872	0.3842	0.3654	0.3123	0.3871
1795	0.3127	0.2213	0.3111	0.3728	0.3826	0.2903	0.3646	0.3784	0.3346	0.4946	0.3654	0.4956	0.3864	0.4526	0.3404	0.4441	0.4718	0.4146	0.4051	0.3864	0.4004
637	0.519	0.3578	0.3585	0.358	0.4783	0.3239	0.3769	0.5375	0.3861	0.466	0.3495	0.4821	0.424	0.3707	0.4022	0.5121	0.4142	0.306	0.3368	0.397	0.4126
289	0.3726	0.3364	0.3111	0.4292	0.332	0.3784	0.3917	0.4897	0.3872	0.5534	0.3528	0.4236	0.3736	0.3864	0.3404	0.4573	0.3093	0.2846	0.3024	0.3864	0.2752
148	0.4541	0.4177	0.4694	0.5427	0.4914	0.5297	0.3568	0.4742	0.2717	0.4177	0.4998	0.5209	0.4177	0.5617	0.3153	0.4745	0.4837	0.4037	0.3962	0.3695	0.4049
	811	**1850**	**Local 1**	**303**	**199**	**386**	**250**	**740**	**445**	**206**	**1786**	**1857**	**2872**	**569**	**172**	**584**	**539**	**240**	**1332**	**1857**	**203**

Appendix 3.2……..continued

139	0.4806	0.4053	0.4446	0.3637	0.4802	0.4227	0.3826	0.5339	0.3289	0.4969	0.3846	0.4139	0.4306	0.5389	0.2573	0.3305	0.4315	0.4181	0.2991	0.3214	0.3801
145	0.448	0.3838	0.3718	0.4603	0.4074	0.3901	0.5592	0.603	0.4432	0.5248	0.3242	0.4821	0.4378	0.3707	0.3887	0.4696	0.3587	0.3723	0.3765	0.3578	0.3718
2862	0.3076	0.3924	0.4023	0.3841	0.3873	0.5069	0.4799	0.455	0.4499	0.6242	0.4312	0.4848	0.3924	0.4157	0.3957	0.5206	0.4245	0.3758	0.43	0.404	0.4023
kjnj	0.5236	0.4008	0.4487	0.4595	0.3841	0.2769	0.492	0.5328	0.4876	0.4996	0.4784	0.5665	0.5259	0.4867	0.3465	0.5057	0.4222	0.3728	0.3787	0.4996	0.4875
297	0.3588	0.2796	0.3681	0.3885	0.2957	0.3474	0.3692	0.4608	0.4166	0.5217	0.4219	0.4256	0.4177	0.5495	0.4601	0.6098	0.4037	0.3296	0.3594	0.3933	0.3443
163	0.4987	0.3721	0.4631	0.5401	0.3927	0.586	0.4984	0.4964	0.5682	0.5447	0.522	0.5165	0.3968	0.4221	0.4939	0.4931	0.5839	0.5075	0.5108	0.4883	0.4229
629	0.4248	0.3775	0.4744	0.3328	0.4447	0.492	0.5058	0.6351	0.4609	0.4008	0.4407	0.3939	0.5393	0.3548	0.4784	0.3978	0.2699	0.303	0.2877	0.3436	0.4115
260	0.427	0.3393	0.391	0.5596	0.3604	0.4513	0.3961	0.4803	0.4773	0.5748	0.506	0.4715	0.4996	0.4297	0.3066	0.4888	0.3645	0.3512	0.356	0.4297	0.4181
164	0.4973	0.4602	0.4883	0.3054	0.5827	0.3881	0.4804	0.5805	0.3578	0.4346	0.452	0.5418	0.4473	0.5993	0.439	0.4538	0.3955	0.4756	0.3757	0.4096	0.4094
3244	0.5857	0.5331	0.551	0.3985	0.4886	0.5572	0.5153	0.5277	0.4399	0.7704	0.6604	0.5633	0.5191	0.4783	0.4437	0.5648	0.5245	0.5245	0.4852	0.4917	0.522
local 2	0.5595	0.5081	0.5103	0.6215	0.547	0.6351	0.5027	0.6674	0.5557	0.9001	0.4601	0.5067	0.6378	0.4301	0.5844	0.7008	0.5557	0.4699	0.6351	0.5637	0.4965
2012	0.4131	0.2791	0.4237	0.3092	0.4079	0.519	0.3051	0.519	0.4478	0.4126	0.4531	0.4312	0.4867	0.5259	0.3353	0.4931	0.4349	0.3972	0.3668	0.4126	0.3874
243	0.357	0.2656	0.3433	0.3637	0.414	0.4096	0.332	0.4768	0.3661	0.4053	0.3018	0.3879	0.4566	0.3565	0.3132	0.4003	0.3789	0.2812	0.3108	0.2987	0.2735
1983	0.3369	0.4221	0.4229	0.4943	0.3677	0.3382	0.3358	0.4011	0.4504	0.4747	0.4665	0.4875	0.348	0.5447	0.2819	0.6059	0.3203	0.4095	0.4274	0.4094	0.3347
752	0.4253	0.3893	0.2951	0.4096	0.3608	0.4822	0.3049	0.3436	0.3025	0.4013	0.4174	0.4336	0.3093	0.4013	0.2372	0.386	0.5057	0.3495	0.3554	0.3893	0.3882
783	0.3329	0.3191	0.4378	0.3254	0.4951	0.4056	0.4261	0.5558	0.4112	0.383	0.5288	0.547	0.4994	0.4871	0.3857	0.3509	0.4112	0.4353	0.3273	0.394	0.336
1992	0.3507	0.3154	0.4481	0.3298	0.3929	0.4526	0.361	0.5074	0.3334	0.4602	0.439	0.4305	0.3732	0.4473	0.274	0.3032	0.4618	0.3955	0.3158	0.3154	0.3245
1845	0.4068	0.3334	0.3203	0.2904	0.3411	0.3116	0.2969	0.4009	0.3438	0.4084	0.3013	0.34	0.3455	0.3578	0.3013	0.4402	0.331	0.2938	0.2875	0.2749	0.3703
322	0.421	0.3707	0.4698	0.4006	0.4493	0.5375	0.4194	0.5217	0.458	0.3707	0.5318	0.4376	0.5556	0.637	0.3755	0.4978	0.4432	0.4286	0.4179	0.3838	0.3585
1915	0.4233	0.3004	0.3111	0.3195	0.357	0.3784	0.2877	0.4465	0.3872	0.4804	0.391	0.4376	0.4256	0.3364	0.3404	0.4441	0.5482	0.3475	0.3784	0.4123	0.4274
1892	0.4366	0.2791	0.4115	0.4461	0.4573	0.4028	0.3516	0.492	0.4478	0.4996	0.3925	0.538	0.5393	0.474	0.3465	0.4807	0.3972	0.3728	0.2769	0.4246	0.4362
3211	0.4948	0.4438	0.432	0.4329	0.2902	0.3706	0.4518	0.4233	0.5048	0.6024	0.4223	0.428	0.4046	0.4305	0.4355	0.6034	0.3275	0.3528	0.4099	0.4572	0.4186
310	0.3753	0.3513	0.3629	0.3465	0.2648	0.2703	0.3784	0.3795	0.4143	0.4797	0.4051	0.4233	0.4007	0.4526	0.331	0.4978	0.3745	0.3616	0.3174	0.3158	0.3504
736	0.4794	0.3813	0.4562	0.3885	0.401	0.4344	0.3945	0.6194	0.3784	0.5217	0.4864	0.5797	0.4683	0.4427	0.4219	0.5132	0.4037	0.3537	0.3239	0.3462	0.4049
725	0.4461	0.5447	0.3591	0.4649	0.4446	0.4274	0.4004	0.4274	0.4366	0.4747	0.4398	0.4875	0.3968	0.4481	0.3885	0.6209	0.3703	0.423	0.4142	0.2902	0.3469
787	0.3424	0.3769	0.4267	0.4119	0.3483	0.4714	0.391	0.4852	0.4535	0.4783	0.4437	0.5045	0.4013	0.465	0.4834	0.608	0.4673	0.4399	0.3924	0.3769	0.4531
218	0.524	0.3497	0.4747	0.4337	0.4053	0.5074	0.3993	0.6592	0.4482	0.3973	0.5331	0.5418	0.5555	0.4473	0.4917	0.5591	0.3955	0.3334	0.3275	0.442	0.3721
3243	0.5431	0.5331	0.4939	0.5263	0.4886	0.5722	0.5301	0.5133	0.5696	0.6369	0.4055	0.5335	0.5191	0.5762	0.5108	0.6686	0.5851	0.4813	0.4444	0.465	0.5658
226	0.3785	0.4846	0.3416	0.4329	0.3751	0.4507	0.4236	0.3579	0.5506	0.4986	0.4525	0.5596	0.3186	0.3918	0.3585	0.6499	0.392	0.4054	0.4647	0.4438	0.432
154	0.4953	0.4713	0.4208	0.4997	0.5209	0.4503	0.5044	0.5174	0.4858	0.6384	0.4253	0.5512	0.524	0.3854	0.4503	0.5831	0.3818	0.4324	0.3874	0.4336	0.4721
410	0.47	0.3282	0.5124	0.4855	0.3454	0.49	0.3363	0.6045	0.3818	0.5105	0.4009	0.4812	0.4092	0.3854	0.4503	0.516	0.2876	0.3695	0.3282	0.2954	0.3961
3414	0.3103	0.2414	0.3082	0.2904	0.3536	0.3616	0.3093	0.4009	0.306	0.3702	0.4002	0.3028	0.4482	0.3702	0.3013	0.3297	0.331	0.2938	0.2875	0.2636	0.2611
1772	0.3172	0.2714	0.3721	0.3675	0.3565	0.3275	0.3364	0.4394	0.4348	0.5136	0.4013	0.3668	0.4473	0.422	0.4137	0.4538	0.3455	0.3334	0.3275	0.3154	0.3844
	811	**1850**	**Local 1**	**303**	**199**	**386**	**250**	**740**	**445**	**206**	**1786**	**1857**	**2872**	**569**	**172**	**584**	**539**	**240**	**1332**	**1857**	**203**

Appendix 3.2……….continued

139	0.3048	0.3677	0.5247	0.4353	0.5694	0.5657	0.6099	0.4437	0.4779	0.332	0.3938	0.3826	0.5328										
145	0.3723	0.4126	0.4804	0.3624	0.5259	0.5066	0.468	0.3855	0.4194	0.3769	0.3623	0.3228	0.446	0.354									
2862	0.4499	0.4764	0.5016	0.3727	0.626	0.4498	0.5318	0.4584	0.4536	0.4154	0.5061	0.2976	0.5357	0.5242	0.4784								
kjnj	0.3608	0.3638	0.3661	0.3925	0.4651	0.3904	0.4718	0.4712	0.465	0.4004	0.3972	0.4258	0.5611	0.47	0.4772	0.4789							
297	0.3415	0.4175	0.3813	0.385	0.4588	0.6183	0.5996	0.3332	0.4337	0.2626	0.4051	0.3945	0.6156	0.3888	0.4741	0.4597	0.4207						
163	0.4644	0.5947	0.5161	0.4665	0.7006	0.5169	0.6377	0.4066	0.5907	0.4138	0.5304	0.5907	0.5973	0.5136	0.4408	0.6587	0.5141	0.6125					
629	0.4222	0.3994	0.4614	0.4531	0.4651	0.3669	0.5804	0.4298	0.534	0.465	0.2995	0.4517	0.603	0.47	0.3844	0.5283	0.5134	0.5208	0.6593				
260	0.3915	0.4744	0.4996	0.3434	0.4872	0.3599	0.5386	0.4345	0.5297	0.4533	0.5499	0.4243	0.4652	0.5124	0.4101	0.484	0.3072	0.5522	0.489	0.4827			
164	0.2863	0.4883	0.3973	0.6061	0.4507	0.5688	0.6446	0.4178	0.3864	0.3364	0.3707	0.4526	0.4947	0.3565	0.3707	0.5679	0.5667	0.4054	0.6845	0.4126	0.6562		
3244	0.5393	0.5364	0.5473	0.4834	0.6375	0.5471	0.6924	0.6609	0.5452	0.4721	0.5631	0.5452	0.5844	0.5306	0.5318	0.5193	0.6305	0.5698	0.6762	0.5725	0.435	0.5762	
local 2	0.6495	0.7094	0.7517	0.7434	0.7174	0.7378	0.6898	0.4979	0.7082	0.5616	0.5795	0.5923	0.6931	0.5614	0.5637	0.576	0.6468	0.5576	0.6125	0.6619	0.5987	0.7868	0.7434
2012	0.3849	0.3523	0.6538	0.3925	0.4901	0.3689	0.684	0.4998	0.4387	0.3398	0.4498	0.4517	0.4089	0.3608	0.4772	0.491	0.4402	0.4207	0.4487	0.4055	0.3664	0.4996	0.4284
243	0.3411	0.4054	0.4833	0.3483	0.486	0.4253	0.5577	0.3603	0.4779	0.3076	0.3411	0.4223	0.4517	0.2933	0.3671	0.4722	0.4323	0.3766	0.528	0.3381	0.4404	0.3929	0.4484
1983	0.3832	0.4229	0.5303	0.2932	0.3406	0.6135	0.5663	0.4056	0.4984	0.4274	0.5461	0.3612	0.4431	0.4855	0.5304	0.5024	0.3994	0.3925	0.5489	0.5415	0.391	0.5161	0.5658
752	0.3864	0.3761	0.5027	0.3692	0.5322	0.5195	0.6076	0.3931	0.4417	0.2933	0.3999	0.4025	0.3975	0.3261	0.454	0.4086	0.3822	0.3509	0.5183	0.4901	0.3932	0.3775	0.5878
783	0.3203	0.2836	0.4395	0.3857	0.4791	0.3279	0.3979	0.4405	0.3331	0.4261	0.3499	0.4384	0.4021	0.3554	0.4223	0.3838	0.3482	0.4831	0.5379	0.4446	0.4668	0.394	0.5288
1992	0.3334	0.2679	0.3732	0.4013	0.5575	0.4302	0.5248	0.4604	0.3993	0.361	0.4104	0.3242	0.3462	0.2441	0.3578	0.3585	0.3216	0.4177	0.4613	0.4126	0.4571	0.3154	0.4783
1845	0.306	0.3703	0.3955	0.3132	0.3989	0.4516	0.4893	0.2607	0.4008	0.3219	0.2683	0.3475	0.3909	0.3918	0.3861	0.4371	0.4096	0.3659	0.4504	0.349	0.4773	0.3955	0.4955
322	0.4286	0.4408	0.5714	0.4577	0.5562	0.4526	0.6504	0.4306	0.5264	0.5104	0.5306	0.434	0.533	0.5547	0.5996	0.5061	0.5054	0.4741	0.5784	0.4364	0.4247	0.5248	0.7942
1915	0.3606	0.4138	0.439	0.3781	0.5696	0.4174	0.5656	0.3431	0.6046	0.3001	0.2841	0.3646	0.4205	0.3957	0.4639	0.3666	0.4004	0.4337	0.3871	0.4258	0.4243	0.4664	0.4721
1892	0.2699	0.4487	0.3891	0.3808	0.4901	0.5947	0.5482	0.4032	0.3757	0.3636	0.4364	0.4258	0.4207	0.3841	0.4634	0.5157	0.3395	0.3082	0.5697	0.5134	0.4293	0.4008	0.5447
3211	0.4754	0.4875	0.4708	0.3342	0.5289	0.6106	0.6773	0.447	0.4662	0.4099	0.467	0.3962	0.5647	0.5549	0.467	0.4211	0.5522	0.3999	0.5165	0.4312	0.4569	0.5566	0.5335
310	0.31116	0.3504	0.4263	0.2959	0.3319	0.4951	0.575	0.3565	0.3784	0.3271	0.3901	0.3397	0.4215	0.384	0.4179	0.4937	0.3552	0.3009	0.6178	0.4401	0.4094	0.4263	0.4852
736	0.3415	0.4049	0.3813	0.4472	0.5378	0.4501	0.5492	0.4399	0.4337	0.3568	0.3659	0.3324	0.4895	0.4517	0.3919	0.4006	0.3972	0.3659	0.7796	0.3629	0.4378	0.3933	0.4601
725	0.3963	0.4631	0.6044	0.3761	0.329	0.5436	0.5158	0.5104	0.4004	0.5132	0.4126	0.4694	0.4965	0.4855	0.4552	0.4764	0.4744	0.4431	0.6105	0.5415	0.5496	0.5741	0.6117
787	0.3745	0.3517	0.3071	0.3567	0.4424	0.4951	0.4095	0.2795	0.4307	0.3404	0.4577	0.5301	0.5554	0.5024	0.5015	0.4556	0.4531	0.2713	0.6117	0.4913	0.435	0.4917	0.4437
218	0.4084	0.435	0.5274	0.439	0.3428	0.4302	0.5744	0.446	0.4526	0.439	0.424	0.4804	0.5081	0.4699	0.4804	0.5817	0.4246	0.3933	0.6679	0.3548	0.3899	0.3973	0.452
3243	0.5851	0.6434	0.5331	0.6286	0.701	0.6019	0.7118	0.5118	0.6077	0.4173	0.5318	0.4721	0.7608	0.4484	0.5166	0.4934	0.4913	0.3972	0.7101	0.5867	0.4914	0.6061	0.5534
226	0.461	0.3922	0.4572	0.2757	0.5007	0.5674	0.5838	0.4621	0.4518	0.4099	0.3813	0.3828	0.4789	0.5105	0.4091	0.4848	0.4062	0.4519	0.5617	0.4699	0.4005	0.6504	0.5189
154	0.4324	0.5401	0.4973	0.3654	0.5815	0.553	0.8473	0.3747	0.4766	0.4496	0.5796	0.4233	0.6315	0.4676	0.448	0.5516	0.6047	0.4541	0.6284	0.5634	0.4811	0.5105	0.489
410	0.2545	0.3369	0.4586	0.32	0.4747	0.4542	0.3993	0.3365	0.3726	0.463	0.4344	0.3976	0.3363	0.4939	0.3947	0.4756	0.4248	0.4053	0.5124	0.4607	0.4402	0.5376	0.6454
3414	0.2938	0.345	0.4756	0.2896	0.3617	0.3784	0.5226	0.3252	0.4286	0.3346	0.3452	0.2969	0.3784	0.3048	0.332	0.4499	0.4478	0.3537	0.4366	0.349	0.4479	0.3578	0.5245
1772	0.3334	0.3245	0.4602	0.3071	0.4381	0.4908	0.541	0.3125	0.5236	0.3004	0.345	0.361	0.4947	0.3565	0.4104	0.4395	0.4614	0.2904	0.5741	0.4126	0.4711	0.4733	0.5191
	750	**699**	**696**	**193**	**249**	**2027**	**741**	**292**	**403**	**1795**	**637**	**289**	**148**	**139**	**145**	**2862**	**kjnj**	**297**	**163**	**629**	**260**	**164**	**3244**

Appendix 3.2……….continued

2012	0.5889																					
243	0.5188	0.3841																				
1983	0.7265	0.3874	0.4183																			
752	0.6386	0.3822	0.3376	0.4129																		
783	0.6973	0.4559	0.282	0.5119	0.4326																	
1992	0.5495	0.3326	0.333	0.4613	0.3093	0.2399																
1845	0.4563	0.3849	0.3289	0.4366	0.314	0.4112	0.3702															
322	0.6118	0.3719	0.4931	0.4266	0.5259	0.5138	0.446	0.3861														
1915	0.4885	0.3516	0.357	0.4838	0.3166	0.3902	0.3242	0.2725	0.3769													
1892	0.5889	0.464	0.305	0.4362	0.3597	0.3181	0.3436	0.3728	0.5344	0.413												
3211	0.6423	0.4966	0.5105	0.4594	0.4079	0.5884	0.5418	0.3528	0.5446	0.4376	0.3939											
310	0.5587	0.4528	0.3346	0.3754	0.3674	0.394	0.3393	0.3363	0.4179	0.3917	0.3209	0.3579										
736	0.6931	0.5341	0.4914	0.4562	0.4588	0.4831	0.3695	0.4037	0.5482	0.4337	0.4571	0.5067	0.3715									
725	0.6438	0.4875	0.4183	0.3591	0.5045	0.4498	0.5447	0.3326	0.4552	0.5435	0.5415	0.5314	0.4111	0.5528								
787	0.6295	0.5177	0.3971	0.4011	0.3692	0.4082	0.465	0.3251	0.5631	0.4173	0.3925	0.4903	0.343	0.338	0.4939							
218	0.5495	0.4008	0.3447	0.4747	0.4894	0.4052	0.4346	0.3578	0.466	0.4946	0.3001	0.4986	0.4134	0.3235	0.5021	0.3531						
3243	0.5554	0.601	0.545	0.7632	0.4552	0.6089	0.4917	0.4002	0.5318	0.4444	0.5447	0.4625	0.4578	0.4472	0.5658	0.497	0.5616					
226	0.5647	0.4312	0.4961	0.3666	0.3829	0.4566	0.4572	0.3788	0.4376	0.4376	0.4966	0.428	0.3706	0.4256	0.3922	0.4763	0.5128	0.4763				
154	0.5187	0.6627	0.3925	0.6132	0.4374	0.6138	0.5795	0.3942	0.5641	0.5624	0.5107	0.4812	0.3168	0.5877	0.5543	0.489	0.5376	0.5293	0.4545			
410	0.5321	0.4607	0.4806	0.4084	0.4374	0.4292	0.3972	0.3336	0.5488	0.5044	0.4015	0.5368	0.3874	0.3363	0.4853	0.3654	0.4213	0.6454	0.4415	0.4576		
3414	0.4837	0.3373	0.2582	0.345	0.3257	0.2777	0.2979	0.2461	0.3723	0.2969	0.3972	0.48	0.2875	0.4166	0.3576	0.4399	0.3828	0.5393	0.392	0.4195	0.3942	
1772	0.5217	0.4126	0.31	0.4094	0.3893	0.383	0.4346	0.2979	0.495	0.3242	0.4367	0.4846	0.2815	0.4177	0.3968	0.3414	0.4346	0.5191	0.4438	0.4213	0.3854	0.2636	
	local 2	**2012**	**243**	**1983**	**752**	**783**	**1992**	**1845**	**322**	**1915**	**1892**	**3211**	**310**	**736**	**725**	**787**	**218**	**3243**	**226**	**154**	**410**	**3414**	**1772**

699	0.3203											
696	0.4084	0.4221										
193	0.3495	0.3162	0.389									
249	0.3495	0.5323	0.6006	0.4299								
2027	0.4143	0.5038	0.4784	0.3872	0.4945							
741	0.5226	0.5492	0.5088	0.4693	0.5905	0.5311						
292	0.286	0.4208	0.4178	0.3424	0.4765	0.4866	0.4315					
403	0.3093	0.3612	0.4256	0.4307	0.4825	0.5078	0.4966	0.3709				
1795	0.2969	0.4138	0.3864	0.3528	0.4551	0.4812	0.5656	0.3028	0.4336			
637	0.306	0.3455	0.4104	0.4022	0.454	0.4139	0.537	0.3423	0.4639	0.2715		
289	0.3219	0.3484	0.4664	0.2575	0.4965	0.4944	0.5656	0.414	0.4774	0.4195	0.4194	
148	0.3537	0.3096	0.5026	0.385	0.5378	0.5115	0.5492	0.4831	0.4205	0.5317	0.5031	0.4337
	750	**699**	**696**	**193**	**249**	**2027**	**741**	**292**	**403**	**1795**	**637**	**289**

Appendix 4.1: Grain yield, grain type and levels of resistance

		Maize weevil		Larger grain borer		
Variety	**Grain yield (tons/ha)**	**Grain damage (%)**	**Resistance level**	**Grain damage (%)**	**Resistance level**	**Grain type**
139	2.32	3.75	Moderately susceptible	18	Highly susceptible	semi-flint
145	3.43	3.50	Moderately susceptible	18.25	Highly susceptible	semi-flint
148	1.51	2.00	Resistant	16.25	Highly susceptible	semi-flint
154	2.77	3.75	Moderately susceptible	25	Highly susceptible	dent
163	4.15	3.75	Moderately susceptible	14.25	Highly susceptible	dent
164	2.19	2.75	Moderately resistant	20.75	Highly susceptible	semi-flint
172	1.80	1.75	Resistant	13	Highly susceptible	semi-flint
1772	3.76	1.25	Resistant	22.75	Highly susceptible	semi-flint
1786	4.81	1.50	Resistant	17.75	Highly susceptible	semi-flint
1795	3.49	3.25	moderately susceptible	13.25	Highly susceptible	dent
1845	1.93	3.00	moderately susceptible	18.25	Highly susceptible	dent
1850	1.70	1.50	Resistant	7.75	Highly susceptible	dent
1857	1.29	3.25	Moderately susceptible	22.5	Highly susceptible	dent
1892	2.80	2.25	Moderately resistant	20.75	Highly susceptible	semi-flint
1915	2.28			12.75	Highly susceptible	dent
193	2.79	2.00	Resistant	18.25	Highly susceptible	semi-flint
1983	3.16	2.25	Moderately resistant	5.00	Highly susceptible	dent
199	2.22	1.25	Resistant	13.25	Highly susceptible	dent
1992	2.88	2.00	Resistant	6.75	Highly susceptible	dent
2012	4.57	0.50	Resistant	6.75	Highly susceptible	semi-flint
2017	1.01	2.75	Moderately resistant	7.75	Highly susceptible	dent
2027	3.17	4.00	Susceptible	10.00	Highly susceptible	dent
203	2.42	4.50	Susceptible	19.00	Highly susceptible	semi-flint
206	2.39	3.50	Moderately susceptible	14.00	Highly susceptible	semi-flint
218	0.99	0.50	Resistant	20.00	Highly susceptible	semi-flint
226	2.06	2.00	Resistant	17.5	Highly susceptible	semi-flint
240	1.40	3.00	Moderately susceptible	18.00	Highly susceptible	semi-flint
243	2.61	1.75	Resistant	23.75	Highly susceptible	semi-flint
249	2.20	1.75	Resistant	9.85	Highly susceptible	dent
250	1.79	1.00	Resistant	18.5	Highly susceptible	dent
260	2.41	3.00	Moderately susceptible	30.75	Highly susceptible	semi-flint
2862		2.75	Moderately resistant			dent
2872	2.49	3.5	Moderately susceptible	11.25	Highly susceptible	semi-flint
289	2.43	2.75	Moderately resistant	8.25	Highly susceptible	semi-flint
292	2.76	4.00	Susceptible	21.5	Highly susceptible	semi-flint
297	1.54	2.25	Moderately resistant	19.00	Highly susceptible	semi-flint
303	0.26	2.75	Moderately resistant	31.00	Highly susceptible	semi-flint
310	1.02	2.50	Moderately resistant	32.00	Highly susceptible	semi-flint
315	2.35	3.25	moderately susceptible	10.75	Highly susceptible	semi-flint
322	1.74	1.75	Resistant	13.5	Highly susceptible	semi-flint
3243	2.85	2.25	Moderately resistant	10.75	Highly susceptible	dent
3244	3.91	0.75	Resistant	7.25	Highly susceptible	dent
332	2.44	3.25	susceptible	13.25	Highly susceptible	dent
3411	2.60	4.00	Susceptible	8.75	Highly susceptible	semi-flint
3414	2.30	4.00	Susceptible	12.5	Highly susceptible	semi-flint
386	1.60	1.00	Resistant	8.00	Highly susceptible	semi-flint
403	2.49	2.25	Moderately resistant	8.00	Highly susceptible	semi-flint
410	1.65	5.75	Highly susceptible	13.5	Highly susceptible	semi-flint
445	3.81	0.25	Resistant	8.25	Highly susceptible	semi-flint
539	1.35	2.75	Moderately resistant	12.50	Highly susceptible	semi-flint
569	4.09	2.00	Resistant	12.25	Highly susceptible	dent
584	2.13	3.50	Moderately susceptible			semi-flint
629	2.41	3.75	Moderately susceptible	18.25	Highly susceptible	semi-flint
637	2.58	1.50	Resistant	11.00	Highly susceptible	semi-flint
696	2.24	3.75	Moderately susceptible	21.00	Highly susceptible	dent
699	2.29	1.50	Resistant	8.50	Highly susceptible	semi-flint
725	1.48	2.25	moderately resistant	13.75	Highly susceptible	semi-flint

Table 4.1…….continued

		Maize weevil		Larger grain borer		
Variety	**Grain yield (tons/ha)**	**Grain damage (%)**	**Resistance level**	**Grain damage (%)**	**Resistance level**	**Grain type**
736	2.45	3.25	Moderately susceptible	17.75	Highly susceptible	semi-flint
740	3.84	3.25	Moderately susceptible	13.75	Highly susceptible	semi-flint
741	1.81	1.75	Resistant	10.25	Highly susceptible	semi-flint
750	3.52	4	Susceptible	22.25	Highly susceptible	dent
752	4.18	1.5	Resistant	11.25	Highly susceptible	semi-flint
783	2.81	2.5	Moderately resistant	26.5	Highly susceptible	semi-flint
787	1.46	1.5	Resistant	14.25	Highly susceptible	semi-flint
811	1.71	1.25	Resistant	15	Highly susceptible	dent
Knjnj	2.31	2.5	Moderately resistant	10.75	Highly susceptible	semi-flint
Local 1	1.72	2.25	Moderately resistant	14.5	Highly susceptible	dent
Local 2	2.66	1.5	Resistant	17.25	Highly susceptible	semi-flint

Appendix 5.1: Correlation between resistant parameters for maize weevil (set a)

Total_number_of_insects	1	-			
weight_loss_%	2	0.5226*	-		
Grain_damage_%	3	0.4653*	0.6965	-	
Adult_mortality	4	0.464*7	-0.0425	0.0082	-
		1	2	3	4

*Highly significant at P<0.001

Appendix 5.2: Correlation between resistant parameters (set b)

Grain_damage_%	1	-			
Weight_loss_%	2	0.6364**	-		
Adult_mortality	3	-0.0629	-0.0186	-	
Total_number_of_insects	4	0.1959	0.2544	0.5294*	-
		1	2	3	4

*significantly correlated at p<0.01, **significantly correlated at p<0.001

Appendix 5.3: Correlation between resistant parameters in set c

Adult_mortality	1	-				
Flour_g	2	-0.1844	-			
Grain_damage_%	3	-0.2975	0.9025*	-		
Total_number_of_insects	4	-0.0288	0.8761*	0.8187*	-	
weight_loss_%	5	-0.2206	0.8925*	0.9847*	0.8287*	-
		1	2	3	4	5

*Significantly correlated at p<0.001

Appendix 5.4: Correlation between resistant parameters in set d

Adult_mortality	1	-				
Grain_damage_%	2	-0.5657**	-			
Total_number_of_insects	3	-0.3992*	0.5174**	-		
Weight_loss_%	4	-0.622***	0.7658***	0.3246	-	
flour_g	5	-0.4112*	0.7971***	0.5895**	0.5978**	-
		1	2	3	4	5

*significantly correlated at p<0.05, **significantly correlated at p<0.01, ***significantly correlated at p<0.001

Appendix 5.5: Correlation between resistant parameters for maize weevil (set e)

Insect mortality	1				
Grain_damage_%	2	-0.1735	-		
Total_number_of_insects	3	0.9909*	-0.117	-	
Weight_loss_%	4	-0.0249	0.9324*	0.0277	-
		1	2	3	4

*significantly correlated at p<0.001

Appendix 5.6: Correlation between resistant parameters for LGB resistance (set e)

Grain_damage_%	1	-				
Insect_mortality	2	-0.2272	-			
Total_number_of_insects	3	0.6685*	0.1982	-		
Weight_loss_%	4	0.9726*	-0.1666	0.6771*	-	
flour_g	5	0.9171*	-0.1856	0.7753*	0.8798*	-
		1	2	3	4	5

*significantly correlated at p<0.001

Appendix 5.7: Ranking of F_1 hybrids using percent grain damage due to maize weevil across sets

Variety	Grain damage (%)	Rank	Set	Variety	Grain damage (%)	Rank	Set	Variety	Grain damage (%)	Rank	set
MWA06A	0.72	1	A	MWMW11940	1.72	29	B	lgMW087940	2.169	57	E
MWA12395	0.794	2	A	MWMW446939	1.772	30	B	MWlg06021	2.23	58	E
MWA08202	1	3	A	MWA446A	1.797	31	A	MWlg11176	2.23	59	E
MWA11312	1.042	4	A	MWMW13940	1.802	32	b	MWlg939164	2.23	60	E
MWA10A	1.086	5	A	MWA151395	1.812	33	A	MWMW674939	2.242	61	B
MWA11A	1.138	6	A	MWMW446940	1.819	34	B	lgMW08710	2.282	62	E
MWA122W	1.192	7	A	MWMW1313	1.873	35	B	MWlg06264	2.291	63	E
MWA151273	1.212	8	A	MWMW67410	1.912	36	B	MWMW13939	2.308	64	B
MWA11273	1.221	9	A	MWMW13940	1.955	37	B	MWlg13074	2.326	65	E
MWA44606	1.296	10	A	MWMW151675	1.958	38	B	MWlg13218	2.387	66	E
MWA676202	1.312	11	A	MWA12202	1.972	39	A	MWlg13089	2.401	67	E
MWA10273	1.328	12	A	MWA06273	1.976	40	A	MWMW674675	2.412	68	B
MWA151A	1.357	13	A	MWA11403-3	1.989	41	A	lgMW26411	2.426	69	E
MWA12A	1.381	14	A	lgMW007940	2.021	42	E	MWMW1306	2.435	70	B
MWA15175	1.383	15	A	lgMW089151	2.021	43	E	lgMW17606	2.438	71	E
MWA10395	1.396	16	A	MWlg08007	2.021	44	E	MWMW13676	2.469	72	B
MWA6760020	1.418	17	A	MWlg08164	2.021	45	E	lgMW02111	2.478	73	E
MWMW15106	1.497	18	B	MWA1512W	2.063	46	A	lgMW16410	2.501	74	E
MWMW446937	1.497	19	B	MWMW11937	2.071	47	B	MWMW44606	2.514	75	B
MWA4462W	1.505	20	A	lgMW021151	2.095	48	E	MWMW446675	2.537	76	B
MWA112W	1.506	21	A	lgMW16413	2.095	49	E	MWMW69006	2.563	77	B
MWA67406	1.584	22	A	MWlg151264	2.095	50	E	MWMW151940	2.581	78	B
MWMW0611	1.609	23	B	MWA06395	2.108	51	A	MWMW11675	2.592	79	B
MWMW674937	1.609	24	B	MWMW1106	2.126	52	B	lgMW16411	2.596	80	E
MWMW151939	1.637	25	B	MWMW1110	2.141	53	B	MWMW13675	2.629	81	B
MWA062W	1.662	26	A	MWMW1210	2.141	54	B	MWMW151937	2.781	82	B
MWA11202	1.684	27	A	lgMW08812	2.156	55	E	lgMW26410	3.138	83	E
MWMW12939	1.7	28	B	MWA06403-3	2.162	56	A	lgMW08711	3.232	84	E

CV = 19.27, SE= 0.3785, LSD= 0.9394, P<0.001, Note: **Set A**: Crosses between adapted Malawi lines and maize weevil resistant lines, **Set B:** Crosses between maize weevil resistant lines**, Set E**: Crosses between maize weevil resistant lines and larger grain borer resistant lines

Appendix 5.8: Ranking of F_1 based on percent grain damage due to LGB across sets

Variety	Grain damage (%)	Rank	Set	Variety	Grain damage (%)	Rank	Set	Variety	Grain damage (%)	Rank	Set
LGA089116	0.998	1	C	LGA074I83	1.861	29	C	LGLG081218	2.363	57	d
LGA087I83	1.266	2	C	lgMW08710	1.894	30	E	LGA089444	2.394	58	C
LGA088A	1.278	3	C	LGLG088176	1.908	31	D	LGLG089074	2.41	59	D
LGA0740020	1.317	4	C	LGA074158	1.909	32	C	MWlg08164	2.412	60	E
LGA264202	1.357	5	C	LGLG087176	1.915	33	D	LGLG007074	2.413	61	D
LGLG089218	1.365	6	D	lgMW08812	1.945	34	E	LGA2640020	2.416	62	C
LGLG021074	1.369	7	D	LGA089A	1.962	35	C	LGA074116	2.423	63	C
LGA0870020	1.425	8	C	lgMW021151	2.02	36	E	MWlg13218	2.479	64	E
MWlg06264	1.432	9	E	LGLG088264	2.042	37	D	LGA088444	2.58	65	C
lgMW087940	1.493	10	E	LGLG089089	2.059	38	D	LGLG021264	2.586	66	D
LGLG007164	1.549	11	D	LGA089716	2.07	39	C	lgMW16413	2.587	67	E
LGA021I83	1.579	12	C	LGLG089176	2.081	40	D	LGA0210020	2.597	68	C
LGA164116	1.589	13	C	LGA164I83	2.085	41	C	LGA218I83	2.598	69	C
LGA176291-4	1.625	14	C	MWlg06021	2.09	42	E	LGA021A	2.626	70	C
LGLG087264	1.644	15	D	LGA264444	2.106	43	C	lgMW02111	2.626	71	E
LGLG007176	1.679	16	D	LGA164A	2.128	44	C	lgMW08711	2.626	72	E
lgMW089151	1.685	17	E	LGA218444	2.152	45	C	lgMW26410	2.714	73	E
LGLG087218	1.696	18	D	LGA074A	2.168	46	C	LGA264216	2.743	74	C
LGLG088218	1.696	19	D	LGLG087074	2.186	47	D	lgMW007939	2.761	75	E
LGA089I83	1.714	20	C	LGA264I83	2.236	48	C	LGLG088007	2.794	76	D
LGA074444	1.726	21	C	LGLG021176	2.244	49	D	LGA164444	2.863	77	C
LGLG074007	1.741	22	D	LGA264158	2.253	50	C	lgMW17606	2.947	78	E
LGA021158	1.798	23	C	LGLG088164	2.27	51	D	LGA264A	2.995	79	C
LGLG007218	1.818	24	D	LGLG089007	2.272	52	D	MWlg13074	3.081	80	E
LGA0890020	1.822	25	C	lgMW16410	2.277	53	E	MWlg11176	3.111	81	E
LGA089118	1.847	26	C	LGLG164007	2.308	54	D	LGLG007088	3.114	82	D
LGA264116	1.854	27	C	LGLG007264	2.32	55	D	lgMW16411	3.165	83	E
LGA021116	1.86	28	c	MWlg13089	2.356	56	E	lgMW26411	3.295	84	E

CV= 22.48, se= 0.4806, lsd =0.9241, $p<0.001$, **Note**: **Set c**: Crosses between adapted Malawi lines and larger grain borer resistant lines, **Set d**: Crosses between larger grain borer resistant lines **Set e**: Crosses between maize weevil resistant lines and larger grain borer resistant lines,