BREEDING INVESTIGATIONS ON RESISTANCE TO FALL ARMYWORM IN TROPICAL MAIZE GERMPLASM

By

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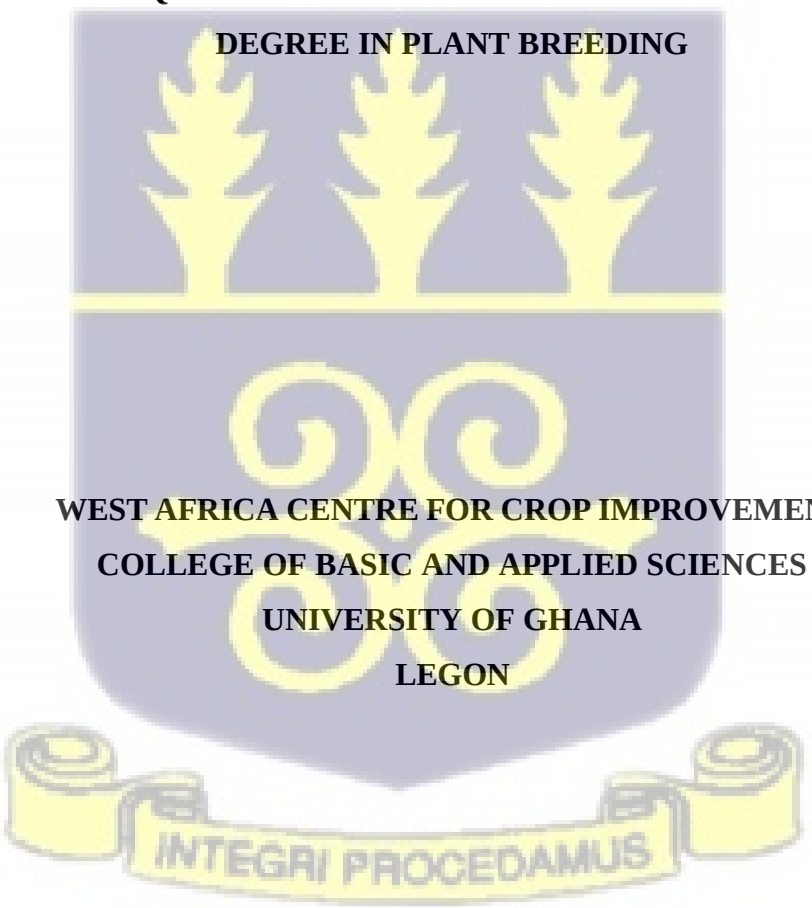
THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING



WEST AFRICA CENTRE FOR CROP IMPROVEMENT

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DECLARATION

I hereby declare that the content of this thesis has not been submitted for any degree, and I further certify that all references and any help received in preparing this thesis have been duly acknowledged.

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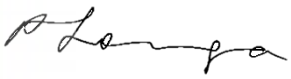

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ABSTRACT

Maize is the main staple food in Kenya and contributes significantly to the country's food and nutrition security. However, significant economic yield losses attributed to fall armyworm (FAW) infestation have been reported in the country. Limited genetic studies have been conducted on FAW resistance in tropical maize. This study was carried out to i) determine the genetic diversity within tropical maize germplasm for resistance to FAW, ii) estimate gene action for fall armyworm resistance and agronomic traits in tropical maize inbred lines, and iii) identify genomic regions and candidate genes associated with resistance to FAW in tropical maize germplasm using genome-wide association studies (GWAS). In objective i), the genetic diversity and population structure of 140 tropical maize inbred lines for resistance to fall armyworm were determined using phenotypic traits and DArTseq-based single-nucleotide polymorphisms (SNP) markers. One hundred and forty maize inbred lines were evaluated for their agronomic traits and response to FAW under artificial infestation in a 14 x 10 alpha lattice design with two replications for two seasons at KALRO Kiboko station in Kenya. Highly significant differences (< 0.001) were observed among the genotypes for FAW leaf and cob damage scores, plant height, ear height, number of ears, ear aspect, and grain yield (t ha^{-1}). Genotype and genotype by environment interaction (GEI) variances were highly significant for all traits. Significant negative correlation was observed between FAW damage parameters and grain yield/yield-related parameters. The variation in performance of the genotypes signified that the tropical inbred lines were genetically diverse and may be used in maize breeding programs for crop improvement. The inbred lines were further genotyped using 24,741 SNP markers. The gene diversity (GD) recorded a mean of 0.35 with a range of between 0.1 and 0.5. The polymorphism information content (PIC) ranged from 0.10 to 0.38 with a mean of 0.28. Observed

heterozygosity (Ho) ranged between 0.18 and 0.49, with a mean value of 0.25. Population pair-wise fixation index ranged from 0.02 to 0.15. Genetic variation within the subpopulation (65%) was higher than among the subpopulation (35%). Population structure analysis based on cross-entropy criteria and neighbor joining hierarchical cluster analysis grouped the inbred lines into nine subpopulations (K=9) and 9 clusters, respectively. The wide genetic variability observed in this study indicated that the inbred lines were genetically diverse and may be important sources of beneficial genes for maize breeding programs. The second objective involved the estimation of gene action for FAW resistance and agronomic traits in tropical maize inbred lines. Combining ability analysis of 123 hybrids generated by North Carolina Design II (NCDII) mating design and 5 checks was performed in a 16 x 8 alpha lattice design with 2 replications, across 3 locations (Kiboko, Kirinyaga and Kakamega) for two seasons. Result showed that FAW leaf damage traits, cob damage and ear aspect were controlled by additive gene effects while plant height, ear height and ear rot were regulated by both additive and non-additive gene effects. Eleven lines in leaf damage score 1 (LD1), 9 in leaf damage score 2 (LD2), 6 in leaf damage score 3 (LD3) and 13 entries in cob damage exhibited significant negative general combining ability (GCA) male effects and 14 entries in LD1, 7 in LD2 and 13 in cob damage had significant negative female GCA effects for fall armyworm damage traits. Ten inbred lines recorded a significant negative GCA effect for ear rot. Lines with significant negative GCA effects for FAW and ear rot from the study can contribute favorable alleles for improvement of resistance to FAW and ear rot in maize, respectively. Twenty-three new superior hybrids with significant specific combining ability (SCA) effect in maize ear rot disease, 42 hybrids in plant height, 42 hybrids in ear height, and a single hybrid with significant SCA effect for grain yield were identified. The selected hybrids were recommended for further evaluation for improvement of their respective traits and preparation for release. In the third objective, 137 tropical maize inbred lines were

evaluated for FAW leaf and cob damage, grain yield and ear rot infection. The lines were evaluated under artificial FAW infestation for two seasons and genotyped with the DArTseq platform. Genome-wide association study (GWAS) revealed that 8 SNPs were highly significantly associated with resistance to FAW damage traits, 10,950 candidate genes associated with FAW leaf damage, and 4,495 candidate genes associated with cob damage in maize. Thirty-four pathways linked with FAW resistance and biological processes governed by 20 most significantly enriched genes associated with FAW damage were identified. The SNPs, candidate genes, pathways, and biological processes identified provided extensive genomic information that can be further investigated for FAW resistance in maize

DEDICATION

I dedicate this work to God Almighty, My Father Francis Wesonga, Mother Lydia Wesonga, brothers Bramwel Wesonga and Denis Oyando, sisters Emily Asoma and Dorothy Ayuma, and friends

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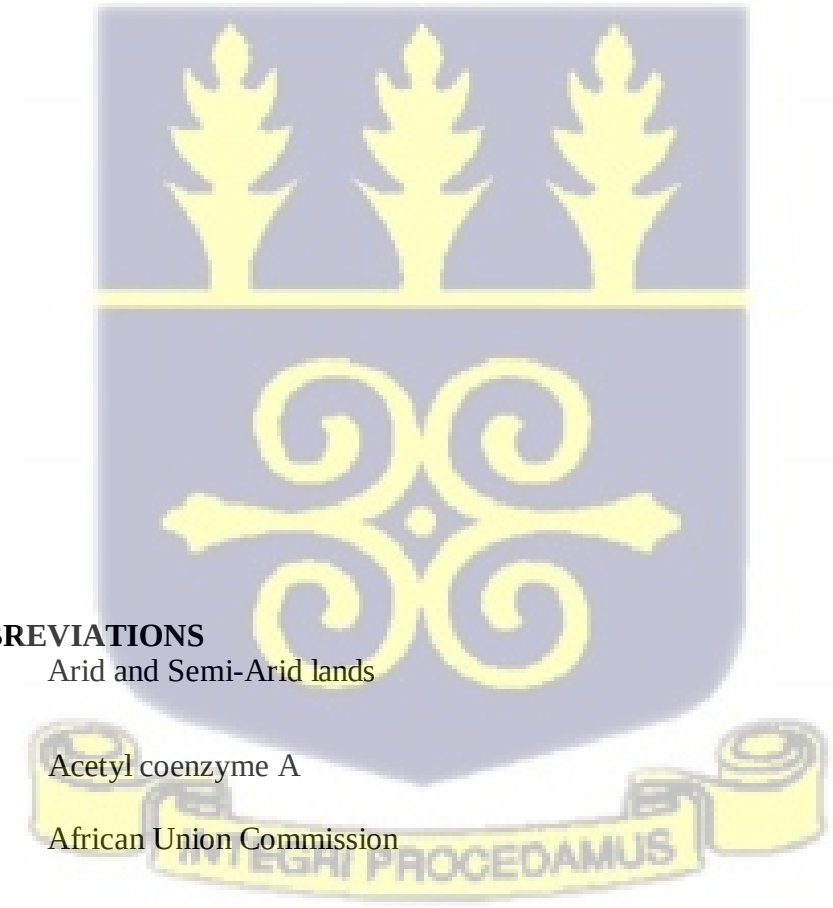


LIST OF ABBREVIATIONS

ASAL	Arid and Semi-Arid lands
Acetyl-CoA	Acetyl coenzyme A
AUC	African Union Commission
BCG	Boston Consulting Group
BGSL	Bonferroni genome-wide significance level

CIMMYT	International Maize and Wheat Improvement Center
DArTseq	Diversity Array Technology sequencing
DIBOA	2,4-dihydroxy-1,4-benoxazin-3-one
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
F1	First Filial Generation
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
FarmCPU	Fixed and random model Circulating Probability Unification,
GAPIT	Genome Association and Prediction Integrated Tool
GBS	Genotyping by Sequencing
GCA	General combining ability
GD	Gene Diversity
GEI	Genotype by Environment Interaction
GLS	Grey leaf spot
GO	Gene Ontology
GWAS	Genome-Wide Association Study
HPR	Host Plant Resistance
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
IPM	Integrated pest management
IRMA	Insect-Resistant Maize for Africa
KALRO	Kenya Agricultural and Livestock Research Organization

KAOP	Kenya Agricultural Observatory Platform
LEA	Landscape and Ecological Association Studies
MAF	Minor allele frequency
MLM	Mixed Linear Model
MLMM	Multi-loci mixed model
MSV	Maize Streak Virus
NAD	Nicotinamide Adenine Dinucleotide
NCDII	North Carolina 11 mating design
OPVs	Open-Pollinated Varieties
PC	Principal Components
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
Q-Q	Quantile–quantile
QTL	Quantitative Trait Locus
SCA	Specific Combing Ability
SSA	Sub-Saharan Africa
SWEEP	Window Extraction of Explicit Polymorphisms
TLB	Turcicum Leaf Blight
USAID	United States Agency for International Development
WACCI	West Africa Centre for Crop Improvement
WEMA	Water Efficient Maize for Africa
WFP	World Food Programme

CHAPTER ONE

1. GENERAL INTRODUCTION

Maize is ranked third in cereal world production and is a source of food, feeds, and raw material for industrial use (Badu-Apraku *et al*., 2021). Maize grain can be consumed as porridge, baby corn, roasted and boiled ears, and popcorn or used to produce cooking oil and high amylose corn for baking and production of snacks (Hossain *et al*., 2021). The crop is rich in most macro and micro nutrients (Rouf Shah *et al*., 2016). Globally, maize production is estimated to be 1040 million MT (Erenstein *et al*., 2022). Maize is a staple source of calories in sub-Saharan Africa (SSA), and it is cultivated on an overall area of 40 million hectares (van den Berg *et al*., 2021). Cultivation of maize in Kenya is carried out on 48.5% of the total arable land (Kang'ethe *et al*., 2020) with a consumption of 103 kg/person/yr (Naseem *et al*., 2018). About 3% of Agricultural GDP in Kenya and 21% of the overall value of the main agricultural commodities is contributed by maize. Maize production directly influences the country's strategic food reserve and national food security (Kang'ethe *et al*., 2020). The trend of maize farming in Kenya shows fluctuation in harvested area and production within 10 years (FAOSTAT, 2024). Climate change, lack of mechanization, and abiotic and biotic stresses have greatly contributed to the decline in maize cultivation in SSA (Mulungu and Ng'ombe, 2020).

Fall armyworm is an important pest in maize farming because of the massive yield losses attributed to its infestation. Damage from FAW causes substantial reductions in maize yields (Midingoyi *et al*., 2021). In addition, FAW epidemics interfere with the local, regional, and global maize trade, enhancing economic risks (Togola *et al*., 2025). The pest causes global yield loss of up to 90% and annual yield loss of about $9.4 billion in Africa (van den Berg *et al*., 2021). An estimated maize yield loss of about US$500 million resulting from FAW has been reported in the USA. In Brazil, approximately US$600 million was spent managing FAW in

2009 (Overton *et al*., 2021). Fall armyworm infestation is also a huge threat to European agriculture, with an estimated yearly economic impact of €12.6 billion (Kafle and Joshi, 2025). Maize farmers in China experienced a rise in the cost of pesticides used for controlling FAW from US$81 in 2018 to US$276 per hectare in 2020, increasing the maize production cost (Prasanna *et al*., 2022).

Fall armyworm is a major threat to the cultivation of maize in Africa (Sisay et al., 2019). In SSA, an estimated maize quantity of 13.5 million tons worth $3 billion is prone to destruction from FAW (Abrahams *et al*., 2017). Annual economic maize yield losses of between 8.3 and 20.6 million tons, i.e., approximately between 21 to 53% of the overall maize produced in SSA, were caused by FAW infestation (De Groote *et al*., 2020). The infestation rates varied within countries in SSA. For instance, in 2017, the range of infestation in the maize fields was between 22% and 67% in Ghana, 25% and 50% in Zambia (Day *et al*., 2017), 93% and 100% in Tanzania, 33% to 100% in Ethiopia, and 100% in Kenya (Sisay *et al*., 2019). In Kenya, maize damage from FAW results in an estimated annual yield reduction of up to 1381 kg $ha^{-1,}$ totaling 47% of the total maize produced in the country (De Groote *et al*., 2020).

Conventionally, maize germplasms are classified by breeders based on the environmental conditions to which they're adapted: tropical maize grows in altitudes of between 26° north and 26° south, while temperate germplasm is cultivated in latitudes above 26° north and below 26° south (Taba and Twumasi-Afriyie, 2008). Tropical maize is adapted to shorter day lengths, while temperate maize is adapted to longer day lengths (Taba and Twumasi-Afriyie, 2008). Temperate maize germplasm is usually susceptible to pests and diseases, while tropical germplasm has a diverse source of resistant genes for biotic stress, including FAW (Abadassi, 2014). Additionally, breeders rarely use temperate maize varieties in the tropical zones because of their low

adaptability (Abadassi, 2014). However, breeders have developed temperate maize inbred lines with resistance to FAW (Prasanna et al., 2018). Tropical inbred lines were used in the study because they are adapted to the local environmental conditions and can be immediately incorporated into pre-breeding programs for crop improvement, reducing the time needed for adaptation (Carena, 2021).

Different approaches have been used to manage FAW, including pesticide use, cultural methods, mating disruption technology, biological methods, agroecological management, and host plant resistance (HPR) (Prasanna *et al*., 2022). Though no single approach has been successful, an effective and sustainable integrated pest management (IPM) strategy is needed for efficient control of FAW. When combined with other management methods in the IPM strategy, native HPR provides significant, sustainable, cost-effective, and environmentally friendly protection against FAW infestation and reduces yield losses attributed to the pests. For an effective IPM strategy for FAW to be achieved, there is a need for plant breeders to develop cultivars with HPR (Prasanna *et al*., 2018). Efforts have been made in developing FAW-resistant maize genotypes with native host resistance (Prasanna *et al*., 2018). Cultivation of resistant varieties has been shown to minimize yield losses attributed to FAW (Blanco et al., 2014). However, there's limited information on African-adapted maize germplasm that confer resistance to FAW, and hence the need to identify genotypes with native resistance to FAW.

The current study involved the identification of sources of native genetic resistance to FAW through artificial and natural screening of germplasm. Identification of the source and strength of resistance is crucial when breeding for FAW resistance in Africa-adapted maize germplasm (Prasanna *et al*., 2018). Breeding progress has been made by the International Maize and Wheat Improvement Center (CIMMYT) through the development of Multiple Insect-Resistant Tropical

(MIRT) and Multiple Borer Resistant (MBR) maize populations (Prasanna *et al*., 2022). The populations provided the foundation for developing improved inbred lines with partial resistance to FAW. Research carried out by CIMMYT in Mexico, Africa, and Asia revealed that there was genetic variation in the MBRT and MBR maize populations to support native host plant resistance breeding for maize insect pests such as the corn borers, stemborers, weevils, larger grain borers, and FAW (Prasanna *et al*., 2022). The study involved screening MBRT and MBR germplasm for resistance to FAW to identify resistant lines.

Progress in resistance breeding for FAW depends on the level of genetic diversity amongst germplasm. Genetic diversity can be assessed through screening and characterization of available germplasm (Kasoma *et al*., 2020). The greater the diversity for a particular trait, the more the expected genetic gain from the selection process. There is little information documented on the genetic diversity of African-adapted germplasm for resistance to FAW. For successful breeding of maize varieties for FAW resistance, there is a need for extensive evaluation of available germplasm for resistance to FAW to identify the existing genetic diversity (Kasoma *et al*., 2020; Prasanna *et al*., 2018). The identified FAW-resistant germplasm will be utilized as sources of resistant genes for the formation of new high-yielding resistant varieties and for the improvement of farmer-preferred high-yielding commercial varieties that are susceptible.

Selection of inbred lines with desirable phenotypes is critical for all hybrid development. Selection can be carried out through morphological characterization or based on the combining ability of the inbred lines (Latha and Lone, 2020). Analysis of combining ability generates information used by breeders to select parental lines for hybrid formation (Arifin *et al*., 2018). The main goal of hybrid formation is to achieve maximum heterosis (hybrid vigor) by crossing genetically diverse inbred lines from different heterotic classes (Latha and Lone, 2020). Combining ability

analysis determines the type of gene action and extent of heterotic expression within the hybrids (Fasahat, 2016). General performance of parental lines does not, *per se*, identify the combining abilities of the lines. F1 Hybrid improvement is mainly based on the selection of parents that are good combiners and from different heterotic groups (Badu-Apraku *et al*., 2021). Therefore, it is important to estimate the general and specific combining abilities of inbred lines before their introduction into a maize breeding program. Identification of gene action controlling FAW resistance is important in determining the genetic mode of inheritance of the trait (Murenga *et al*., 2015).

Knowledge of the genetic basis of FAW resistance is important for achieving an effective HPR (Badji *et al*., 2018). Genome-wide association studies (GWAS) were used to investigate the genetic basis of insect resistance (Hanson *et al*., 2018). Breeding for FAW host plant resistance (HPR) requires the identification of genomic regions associated with resistance to the pest through Genome-wide association studies (Adewale *et al*., 2020). GWAS using single-nucleotide polymorphism (SNP) markers was used to investigate the genetic basis of resistance to FAW in tropical maize germplasm by identifying SNPs and candidate genes regulating the resistance (Badji *et al*., 2020).

Problem statement

The study investigates FAW infestation, a critical issue in maize production, due to substantial economic yield losses associated with the pest in Kenya. Fall armyworm is a destructive pest that feeds on maize leaves and cobs, significantly reducing crop productivity. Given maize's role as Kenya's primary staple food, addressing FAW resistance is essential for national food security and nutrition. Despite the economic and agricultural importance of maize in Kenya, limited genetic studies have focused on breeding tropical maize germplasm resistant to FAW. Previous

research has largely concentrated on pest management strategies, but breeding FAW-resistant maize varieties remains an underexplored area. Given the window period between developing and the adoption of new maize varieties, breeding programs must focus on the development of improved climate-adapted varieties for ease of farmer access and adoption. This study aims to fill that gap by investigating the genetic diversity, gene action governing FAW resistance traits, and genomic regions associated with FAW resistance in African Adapted Tropical Inbred lines.

Rationale for the Study

The study is justified by the urgent need to develop sustainable solutions for FAW infestation in maize. Current pest control methods, such as chemical pesticides, pose environmental and economic challenges. Breeding maize varieties with inherent FAW resistance (HPR) offers a long-term, cost-effective, and environmentally friendly solution. The study evaluated multiple insect resistance tropical (MIRT) and multiple borer resistance (MBR) maize germplasm for resistance to FAW, which increased the chances of detecting sources of resistance genes within the population under study. The use of tropical-adapted maize germplasm ensured that the resistant lines identified in the study could be directly incorporated into pre-breeding programs for crop improvement by eliminating the time required for adaptation. Identifying the source and strength of resistance is crucial when breeding for FAW resistance in Africa-adapted maize germplasm. The sources of resistant genes identified in the study will be used to develop new inbred lines, form resistant hybrids, and improve FAW-susceptible farmer-preferred commercial varieties. By identifying resistant genetic lines, gene action governing FAW resistance traits, and uncovering the genomic regions associated with FAW resistance, the research paves the way for improved maize breeding programs, ensuring higher yields and enhanced food security in Kenya. In this regard, the main objective of the study was to identify tropical maize germplasm with resistance to FAW for high and sustainable maize production.

The specific objectives of the study were:

i. To evaluate the genetic diversity within tropical maize germplasm for FAW resistance using phenotypic traits and molecular markers (DArTseq-based SNP markers)

ii. To determine the inheritance patterns of FAW resistance and important agronomic traits in tropical maize by estimating the gene action governing the traits.

iii. Identify genomic regions and candidate genes associated with resistance to FAW through Genome-wide association studies and metabolic pathway analysis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, distribution, and production of maize

2.1.1 Origin of maize Origin

Maize (2 n = 20) belongs to the *Poaceae* (grass) family, genus *Zea*, and *may* species (ICAR, 2012). Identifying the center of origin for maize was based on research findings on the geographical patterns of the crop species diversity (Orozco-Ramírez *et al*., 2017). Maize originated from the subtropics of Central America between Guatemala and Mexico more than 9000 years ago (Erenstein *et al*., 2022). Research shows that maize originated from its wild relative teosinte as a result of evolutionary forces like human selection, hybridization, genetic drift, and mutation (Awata et al., 2019). Distribution of teosintes and domestication of maize in Mexico further confirms the region as the center of origin for maize (Hernández, 2009). However, the low rate of gene flow between maize and teosinte has enabled the two crops to co-exist separately (Awata *et al*., 2019). Maize was domesticated in Mesoamerica (Mexico) over 9000 years ago and spread globally after the colonization of America (Ramirez-Cabral *et al*., 2017). Maize was spread over South and North America, generating an extensive diversity of landraces adapted to tropical and temperate environments (Camus-Kulandaivelu et al., 2006).

2.1.2 Distribution and production of maize

Maize has been distributed globally from the center of origin (Central America) to almost all the temperate regions and to a smaller extent in sub-tropics and tropical zones (Gumbe, 2020). An increase in global maize production was experienced in the past eras, driven by a rise in demand, technological advancement, expansion of cultivation areas, and yield improvement (Erenstein *et al*., 2022). Maize is among the most cultivated crops in the world, and is grown in about 193.7 million

hectares in over 170 countries, generating approximately 1147.7 million tons of maize. However, America and Asia produce over 80% of the global maize yields (Matos *et al.*, 2024). China is the second major maize producer globally, with an annual production of 2260.95 million tons, accounting for 22.72% of world maize production (Peng *et al*., 2023). Africa produces 7.4 % of the global maize and is ranked fourth after America, which produces half (49.6%) of the maize globally, Asia (32%), and Europe (10.0%) (Erenstein *et al*., 2022).

The largest maize producer in Africa is South Africa, followed by Nigeria (Abayomi, 2023). In Kenya, maize is grown in 2.196 million ha of land and cultivated by over 3 million subsistence farmers producing 3.897 million tons annually (Njeru *et al*., 2022). Maize is the main staple food in Kenya; it contributes significantly to the country's food security and nutrition. Maize farming offers food, feed, income, and employment to 90% of Kenyans (Njeru *et al*., 2023). Seventy-five percent of maize farming in Kenya is carried out by small-scale farmers who produce over sixty-five percent of the total maize consumed (Tarus, 2019). Maize demand in Kenya is increasing with a rise in population (at a rate of 2.2% annually), economic growth, and urbanization (Aoko et al., 2024). A slight increase in maize production was registered in 2015, 2018, and 2020; however, this was followed by a decline in the subsequent years (Figure 2.1). Kenya has also continued to produce low maize yields of less than 2 t ha^{-1} for 10 years (Figure 2.1), which is far below the expected yield of 6t ha^{-1} (Akuriba, 2021).

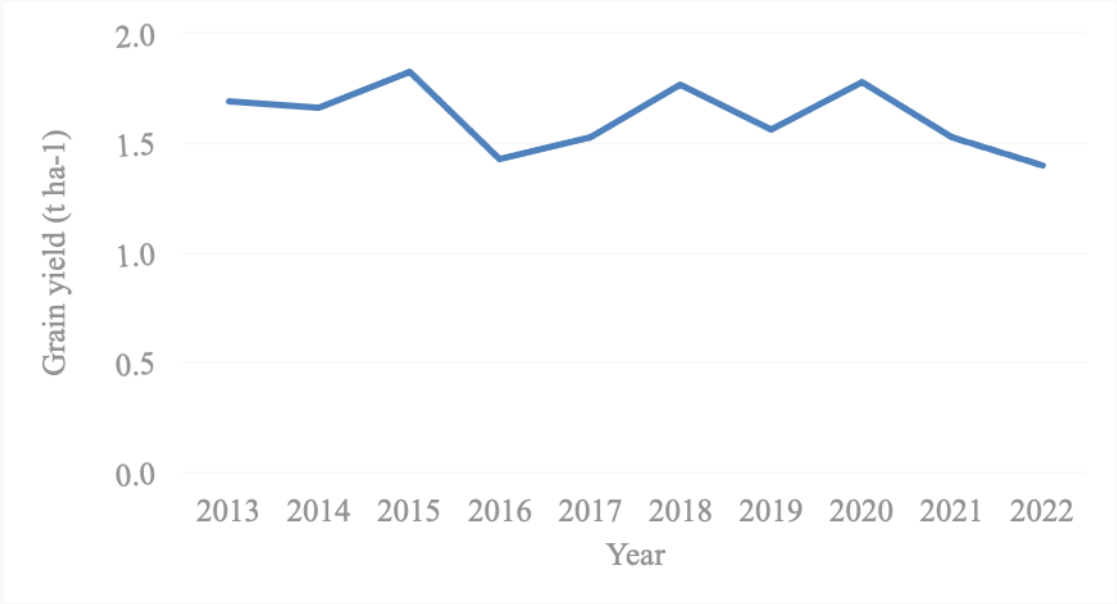


Figure 2.1. Maize yield (t ha^{-1}) in Kenya between 2013 and 2022.

Source: (FAOSTAT, 2024)

Similar reports indicate that maize production reduced by 6.5% from 2021 to 2022 (from 36.7 to 34.3 million bags) (EPZ, 2022), indicating the need to increase maize production in Kenya without focusing on expanding the production area or relying on costly maize importation (Aramburu-Merlos *et al.*, 2024). Over time, fluctuation in maize production coupled with rapid population growth experienced in Kenya makes the supply lower than demand, prompting the country to import maize to meet the local consumption trends (Kang'ethe *et al.*, 2020). Increased maize importation may hurt Kenya's economy since resources meant for development may be used to procure the maize (Kang'ethe *et al.*, 2020). To ensure that the supply meets the demand, plant breeders need to produce high-yielding stress-resistant maize varieties (Njeru *et al.*, 2023).

In Kenya, there are six agro-ecological maize growing zones. These are tropics, dry mid-altitude, moist mid-altitude, dry transitional, moist transitional, and highland tropical zones (Figure 2.2) (Ouma and De Groote, 2011). The six zones were further grouped into three based on their yield production (De Groote *et al.*, 2020).

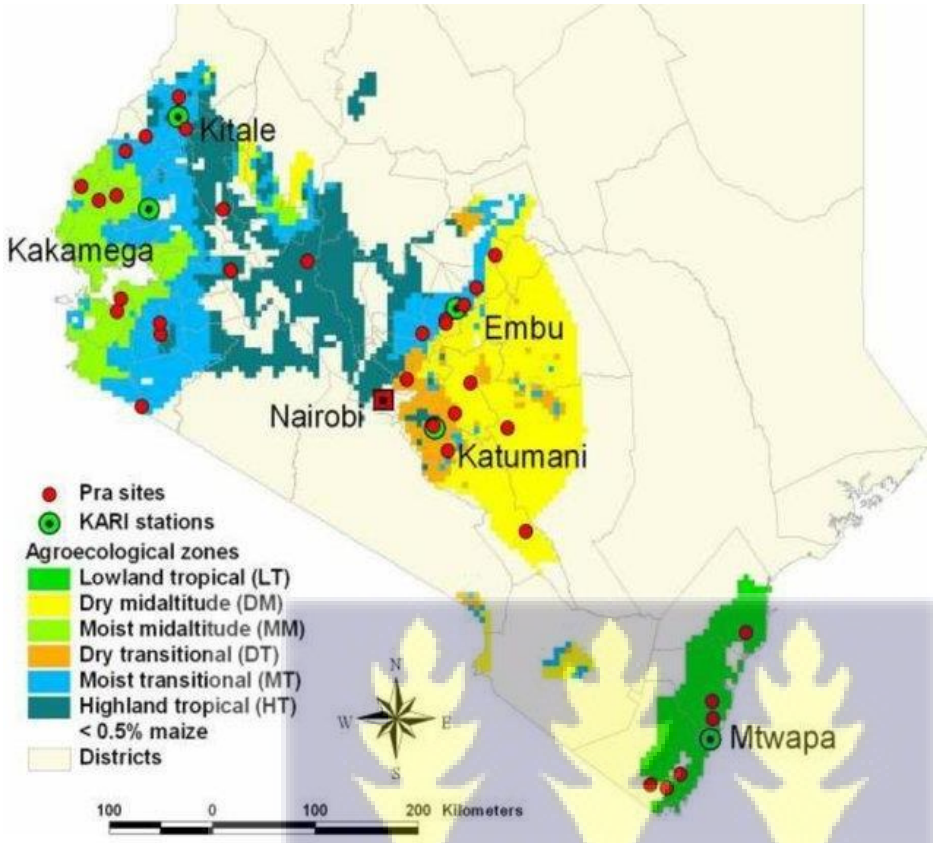


Figure 2.2. Agro-ecological map of Kenya showing the major maize-growing locations.
Source: Ouma and De Groote (2011).

Data from national survey carried out on maize farmer based on adoption of recommended varieties (late maturing, medium maturing, dryland materials (drought tolerant) and lowland tropical materials), length of growth season and farmer's planting strategies combined with spatial data on levels of precipitation, temperature and elevation were used to group the 6 agroecological zones into 3 categories (De Groote *et al.*, 2020). These groups are: i) lowland tropics found in the coast, mid altitude, and dry transitional zones located within Machakos, ii) highland tropics found in western and central parts of the country, and iii) the moist transitional zones bordering them on the west and east and the Lake Victoria moist mid altitude zones (De Groote *et al.*, 2020).

The first group, consisting of the lowland tropics, is found in the coast, mid-altitude, and dry transitional zones located around Machakos and covers 50% of the total maize production area. These zones are generally low-yielding (around 1 t ha^{-1}) and contribute only 30% of the total maize harvested. The second group, composed of the highland tropics, is found in the central and western parts of Kenya and the moist transitional zones. These zones are characterized by high yields of above 2.5 t $ha^{-1,}$ accounting for about 50% of the total maize harvested in Kenya, and cover 30% of the total maize production area. The third group is the Lake Victoria moist mid-altitude zones, which are characterized by moderate yields of 1.5 t ha^{-1} (De Groote *et al*., 2020).

Kenya has 2 maize cultivation seasons determined by the rainfall pattern experienced by the country. The main season, which is defined by long rains, occurs in March, April, and May, and the minor season is characterized by short rains in October, November, and December. The amount of rainfall received varies from one agro-ecological zone to another. Different agro-ecological zones produce maize during different seasons. In the highlands, 99% of the maize is cultivated during the main season, while 49% of maize in the moist transitional zones is grown in the minor season (De Groote *et al.*, 2020). A decline in maize production has been observed in these zones due to constraints faced by the farmers.

2.1.3 Tropical versus temperate maize

Tropical maize is cultivated in altitudes of between 26° north and 26° south, while temperate germplasm grows in latitudes above 26° north and below 26° south (Taba and Twumasi-Afriyie, 2008). Tropical maize is adapted to shorter day lengths, while temperate maize is adapted to longer day lengths (Taba and Twumasi-Afriyie, 2008). Tropical germplasms are generally high-yielding, producing an average of 7.2 t ha^{-1}, compared to tropical germplasms whose average yield is 3.3 t ha^{-1}. However, a maize yield increase of 2.3% per annum has been observed in the

tropical regions and 1% in temperate zones (Campos and Caligari, 2017). Additionally, maize genotypes with a temperate background are stable compared to those from the tropics, and can be used to improve maize stability during the breeding process (Nyoni *et al.*, 2023). Tropical maize landraces have native host plant resistance to pests and diseases due to their evolution under natural selection. They have morphological adaptations, such as having numerous leaves with heavy husks to protect them against insects and birds (Campos and Caligari, 2017). This makes tropical germplasm a diverse source of genes for resistance to biotic stresses and could be used to improve Temperate maize germplasms, which are usually susceptible to pests and diseases (Abadassi, 2014).

Tropical maize varieties are usually taller than temperate germplasm, have an ear height/plant height ratio of 0.65, and have huge tassels to ensure enough pollen production. This makes them unstable and more susceptible to lodging (Campos and Caligari, 2017). Tropical maize produces a low-moderate number of kernels (300–400) per plant, and rapidly germinates when sown. Furthermore, they have a harvesting index of between 0.25 and 0.40, and the plants may become unfertile when they encounter stress during the flowering phase (Campos and Caligari, 2017). On the other hand, temperate maize hybrids have a harvesting index of 0.5–0.55, display vigorous ear growth, and have strong stalks, making them stable and resistant to lodging. They have smaller but more erect leaves, small tassels, and big ears with approximately 500–600 kernels per ear, hence high yielding and very fertile (Campos & Caligari, 2017). Tropical germplasms are late maturing and can be used to prolong maturity in a breeding program, while temperate germplasms are early maturing and can introduce earliness in flowering during crop improvement (Nyoni *et al*., 2023). Genetic diversity is important for crop improvement; breeders have attempted to broaden the genetic base of temperate germplasm using tropical maize, but are faced with a major limitation of photoperiodic sensitivity, slowing down the process (Campos

and Caligari, 2017). However, temperate maize has been used to improve important agronomic traits such as yield increment, ear size, and characteristics of the tassels (Kosgei *et al*., 2025).

2.1.4 Limitations to maize cultivation in Kenya

Maize yield produced by smallholder maize farmers is approximately 1.8 t ha^{-1}, which is far below the expected potential yield of six tons per hectare (Munialo *et al*., 2020). The difference in yield gaps observed is over 50% making most families food-insecure (Munialo *et al*., 2020). Constrains faced by maize farmers in Kenya include: high cost and low availability of farm inputs, decreasing soil fertility, invasion of pests and diseases, poor coordination of extension services, climate change, use of uncertified seed by smallholder farmers, and lack of appropriate irrigation schemes (Kipkulei *et al*., 2024). A reduction in maize yield produced due to rising cost of fertilizers, drought, and other factors was estimated to result in a total of 4.1 million people in Kenya experiencing acute food insecurity, more so in Arid and Semi-Arid lands (ASAL) in 2022 (WFP and BCG, 2022).

Lack of proper timing of farm operation, such as pest and disease control, weeding and fertilizer application, and harvesting, may also contribute to reduced yields. This can be solved by adopting modern agricultural technologies such as Agricultural automation using Artificial Intelligence (AI) to identify nutrient deficiencies, pests and diseases, and weed infestations, resulting in efficient crop health management (Sow *et al*., 2024). In addition, smallholder maize farmers in Kenya have challenges in accessing credit facilities, failure of agricultural policies, poor infrastructure, a deficiency of appropriate skills, and a lack of manpower. These may be solved using digital (precision) agriculture (Gumbi *et al*., 2023). Farmers can use a weather mobile application on their phones to assess the weather conditions and determine the appropriate time for farm operations such as land preparation, planting, and harvesting. Kenya Agricultural and Livestock Research Organization (KALRO) has developed mobile applications that assist

farmers with systematic information on crop management. For example, the Organization has a Kenya Agricultural Advisory Platform (KAOP), an integrated online application using geographical data sourced from satellites to produce real-time information and advice for farmers based on their geographical location (Akuku, 2018). KAOP predicts temperature and rainfall levels and uses the data to generate plots based on the geographical information and provide weather observations. The application provides farmers with access to current information and recommendations for the exact place, for instance, on which crop to grow, how to prepare the soil, when to plant, how to water the crop, fertilization, methods of controlling diseases and pests, time to harvest, and proper storage facilities (Kropff *et al*., 2023). Climate change has adversely affected maize production in Kenya. Production is affected by unreliable rainfall, prolonged drought, erratic floods, and unpredictable temperatures experienced within the country during maize growing seasons (Mulungu and Ng'ombe, 2020). Most maize farmers depend on rainfed production; the crop is very sensitive to water deficiency throughout its productive phase (flowering and grain filling) (Woomer *et al*., 2024). A positive correlation between the amount of water available at this stage and grain yield harvested has been documented (Omoyo *et al*., 2015). Flooding results in nutrients leaching and waterlogging, which lead to low soil fertility, particularly for phosphorus and nitrogen. Maize yield losses also occur from reduced seed viability due to rotting during planting, which affects germination. Inadequate drying during the harvesting period results from high humidity, which is accelerated by rainfall, and enhances aflatoxin contamination of the grains (Woomer *et al*., 2024).

Key biotic factors affecting maize farming in Kenya are: a) maize diseases include maize lethal necrosis, turcicum leaf blight (TLB), grey leaf spot (GLS), common leaf rust, and maize streak virus (MSV), b) parasitic weeds, mostly striga, and c) insect pests like desert locusts, weevils, stemborer, and fall armyworm (Keno *et al*., 2018; Njeru *et al*., 2023). These biotic stresses can

cause up to 100% yield losses (Njeru *et al*., 2023).

2.2 Fall armyworm distribution and biology

2.2.1 Fall armyworm distribution

Fall armyworm is a Noctuidae pest native to North and South America and foreign to Africa (Keno *et al*., 2018). It is polyphagous in nature, infesting different crops such as sorghum, rice, forage grasses, sugarcane, cotton, turf grasses, and peanuts, but has a high preference for maize (Keno *et al*., 2018). The fall armyworm was first identified in 2016 in Nigeria, then spread rapidly within Africa (Table 2.1; Figure 2.3) (FAO, 2022).

Table 2.1. Distribution of fall armyworm in Africa

Country	Year detected	Country	Year detected
Benin	2016	Republic of Congo	2017
Nigeria	2016	Rwanda	2017
São Tomé et Príncipe	2016	South Africa	2017
Togo	2016	South Sudan	2017
Cameroon	2017	Swaziland	2017
Chad	2017	Uganda	2017
Tanzania	2017	Zambia	2017
Ethiopia	2017	Zimbabwe	2017
Ghana	2017	Angola	2017
Guinea	2017	Burundi	2017
Kenya	2017	South Africa	2017
Malawi	2017	Gabon	2018
Mozambique	2017	Mali	2018
Namibia	2017	Sudan	2018
Niger	2017	Somali	2018
Botswana	2017	Egypt	2019
Sierra Leone	2017	Mauritania	2020

Source: FAO (2020)

Figure 2.3. Fall armyworm status and distribution.
Source: FAO (2020).

2.2.2 Factors influencing FAW distribution and invasion in Kenya

Fall armyworm has an invasive nature, which helps the pest to spread to all the key maize-growing regions within the country, with variation in losses experienced depending on the agro-ecological zones (Mutyambai *et al*., 2022). High FAW infestation rates have been recorded in the lowlands and the lowest levels in the highlands (Mutyambai *et al.*, 2022). The amount of infestation and damage depends on: environmental conditions, the cultural practices in the area, topographical site of the farm, type of cultivar planted, and planting season (Kebede and Shimalis, 2021). Temperature is key for FAW mobility, metabolism, and host availability, thus determining the changes in the pest population (Kareem *et al*., 2022). Increased temperature enhances the developmental rate and number of generations of FAW, leading to a rise in the pest population (Nurzannah *et al*., 2020). Rainfall distribution and frequency may affect FAW reproduction, development, and survival. Heavy rainfall leads to a reduction in the FAW population since they may be washed away or drowned (Nurzannah *et al.*, 2020). FAW infestation decreases during rainfall seasons and increases when the rains reduce (Niassy *et al*., 2021). FAW is polyphagous in nature and feeds on above 80 species of plants (Nayyar *et al*., 2021). During rainy seasons, farmers tend to plant varieties of crops that provide a wide range of host plants and promote the spread of the pest (Nurzannah *et al*., 2020). A relationship has been established between maize crop phenology and fall armyworm infestation; there is a significant variation of adult FAW population density at all maize developmental stages (Niassy *et al*., 2021). However, larval infestation occurs mainly at the vegetative and reproductive phases (Niassy *et al*., 2021). In addition, agronomic and management practices used by farmers and the cultivated maize variety determine the level of FAW infestation (Mutyambai *et al.*, 2022). Variation in the amount of cellulose among different maize varieties may cause differences in germplasm resistance to fall armyworm (Rojas *et al*., 2018). Resistant genotypes contain more cellulose content than susceptible genotypes (Rojas *et al*., 2018). Cellulose contributes to making

the plant less palatable to the larvae by making it tougher and not easily digested or metabolized by the insect and as a result, fall armyworm larvae attracted to susceptible genotypes weigh twice as much as those attracted to resistant genotypes (Rojas *et al.*, 2018).

2.2.3 Life cycle of fall armyworm

Fall armyworm undergoes complete metamorphosis (Figure 2.4). These growth stages include egg, instar, pupa, and moth (adult) (Naharki *et al.*, 2020) and take 30 days to complete in warm temperatures and 60-90 days in cooler temperatures (Kebede and Shimalis, 2021).

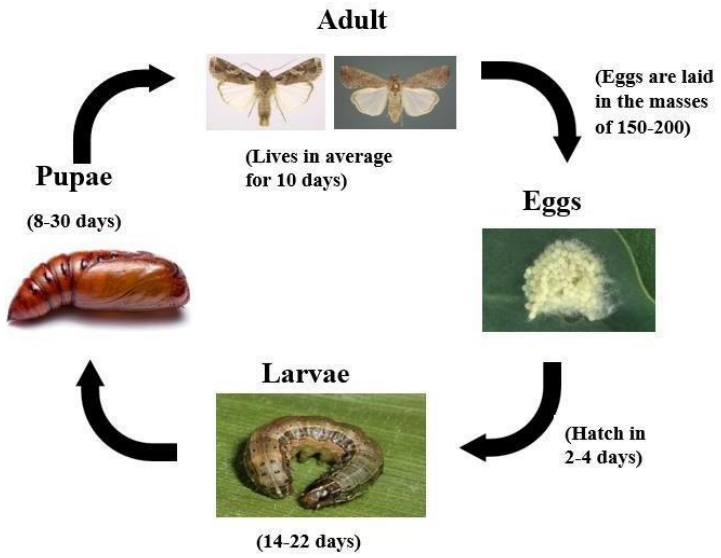


Figure 2.4 Life cycle of fall armyworm

Source: Naharki *et al*. (2020)

FAW does not experience a resting period, and its infestation occurs throughout the year in endemic regions. In non-endemic zones, fall armyworm migrates and invades when the environmental conditions are favorable and may undergo just one life cycle, then become extinct (Kebede and Shimalis, 2021). The fall armyworm adult moth lays about 100 to 200 eggs underneath the plant leaves. The eggs are protected by scales from the abdomen of the moth after oviposition and hatch into larvae, which cause major destruction to the maize plant (Naharki *et al*., 2020).

Young FAW larvae feed superficially, resulting in slightly transparent windows. The movement of the young larvae from one plant to the next can be facilitated by a silken thread woven by larvae, which, when blown by wind, transports them to the new plant (Harrison *et al*., 2019). The larvae feed on either young maize leaf whorls, tassels, or ears, while mature larvae can penetrate the maize plant stem and damage the whole plant. However, FAW larvae prefer foliar whorls in

tender shoots and cob silks in old plants and actively feed at night (Harrison *et al*., 2019). FAW leaf destruction indirectly influences the grain yield because it destroys leaves, which are responsible for photosynthesis (Prasanna *et al*., 2018). The larvae can burrow into the soil (2-8 cm) during pupation and transform into a 20- 30 cm oval-shaped cocoon composed of loose silk. The pupal stage takes between 8 and 9 days to transform into an adult moth (FAO, 2020). The growth rate of fall armyworm larvae in resistant maize genotypes is significantly lower than in susceptible genotypes (Rojas *et al*., 2018). Early maturing maize cultivars experience less damage from the pest through the escape mechanism (Rojas *et al*., 2018). As the maize plant matures, its level of hemicellulose increases, resulting in reduced feeding rates, oviposition, and survival rates (Abrahams *et al*., 2017).

2.3 Economic effect of fall armyworm on Maize in Kenya

Maize infestation by FAW causes economic yield losses (Overton *et al*., 2021). The pest distorts crop growth, reduces yield, and may result in entire crop failure if no effective control measures are undertaken (Kareem *et al*., 2022). FAW infests plants at all their developmental stages (Kebede and Shimalis, 2021). However, severe damage is experienced at the early growing phase, and maize ears can be extremely damaged under heavy infestation, leading to massive yield loss (Kareem *et al*., 2022). Fall armyworm destruction of the ears may expose the grains to damage by other pests, pathogens, and diseases. Mature larvae can cut the entire base of the stem, destroying the maize plant (Kebede and Shimalis, 2021). Fall armyworm can cause yield losses of between 8.3 and 20.6 million metric tons annually if left uncontrolled (Kebede and Shimalis, 2021; De Groote *et al*., 2020). In Africa, FAW infestation in maize farms results in 11-58% yield losses, causing an annual revenue loss of up $9.4 billion, while in SSA, crops worth approximately $13 billion annually are at risk of being damaged by fall armyworm (Kansiime *et*

al., 2023). In Kenya,

yield losses of 1381 Kg $ha^{-1,}$ translating to 47% of the total yield have been reported (De Groote et al., 2020). Fall armyworm interferes with regional and international trade due to the phytosanitary measures required by maize-importing countries (Overton *et al*., 2021). High economic losses are experienced because small-scale farmers have limited knowledge of good farming practices, farm inputs, technologies, FAW control methods, and inadequate financial resources needed for scouting and response to invasion (Kansiime *et al*., 2023).

2.4 Fall armyworm management practices

Fall armyworm is a significant pest in maize farming, and it is important to develop a well-coordinated, flexible, and effective method for managing the pest. These can be achieved by incorporating the Integrated Pest Management (IPM) approach (Prasanna *et al*., 2018). An effective IPM method incorporates cultural, biological, chemical, and plant host resistance methods for FAW management (Prasanna *et al*., 2018). Small-scale maize farmers experience financial constraints that predispose them to rely on cultural approaches for management of FAW (Hruska, 2019). These cultural approaches include early planting, intercropping, use of early maturing cultivars, rotational farming, exhaustive ploughing, hand picking of the eggs and larvae, application of soil, sand, tobacco extracts, sawdust, ash, or lime in the maize whorl to kill the larvae (Kebede and Shimalis, 2021; Hruska, 2019). Even though cultural methods are easy to use and require less expertise, they are laborious and not practical for small-scale farming.

Biological methods, including using natural enemies like braconids, tachinids, and mermithids, have contributed considerably to the reduction of FAW population in maize farms (Bateman *et al*., 2018). Entomopathogens (nematodes, fungi, and bacteria) have been used to produce bio-

pesticides for fall armyworm control. Biological methods are safe due to their low levels of environmental toxicity. However, their efficacy levels are low and are sensitive to non-target specific chemicals, which lead to their elimination and reduction of natural enemies and beneficial microorganisms (Hruska 2019; Bateman *et al*., 2018). Natural enemies are limited and not affordable to smallholder farmers (Kasoma *et al*., 2020). Chemical control is effective in fall armyworm control; however, its drawbacks outweigh its benefits. However, chemical controls pose a human health hazard caused by pesticide poisoning. Moreover, the cost-effective chemicals present in the market may not be effective due to pest resistance (Nuambote-Yobila *et al*., 2023). *Bacillus thuringiensis (Bt)*- based host plant resistance has been used for the management of FAW in maize (Wan *et al*., 2021). The transgenic maize was developed by introducing *cry* (crystal protein) genes from Bt (*Bacillus thuringiensis*) into the varieties (Prasanna *et al*., 2018). Genetically modified Bt maize has been developed that confers resistance to fall armyworm but faces stringent policy controls for fears and controversies associated with it (Prasanna *et al*., 2018). The Water Efficient Maize for Africa (WEMA) project in some African countries developed improved maize cultivars with *Bt* resistance for smallholder farmers (Prasanna *et al*., 2018). In Kenya, Uganda, Tanzania, South Africa, and Mozambique, the project developed *Bt* maize (MON 810) that confers strong resistance to stem borers and partial tolerance to FAW, waiting for the release (Prasanna *et al*., 2018). However, FAW resistance to *Cry1F* toxins in transgenic maize has been documented in Puerto Rico, southeastern mainland USA, and Argentina (Van Den Berg *et al*., 2021). Fall armyworm biotypes have developed resistance to *CryF1*, *Cry1A*.105, and *Cry2Ab2* in South Africa (Asare *et al*., 2023). The breakdown in resistance is faster in transgenic plants than in plants with native host plant resistance. This results from transgenic insect resistance being either monogenic or oligogenic, hence the pest can evolve to fast overcome the resistance of a few genes compared to native resistance, which is

polygenic and therefore more durable (Prasanna *et al.*, 2022). When breeding for resistance, more preference is given to partial resistance as compared to complete resistance. Since partial resistance confers horizontal resistance, and the pest may take a long time to break during its evolution, hence the durability (Matova et al., 2020). Host plant resistance breeding should seek to find, use, and combine multiple resistant traits to strengthen the resistance (Prasanna *et al.*, 2021).

2.5 Breeding for host resistance to fall armyworm

Host plant resistance (transgenic or native) is an important element of IPM strategy (Prasanna *et al.*, 2018). HPR is the intrinsic ability of a plant to resist, avoid, tolerate, or recover from pest attack in conditions that would have otherwise caused greater economic injury (Kasoma *et al.*, 2020). Host plant resistance may offer a safe, cost-effective, and environmentally friendly method of controlling fall armyworm. Progress has been made over the past decades in developing maize genotypes with natural HPR through germplasm evaluation and selection (Viana *et al.*, 2022). The International Maize and Wheat Improvement Center (CIMMYT) has developed maize germplasms with multiple borer resistance (MBR) and multiple insect resistance tropical (MIRT) (Prasanna *et al.*, 2022). Temperate maize inbred lines with resistance to FAW were developed in the USA. These included Mp704; Mp705, Mp706; Mp707, Mp708, Mp713; Mp714, and Mp716 genotypes (Prasanna *et al.*, 2018). CIMMYT has also identified and developed FAW-resistant lines, comprising inbred lines that had earlier been identified at CIMMYT-Mexico and validated in Kenya through artificial screening, and some CIMMYT lines (CMLs) found to be resistant to FAW in Kenya. These lines were recommended for use in breeding programs as donors for FAW-resistant genes (Prasanna *et al.*, 2022). However, there is limited information on maize cultivars conferring native host resistance to fall armyworm (Matova *et al.*, 2020). And therefore, studies are still ongoing to screen for native FAW resistance within available African maize

germplasm (Prasanna *et al*., 2018). The International Maize and Wheat Improvement Center (CIMMYT) recommends vigorous screening of African-adapted maize inbred lines, commercial hybrids, and improved open-pollinated varieties (OPVs) for resistance to fall armyworm under artificial infestation to identify resistant genotypes (Prasanna *et al*., 2018). Resistant maize varieties have been reported to have higher yields of better quality than susceptible ones under similar levels of fall armyworm infestation (Sandhu and Kang, 2017). There is limited information on FAW resistance among tropical maize germplasm in Africa, and there is a need for large-scale assessment of African-adapted maize germplasm for resistance to the pest (Kasoma *et al*., 2020).

Maize breeders can achieve maximum genetic gain when there is adequate genetic diversity within a locally adapted breeding population, since the local germplasm can be introduced directly into the pre-breeding program, and won't need the extra time required for adaptation to the environment (Carena, 2021). The screening of African-adapted tropical inbred lines will help identify diverse sources for FAW resistance genes that can be used to develop resistant varieties or improve farmer-preferred high-yielding but susceptible varieties.

2.5.1 Sources of genetic diversity for host plant resistance to fall armyworm

Plant breeders use genetic diversity to improve desirable traits in crops. Genetic diversity is the variation of genetic traits within a crop variety or species (Salgotra & Chauhan, 2023). Genetic diversity can be evaluated using phenotypic, biochemical, or molecular markers (Govindaraj *et al*., 2015). Morphological markers are based on assessing the visual traits and are easy to use. However, morphological markers are prone to phenotypic plasticity (Govindaraj *et al.*, 2015). Biochemical markers use isozymes, which are limited in number and also structurally complex, thus limiting their exploration. These limitations can be overcome by using molecular markers

that are stable, available in all tissues, and not influenced by environmental, epistasis, and pleiotropic effects (Govindaraj *et al*., 2015). Assessment of molecular genetic variation can be used to determine if the morphological variation observed reveals the genomic differentiation patterns (Govindaraj *et al*., 2015). Genetic diversity provides a platform for the selection of plants with desirable traits. Varieties with high genetic diversity and a broad genetic base adapt better, are more tolerant to biotic and abiotic factors than others (Bhanu, 2017).

Sub-Saharan Africa is one of the centers of diversity for tropical and subtropical maize germplasm, which has developed stress adaptation genes through evolution (Dube *et al*., 2023). Initial stages of phenotyping include numerous genetically diverse lines, which increases the chances of identifying and selecting desired lines to achieve high response to selection (Kasoma *et al*., 2020). Determining the genetic diversity that exists within tropical maize inbred lines could help in grouping inbred lines into heterotic groups according to their relationship. This enables breeders to select parental lines from different heterotic groups for hybrid formation, for the achievement of maximum heterosis (Dube *et al*., 2023). The discovery of new sources of FAW resistance genes in tropical maize germplasm and the knowledge of the mechanism regulating resistance generate a novel way for exploring host plant resistance for maize improvement (Asare *et al*., 2023).

When screening germplasm for resistance to FAW, an insectarium is required for FAW larvae mass production and facilities for infestation, maize screening, and selection (Kasoma *et al*., 2022). USDA-ARS researchers developed protocols for artificial screening of maize germplasm for resistance to FAW (Prasanna *et al*., 2022). In Kenya, CIMMYT, in collaboration with KALRO, has a FAW mass rearing facility at KALRO Katumani. The facility rears about eight hundred neonates annually, which are used for artificial screening of germplasm for resistance to

FAW under artificial infestation at KALRO Kiboko, Kenya (Prasanna *et al*., 2018). Artificial infestation screenhouses are also available at KALRO Kiboko for FAW evaluation trials for lines and hybrids (Prasanna *et al*., 2018). Screening for resistance to FAW can be conducted under artificial infestation in controlled environments or under natural infestation in hotspot areas. The disadvantage of natural infestation is that pest escape might occur instead of host resistance, leading to overestimation of resistance and confounding bias in selection. As a result, susceptible genotypes might be selected, resulting in a low selection response, thereby increasing the cost of breeding (Kasoma *et al*., 2022). It is therefore necessary to perform artificial screening of FAW in a screenhouse with optimal environmental conditions to enhance host–pest interaction. During screening, an equal number of larvae is placed on each plant to avoid biases. This allows for accurate observations on pest development and the response of the host plant, hence providing great accuracy in selection and efficacy (Kasoma *et al*., 2022).

2.5.2 Mechanism of Host Plant Resistance

Host plant resistance is the ability of a plant to withstand or repel attack from pests (Viana *et al*., 2022). Insect resistance breeding program aims at developing genotypes that are resistant to pests and maintain or enhance agronomic traits such as yield. Thus, one needs to understand the pest's reproductive and development cycle, rearing, infestation, methods of evaluating pest damage on the plant, screening and selection of resistant plants, and the mechanism of inheritance of the resistant genes (Viana *et al*., 2022). Understanding mechanisms involved in host plant resistance helps in the selection of superior genotypes and increases the breeding program efficiency (Nuambote-Yobila *et al*., 2023).

Mechanisms of HPR include antibiosis, tolerance, and non-preference (antixenosis). Antibiosis refers to the effect of resistant plant cultivars on the insect's life cycle and physiology, which includes their survival, growth, and fertility (Sandhu and Kang, 2017). Tolerance is the ability of

the host plant to perform economically well in terms of yield quantity and quality despite insect attack compared to the non-tolerant variety due to their stability (Sandhu and Kang, 2017). Tolerant plants can withstand insect attack and damage with minimal effect on their economic production levels. Non-preference is where the behavior of the insect is influenced by certain host plant traits such that the pest will infest one cultivar and not the other (Sandhu and Kang, 2017). HPR mechanism to fall armyworm was observed in AM 013, RO 009, and MA 002 maize genotypes in Brazil through antibiosis, where low larval viability was observed, and through antixenosis in RR 168 and PA 110 genotypes, which recorded low larval leaf consumption (Ligia *et al*., 2016). Maize crops recover from FAW damage during the rapid growth phase, yet late detection of the pest infestation and severe damage levels may be irreversible (Mutyambai *et al*., 2022). Maize varieties have been reported with foliar damage scores ranging from 3.2 to 5.3 on the Davis damage scale (Mutyambai *et al*., 2022). For an effective HPR to FAW to be achieved, there is a need to determine the gene action involved in the resistance mechanism.

Mechanisms of FAW resistance may vary on the maize leaves, silk, and ear. For instance, CIMMYT- Kenya identified inbred line CML71 to have shown significant FAW resistance to leaf damage through antibiosis and antixenosis mechanisms, but no resistance was observed on the silk and ear. Whereas inbred line CKSBL10008 showed significant resistance through antibiosis on leaves and some antixenosis for feeding on leaves, silks, and ears (Nuambote-Yobila *et al*., 2023). Host plant resistance is only important if it is heritable, polygenic, durable, and measurable by a standard enabling distinguishing resistant cultivars from susceptible ones (Prasanna et al., 2018). This shows the need to determine the mode of inheritance of the genes governing the FAW resistance trait.

2.6 Gene Action, combining ability

Gene action refers to the performance or expression of genes in a breeding population and can be

classified into additive or non-additive (dominance and epistasis) components (Acquaah, 2012). Knowledge of gene action, the number of genes governing a certain trait, and their inheritance is key for any crop improvement breeding program to succeed (Rather and Deo, 2018). Quantitative traits are controlled by polygenes with minor additive effects and are defined by their gene action instead of the number of genes coding for the trait (Acquaah, 2012). Additive gene action occurs when the addition of a single gene in the same locus increases the phenotypic expression of a trait and can be measured (Acquaah, 2012). Breeders mainly focus on quantitative traits with additive gene effects because they are heritable and can be fixed. Dominance component of non-additive gene action is experienced when heterozygotes have more resemblance to one parent than the other, making it difficult for breeders to distinguish phenotypically between homozygotes and heterozygotes (Acquaah, 2012). Epistatic gene action is brought about by the interaction of alleles from different loci, leading to the phenotypic expression of one gene at a particular locus being masked by that of another gene at a different locus (Acquaah, 2012).

Combining ability is an evaluation of the value of parental genotypes based on their progeny's performance (Fasahat, 2016). It is the ability of parents to combine during crossing and allow the transmission of desirable genes to the offspring (Fasahat, 2016). Combining ability is classified as general and specific combining ability (Tesfaye *et al*., 2019). General combining ability (GCA) is the average performance of lines in all possible crosses. In contrast, SCA measures the deviation of hybrid performance from the expected average performance in a hybrid combination. GCA results from additive gene action, while SCA is due to non-additive gene action (dominance and epistatic gene effect). GCA is important in hybridization and selection of germplasm to be used in a breeding program for population improvement, whereas SCA is crucial in hybrid formation (Temesgen, 2021).

Determination of the mode of gene inheritance will allow breeders to maximize the genetic potential of inbred lines for optimal heterosis. Performance of hybrids is determined by genetic variation, mode of gene action governing the trait of interest, and its heritability (Azmach *et al*., 2021). Maize inbred lines may be classified into heterotic groups based on the SCA using information from either the line pedigree or the hybrid. Maize improvement programs depend on the identification and use of heterotic groups and patterns (Bari *et al*., 2016). Heterosis is maximized when inbred lines from different heterotic groups are crossed (Bari *et al*., 2016).

The gene action governing insect pest resistance in maize is mainly additive. Therefore, breeding methods like recurrent and mass selections can be used to accumulate the genes desirable for insect resistance (Viana *et al*., 2022). Recurrent selection has been used for increasing levels of phenolic concentrations, causing resistance to the Mediterranean corn borer and Stemborers in Africa, whose main gene effect is additive. Both additive and non-additive gene action have been described to

condition resistance to maize weevil due to highly significant GCA and SCA observed for the trait (Amissah *et al*., 2019). Maize inbred lines with desirable GCA effect for FAW resistance have been reported, indicating the trait is partly regulated by additive gene action and therefore can be used for population improvement and development of FAW-tolerant maize hybrids (Kamweru *et al*., 2023; Job *et al*., 2022). On the contrary, FAW damage-related traits have been reported to be governed by non-additive gene action and can be improved by heterotic breeding. It was observed that genetic recombination among susceptible genotypes may give rise to progenies with moderate resistance (Kasoma *et al*., 2021). However, there is limited information on the gene action governing resistance to FAW in tropical maize inbred lines.

Gene action can be deduced from combining ability analysis of the inbred lines using a mating design such as biparental mating, triple testcross, pure line progenies, polycross, diallel mating

designs, line x tester North Carolina designs (Awata *et al*., 2018). The current study used North Carolina Design II (NCDII) to estimate the gene action regulating FAW resistance. The North Carolina (NC) mating designs allow the determination of additive and non-additive variance components of gene action using the information derived from half-sib (HS) families (Awata *et al*., 2018). North Carolina Design II is a factorial mating design where every group of males is mated to every single group of females, generating half-sib families (Muthoni and Shimelis, 2020)

every progeny family has half-sib relationships through both a common male and a common. In this mating design, the variance components of the males and female parents are equivalent to the GCA effects, while those of the cross (males x females) represent the SCA effect (Muthoni and Shimelis, 2020). Consequently, parental mean squares (males and females) estimate the additive variance, while the mean square for interaction (males x females) determines the non-additive (dominance) variance component (Awata *et al*., 2018). The advantages of NCDII are: more parents can be used in the breeding nurseries by dividing them into subsets depending on the availability of resources, dominance variance can be estimated directly from the mean square, and two independent additive variance components from the males and females can be determined (Awata *et al*., 2018). Breeding value is the GCA effect of a trait. When determining the gene action regulating FAW resistance in maize, parental lines with significant negative GCA effects are selected for further improvement or introduction into a breeding program as sources of FAW-resistant genes. Negative GCA effect indicates that the damage due to FAW infestation will be low, showing that the plants are resistant to the pest (Williams *et al*., 2018). Conversely, highly significant positive GCA and SCA effects for FAW show that the genotypes

are susceptible to the pest and should be selected against (discarded) (Karaya *et al*., 2009).

2.7 Heritability

Heritability is the percentage of the overall phenotypic variance attributed to the mean gene effect. phenotypic variance results from the genotypic expression of an organism, environmental effects, and the interaction of the genotype with the environment. The genotypic variance can further be divided into additive + dominance, + epistasis (Schmidt *et al*., 2019). Heritability explains the extent to which a trait is genetically determined and the degree of resemblance between families (Majhi, 2020). Broad-sense heritability is estimated from the genotypic value, while the breeding value (additive value) is used to determine the narrow-sense heritability (Schmidt *et al*., 2019). Heritability is classified into low (below 30%), moderate (30–60%), and high (above 60%). Low narrow-sense heritability estimates indicate that the non-additive gene action is the most predominant in regulating the trait, and high heritability shows that the trait is mainly controlled by the additive gene effect (Agbowuro and Salami, 2021). Heritability estimates can be obtained in NCDII by pooling the mean squares of males and females (additive gene effects) (Awata *et al*., 2018). Understanding the genetic control and heritability of genes regulating resistance to FAW is a prerequisite for improving the trait in maize. Previous studies recorded moderate heritability estimates for FAW-resistant traits (Kasoma *et al*., 2021). Similarly, Kamweru *et al*. (2022) recorded similar observations for the FAW damage parameter.

2.8 Genotype by Environment interaction

Genotype by environment interaction (GEI) is the difference in the interaction of a genotype across locations (Yadesa, 2022). Assessment of genotype by environment interaction (GEI) for FAW resistance is key for selecting genotypes across locations. There are two types of GEI,

namely, cross-over and non-cross-over. Cross-over GEI arises when the performance of a genotype varies across different environments, while non-cross-over occurs when the performance is consistent across different environments (Yali, 2022). Genotype by environment interaction may result in some genotypes changing ranks in their performance on quantitative traits like yield and FAW resistance across environments (Teressa *et al*., 2021). Phenotypic variance of a quantitative trait can be partitioned into genotypic variance (δ^{2G}), environmental variance (δ^{2E}), and genotype-by-environment interaction variance ($\delta^{2G \times E}$). GEI variance can further be divided into additive-by-environment interaction variance, dominance-by-environment interaction variance, and epistatic-by-environment interaction variance (Begna, 2022).

Studies by Badji *et al*. (2020) observed a highly significant GEI for resistance to FAW, indicating that the genetic expression of the trait is highly influenced by the environmental factors. Significant GEI lowers the heritability of a trait, implying that the trait is mostly controlled by environmental factors rather than genetic factors (Badji *et al*., 2020). Phenotypic plasticity is usually observed in maize in response to FAW damage in multilocational trials. Inbred lines with natural FAW resistance to leaf damage are often underscored when screening germplasms in different seasons (years) due to the strong influence of GEI (Kamweru *et al*., 2022). Multilocational trials for resistance to FAW could be used to identify stable genotypes across and within the environments (location, year, and season) and the findings used in making breeding decisions (Badji *et al*., 2020). Multilocation trials are necessary for quantifying hybrid stability and adaptability for yield and FAW resistance since the quantitative traits are highly influenced by the environment (Chandel *et al*., 2019). Genotype by environment interaction may limit response to selection because genotypes may respond differently in different environments (Ngailo *et al*., 2019). This shows that determining the GEI is important in maize breeding. However, there is limited information on the genotype-by-environment effect on resistance to

FAW in maize.

2.0. Genome-wide association studies of tropical maize for resistance to FAW

The rapid spread of FAW increases the need to identify the genomic regions and genes governing FAW resistance in tropical maize germplasm (Warburton *et al*., 2023). Understanding the genetic basis of FAW resistance is vital when breeding for HPR to the pest (Badji *et al*., 2020). The efficiency of breeding for crop improvement may be enhanced by the incorporation of linkage mapping, genomic selection, and genome-wide association studies (GWAS) with conventional breeding (Kamweru *et al*., 2022). GWAS is one of the most advanced modern breeding technologies for mapping the genomic regions of maize associated with FAW resistance (Badji *et al*., 2020). The advantage of GWAS over biparental QTL analysis is that the technology incorporates the high differentiation and several recombination histories present in the existing population to narrow down the resolution of the QTL to the nucleotide level, thereby allowing increased statistical power (Badji *et al*., 2020). GWAS is a fast and efficient way to detect genetic diversity within maize germplasm (Ma and Cao, 2021).

GWAS and metabolic pathway analysis have been successfully used to identify genes responsible for multiple insect resistance (Badji *et al*., 2020), corn earworm resistance (Warburton *et al*., 2018), and FAW damage in maize (Warburton *et al*., 2023; Kamweru *et al*., 2022). CIMMYT identified 22 SNPs that were significantly associated with FAW leaf damage and a single SNP associated with ear damage when GWAS was carried out on maize inbred lines for FAW tolerance (B. Prasanna *et al*., 2021). Similarly, GWAS has further identified more SNP markers associated with FAW resistance traits in maize (Warburton *et al*., 2023; Kamweru *et al*., 2022; Badji *et al*., 2020). These studies provide evidence that GWAS can be used to identify the SNP markers and genes governing FAW resistance in tropical maize germplasm. However, few

GWAS have been carried out on FAW resistance, and hence there is limited information on the genetic architecture of native genetic resistance to FAW in tropical maize germplasm (Prasanna *et al.*, 2021). Consequently, more GWAS studies should be carried out on African-adapted maize germplasm to identify the resistant genes

CHAPTER THREE

3.0 GENETIC DIVERSITY AND POPULATION STRUCTURE OF TROPICAL MAIZE INBRED LINES FOR RESISTANCE TO FALL ARMYWORM

3.1 Introduction

Maize (*Zea mays* L., 2n = 20) is a staple food in Africa and provides above 30% of annual caloric intake to over 300 million people in the region (Kasoma *et al.*, 2020). In Kenya, maize contributes significantly to food, feed, nutrition security, and the livelihood of millions of small-scale farmers. Maize is produced on 2.1 million ha of land and grown by over 3 million small-scale farmers in Kenya (FAOSTAT, 2024). However, production is below the expected yield potential of 6 t ha^{-1}, possibly due to challenges such as negative effects caused by climate change, emerging pests, and diseases (Njeru *et al.*, 2022).

Fall armyworm is a key pest in maize farming due to the significant economic yield losses associated with it (Sisay *et al.*, 2019). In Kenya, about 47% of FAW infestation resulted in yield losses of 1381 kg ha^{-1} (De Groote et al., 2020a). Integrated Pest Management (IPM) methods used for controlling FAW consist of biological, cultural, chemical control, and host plant resistance (HPR) (Matova *et al.*, 2020).HPR is an integral part of the FAW IPM strategy because it may offer a safe and affordable way of managing the pest (Viana *et al.*, 2022). The level of genetic diversity existing in a crop determines its ability to resist pests. Determination of the degree of genetic diversity in and between breeding populations is key to the success of any crop improvement program (Adu *et al.*, 2024).

Maize reproduces sexually through cross-pollination, hence creating a high amount of genetic diversity in the morphological characters like flowering days, maturity period, plant height, ear and kernel features, and variation on their response to diverse weather conditions (Patel *et al.*, 2024). Diversity is the basis upon which evolution and breeding originate. Development of a

breeding and conservation program requires a comprehensive understanding of genetic diversity (Kanaka *et al*., 2023). Genetic diversity studies in the maize breeding program will assist in grouping inbred lines into different heterotic groups based on their relatedness. This information is important for the selection of parental lines for crop improvement of different beneficial traits and for genetic base improvement of the breeding population (Ayesiga *et al.*, 2023). The analysis of genetic diversity and population structure of various populations shows the significance of heterozygosity, inbreeding, and crossbreeding on selection.

Genetic diversity in maize can be assessed through phenotypic and genotypic characterization (Aci *et al*., 2018). However, phenotypic polymorphism is unstable because it is prone to environmental changes and variation in a plant's developmental stages (Patel *et al.*, 2024). Genotypic characterization using DNA-based molecular markers is highly preferred for diversity and population structure studies because they are stable, polymorphic, and not influenced by the environment (Aci *et al*., 2018). Different types of molecular markers have been used for diversity studies. These include single-nucleotide polymorphisms (SNPs), random amplified polymorphism DNA (RAPDs), restriction fragment length polymorphism (RFLPs), simple sequence repeats (SSRs), and amplified fragment length polymorphism (AFLPs) (Ayesiga *et al*., 2023). Previous genetic diversity studies were based on low-throughput molecular markers such as RAPDs, RFLPs, SSRs, and AFLPs. However, currently, the high-throughput genotyping platforms, such as genotyping by sequencing (GBS) and single-nucleotide polymorphisms (SNPs), are applied in diversity analysis. This is because they are bi-allelic and are expressed at a much higher frequency in the genome compared to other molecular markers (Adu *et al*., 2024). SNP markers have the potential and are effective in examining genetic diversity, purity, and population structure of different maize varieties, particularly the maize inbred lines (Adu *et al*., 2024).

The study of genetic diversity among populations of inbred lines is crucial in determining the best breeding approaches to undertake. For instance, parental lines are selected for hybrid formation to maximize heterosis and develop new inbred lines (Badu-Apraku *et al.*, 2021). The level of heterozygosity and the inbreeding coefficient of inbred lines determine the genetic purity and degree of homozygosity in a breeding population (Josia *et al.*, 2021). Studies on genetic diversity and population structure of maize inbred lines developed by CIMMYT and IITA reported that most lines had genetic purity of less than 95% and therefore further inbreeding or purification was needed to increase the levels of homogeneity (Wegary *et al.*, 2019).

There is limited information documented on genetic diversity, purity, and population structure of African Adapted maize germplasm, and most of the studies were based on low-density markers and small samples (Wegary *et al.*, 2019). The use of low-density markers reduces the accuracy in identifying the genetic diversity existing within a population and does not give a correct estimate of the heritability of a trait (Kriaridou *et al.*, 2020). Therefore, the current study focuses on using the highest density SNP markers. Genetic diversity of a crop determines how it responds to insect infestation, for example. Consequently, the knowledge of the level of genetic diversity in and between populations is key for the success of a FAW resistance breeding program (Adu *et al.*, 2024).

Most commercial hybrid maize and cultivars in Africa are extremely vulnerable to infestation by FAW; hence, breeding efforts should be geared towards detecting new genes that are resistant to the pest (Singh *et al.*, 2022). Using genetically diverse germplasm in crop improvement is important for achieving durable resistance to FAW. This is because it will enable the development of genotypes with strong horizontal resistance that can cope with continuous pest evolution pressure (Job *et al.*, 2022). Breeding for FAW resistance requires the selection of donor parents that have

broad genetic diversity. Maize lines with moderately high genetic diversity and varying levels of FAW tolerance have been identified (Adu *et al*., 2024). Population structure analysis for FAW resistance in maize has successfully been used to assess genetic diversity among maize inbred lines in Zambia (Kasoma *et al*., 2020). However, information on the genetic diversity of the available maize population in Africa is still limited. Therefore, the objective of this study was to assess the genetic diversity and population structure of 140 tropical maize inbred lines with variable resistance to fall armyworm using phenotypic traits and DArTseq-based single-nucleotide polymorphisms (SNP) markers.

3.2 Materials and methods

3.2.1 Germplasm

A set of 140 maize inbred lines sourced from the International Maize and Wheat Improvement Center (CIMMYT) was selected based on their background of having multiple borer and multiple insect resistance traits. The full list of maize inbred lines used in the study and their pedigree information is indicated (Table 3.1).

Table 3.1. List of genotypes used in the study

Entry	Pedigree code	Entry	Pedigree code	Entry	Pedigree code	Entry	Pedigree code
1	CKSBL1008	36	CKL21597	71	CKL21650	106	CKL201328
2	CKL177012	37	CKL21598	72	CKL21651	107	CKL201330
3	CML610A	38	CKL21599	73	CKL21652	108	CKL201341
4	CML336	39	CKL21600	74	CKL21656	109	CKL201343
5	CML338	40	CKL21601	75	CKL21657	110	CKL201353
6	CML125	41	CKL21602	76	CKL21658	111	CKL201363
7	CML24	42	CKL21603	77	CKL21660	112	CKL201366
8	CKL201115	43	CKL21604	78	CKL21661	113	CKL201368
9	CKL201171	44	CKL21605	79	CKL21662	114	CKL201381
10	CKL201243	45	CKL21606	80	CKL21663	115	CKL201384
11	CKL201281	46	CKL21608	81	CKL21664	116	CKL201385
12	CKL201294	47	CKL21610	82	CKL21665	117	CKL201388
13	CKL201303	48	CKL21612	83	CKL21667	118	CKL201389
14	CKL201314	49	CKL21613	84	CKL21668	119	CKL201391
15	CKL21611	50	CKL21615	85	CKL21670	120	CKL201407
16	CKL21618	51	CKL21616	86	CKL21671	121	CKL201409
17	CKL21625	52	CKL21617	87	CKL21672	122	CKL201410
18	CKL21631	53	CKL21619	88	CKL21681	123	CKL201413
19	CKL201331	54	CKL21621	89	CKL21682	124	CKL201415
20	CKL201346	55	CKL21624	90	CKL21683	125	CKL201417
21	CKL21653	56	CKL21627	91	CKL21686	126	CKL201423
22	CKL21578	57	CKL21628	92	CKL21688	127	CKL201507
23	CKL21580	58	CKL21629	93	CKL21694	128	CKL201512
24	CKL21581	59	CKL21631	94	CKL21696	129	CKL201522
25	CKL21582	60	CKL21632	95	CKL21698	130	CKL201526
26	CKL21583	61	CKL21633	96	CKL201274	131	CKL201528
27	CKL21584	62	CKL21634	97	CKL201279	132	CKL201531

28	CKL21587	63	CKL21638	98	CKL201283	133	CKL201584
29	CKL21588	64	CKL21640	99	CKL201285	134	CKL201586
30	CKL21592	65	CKL21641	100	CKL201286	135	CKL201588
31	CKL21591	66	CKL21644	101	CKL201292	136	CKL201589
32	CKL21592	67	CKL21645	102	CKL201297	137	CKL201590
33	CKL21594	68	CKL21646	103	CKL201307	138	CKL201594
34	CKL21595	69	CKL21648	104	CKL201319	139	CKL201598
35	CKL21596	70	CKL21649	105	CKL201320	140	CKL201599

3.2.2. Experimental site

The inbred lines were evaluated under artificial FAW infestation during season A (March – May, 2023) and season B (October – December, 2023) in Kenya Agricultural and Livestock Research Organization (KALRO), Kiboko station (Table 3.2).

Table 3.2. Description of Kiboko during Season A (March – May, 2023) and Season B (October – December, 2023) in 2023

Name of Location	Geographic location			Mean annual	Temperature (˚C)		Soil type
	Longitude	Latitude	Altitude	Rainfall (mm)	Min	Max	
Kiboko	37˚75˚E	2˚15˚ S	993m	548	17	30.6	Semi-arid with ferrasols to ferric luvisol soils
Seasonal weather	Relative humidity			Rainfall (mm)	Temperature (˚C)		
Season A	Dry Bulb	Wet Bulb	Dew Point Depression		Min	Max	Relative humidity (%)
March	24.23	21.50	2.73	0.96	18.61	33.23	77.68
April	22.90	21.53	1.37	7.53	18.43	31.48	88.27
May	22.71	21.15	1.56	0.13	17.34	31.85	86.55
Mean	23.28	21.39	1.89	2.87	18.13	32.19	84.16
Season B							

October	23.58	21.05	2.53	2.11	18.73	31.92	78.94
November	22.35	21.45	0.90	32.92	18.55	28.65	92.00
December	22.39	20.68	1.71	2.84	18.35	29.40	85.65
Mean	22.77	21.06	1.71	12.62	29.99	18.54	85.53

Source: Kenya Meteorological Department, 2024

3.2.3. Phenotypic characterization

3.2.3.1 Experimental design and crop establishment

The I40 tropical inbred lines were evaluated in a 14 x 10 alpha lattice design with two replications. Two seeds per hill of each inbred line were sown in a 5m single row plot with an inter-row spacing of 0.75m and inter-hills spacing of 0.25m, and later thinned to one. Fertilizer was applied at recommended rates (60kg N and 60kg P_2O_5 ha^{-1}) and the field kept free from weeds. Supplementary irrigation was applied when needed. Fall armyworm larvae mass rearing was carried out in KALRO, Katumani insectary. Artificial infestation of plants with five instar FAW larvae was carried out on the 14th day (V5 stage) after germination. The application was conducted manually using a camel hair brush with the larvae placed on the furl and whorl leaves of each plant. This process was carried out early in the morning to avoid desiccation of the larvae.

3.2.3.2 Data collection

Assessment of FAW resistance

Fall armyworm resistance in the maize inbred lines was assessed based on the FAW feeding on leaf and cob damage. Data were collected on the level of FAW leaf feeding damage for each plant per plot on the 7th, 14^{th,} and 21st day after artificial infestation using a visual rating scale of 1–9,

where 1 is highly resistant and 9 is highly susceptible (Table 3.3, Figure 3.1) (Prasanna *et al*., 2018). Fall armyworm cob damage (CD) score rating was recorded for each ear per plot at harvest on a scale of 1 to 9, where 1 is highly resistant and 9 is highly susceptible (Table 3.4; Figure 3.2).

Assessment of agronomic parameters

Agronomic data were collected on plant height, ear height, number of ears, ear aspect, and grain yield. Plant height (PH) was measured in centimeters by measuring 10 representative plants per plot from the ground to the first tassel branch. Ear height (EH) was determined by measuring 10 representative plants from the ground to the insertion of the topmost ear. The number of ears harvested was recorded by counting the number of ears harvested per plot. Ear aspect (EA) was scored using a visual scale of 1 to 5, where 1 represented well-developed, uniform ears with preferred stature, strength, grain color, and texture, while 5 represented worse with undesirable contrasting traits.

Ear rot % was recorded as a percentage of rot per cob due to infection with ear rot disease. Shelled grain weight was measured by weighing shelled grains per plot and using the results to calculate the grain yield in t ha^{-1} as shown below.

$$\text{Grain yield} = (\text{Field weight} * (\frac{10000}{\text{plot area}}) * (\frac{100 - \text{Grain misture}}{100 - 12.5}) * \text{Shelling percentage})$$

$$\text{The shelling percentage} = (\frac{\text{Grain weight}}{\text{field weight}}) * 100$$

The grain moisture of the sample is measured as a percentage using a moisture meter and adjusted to 12.5 % during the calculation

Table 3.3. Scale for assessment of fall armyworm leaf damage in maize.

Score	Damage symptoms/description	Response
1	No visible leaf-feeding damage	Highly resistant
2	Few pinholes on 1-2 older leaves	Resistant
3	Several shot-hole injuries on a few leaves (<5 leaves) and small circular hole damage to leaves	Resistant
4	Several shot-hole injuries on several leaves (6–8 leaves) or small lesions/pinholes, small circular lesions, and a few small elongated (rectangular-shaped) lesions of up to 1.3 cm in length present on whorl and furl leaves	Partially resistant
5	Elongated lesions (>2.5 cm long) on 8-10 leaves, plus a few small- to mid-sized uniform to irregular-shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves	Partially resistant
6	Several large elongated lesions present on several whorl and furl leaves and/or several large uniform to irregular-shaped holes eaten from furl and whorl leaves	Susceptible
7	Many elongated lesions of all sizes present on several whorl and furl leaves plus several large uniform to irregular-shaped holes eaten from the whorl and furl leaves	Susceptible
8	Many elongated lesions of all sizes present on most whorl and furl leaves, plus many mid to large-sized uniform to irregular-shaped holes eaten from the whorl and furl leaves	Highly susceptible
9	Whorl and furl leaves almost totally destroyed and plant dying as a result of extensive foliar damage	Highly susceptible

Source: (Prasanna et al., 2018)

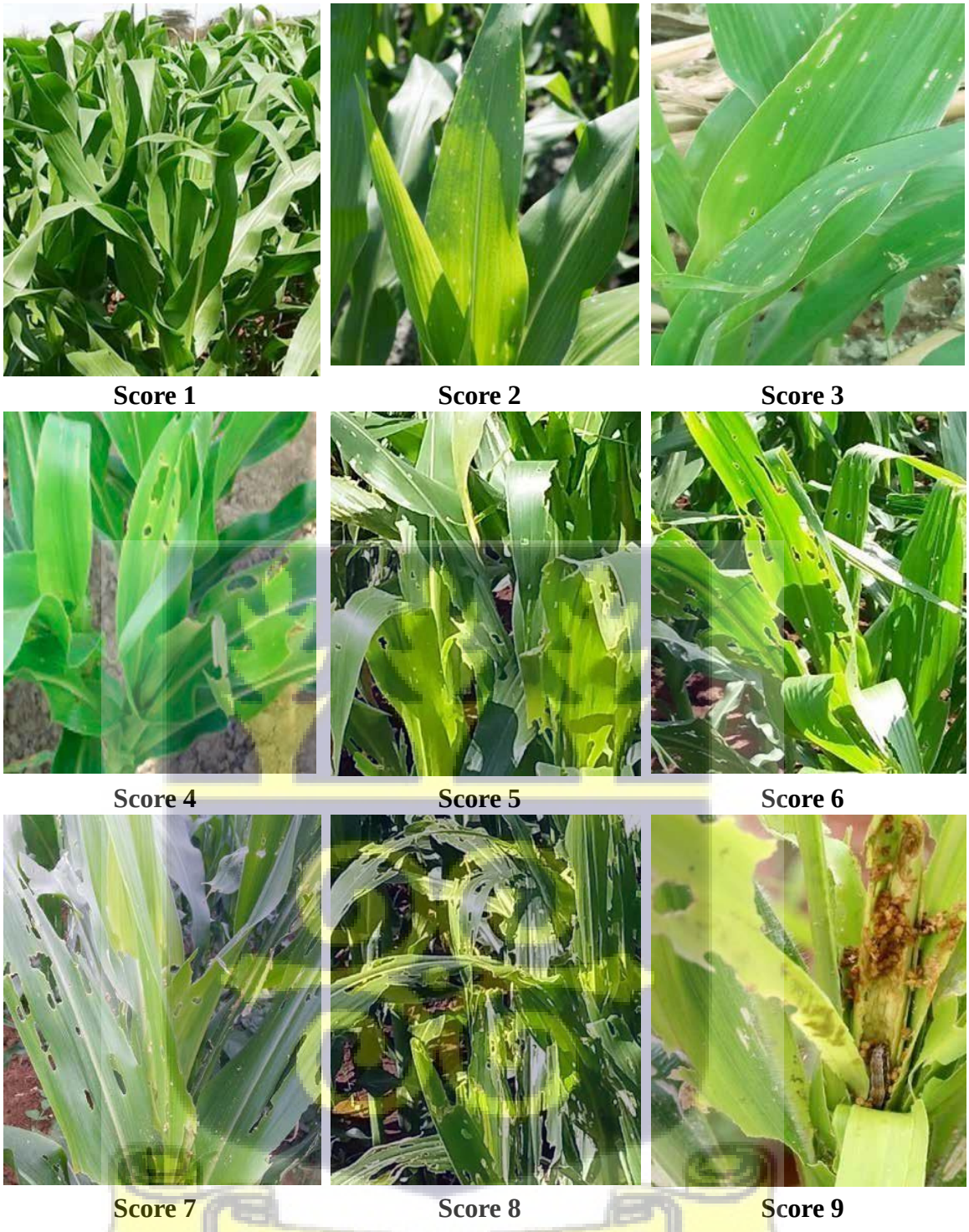


Figure 3.1. FAW leaf damage scores on maize.

Source: (Prasanna et al., 2018)

Table 3.4. FAW ear damage score on maize

Score	Damage symptoms/description	Response
1	No damage to the ear	Highly resistant
2	Damage to a few kernels (<5) or less than 5% damage to an ear	Resistant
3	Damage to a few kernels (6-15) or less than 10% damage to an ear	Resistant
4	Damage to 16-30 kernels or less than 15% damage to an ear	Partially resistant
5	Damage to 31-50 kernels or less than 25% damage to an ear	Partially resistant
6	Damage to 51-75 kernels or more than 35% but less than 50% damage to an ear	Susceptible
7	Damage to 76-100 kernels or more than 50% but less than 60% damage to an ear	Susceptible
8	Damage to >100 kernels or more than 60% but less than 100% damage to an ear	Highly susceptible
9	Almost 100% damage to an ear	Highly susceptible

Source: (Prasanna *et al*., 2018)

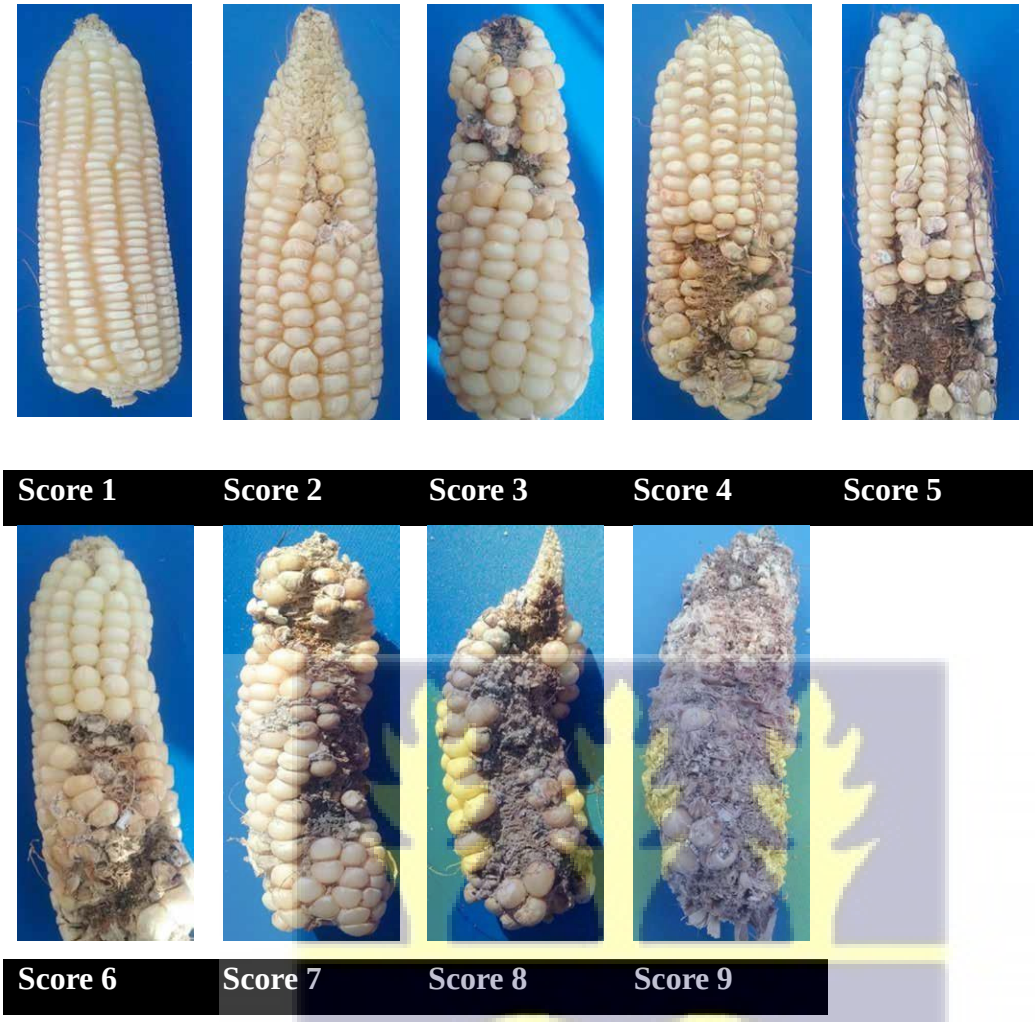


Figure 3.2. FAW scores based on ear damage.

Source: (Prasanna et al., 2018)

3.2.3.3 Analysis of phenotypic data

Collected data was subjected to Analysis of variance (ANOVA) using the Multi-Environment Trial Analysis with R (META-R) program for individual and combined environments. The genotype was considered a fixed effect. The total variance in the ANOVA table was partitioned into phenotypic, genotypic, and genotype by environment (GXE) interaction. The broad sense heritability was estimated using the phenotypic and genotypic variance ratios of the variance component. The phenotypic data were subjected to correlation and principal component analysis using META-R. The eigenvalues were calculated, and the proportion of variance for each trait was presented in a principal component biplot.

3.2.4 Diversity Array Technology Sequence-based SNP markers

3.2.4.1 Genotyping by sequencing

Genotyping of the inbred lines was carried out at SEQART Africa, located at the International Livestock Research Institute (ILRI) in Nairobi, Kenya. Leaf tissue was collected in the 3rd week after germination from the seedlings of the 140 inbred lines grown under screen house conditions. DNA extraction was carried out on the leaf tissues using the Nucleomag Plant DNA extraction kit. The genomic DNA extracted was in the range of 50-100ng/ul. DNA quality and quantity were checked on 0.8% agarose. Gene libraries were constructed according to Kilian et al. (2012). DArTSeq complexity reduction method through digestion of genomic DNA using a combination of (PstI _NspI) enzymes and ligation of barcoded adapters and common adapter followed by PCR amplification of adapter-ligated fragments. Libraries were sequenced using Single Read sequencing runs for 138 cycles. Next-generation sequencing was carried out using NovaseqX.

Genotyping by sequencing (GBS) was carried out using the DArTseqTM technology, which provides rapid, high-quality, and affordable genome profiling, including the most complex polyploid genomes. Scoring of the DArTseq markers was accomplished using an in-house marker scoring pipeline based on algorithms called DArTsoft14. Both the SilicoDArT markers and SNP markers were scored as binary for presence and absence (1and 0, respectively) of the restriction fragment with the marker sequence in genomic representation of the sample. Both markers (SilicoDArT and SNP) were aligned to the reference genomes of Maize_B73_V4.0. assembly, to identify chromosome positions. SNP quality control was carried out using R statistical software version, where the *raw.data* function of the *snpReady* package was used to eliminate monomorphic markers, markers with no chromosomal position, and those whose minor allele frequencies (MAF) were below 5%. Twenty-four thousand, seven hundred and forty-one markers with SNP call rate of more than 95% were used for genetic diversity and population structure analysis.

3.2.4.2 Analysis of genetic diversity parameters

The genetic analysis for 140 inbred lines was carried out using R statistical software version 4.3.0 (R). The popgen function of the *snpReady* package in R was used to compute the polymorphic information content (PIC), minor allele frequency (MAF), heterozygosity (Ho), and gene diversity. The Landscape and Ecological Association Studies (LEA) R package was used for population structure analysis. Results from population structure analysis were used to analyze the molecular variance using the *poppr* package in R software. The *pca* function in the LEA package was used for principal component analysis to validate the generated clusters by constructing a PCA scree plot. The optimal number of sub-populations (K) was calculated using the *snmf* function of LEA by estimating the ancestry coefficient and plotting cross-entropy graphs to

identify the maximum number of subpopulations generated. Neighbor joining hierarchical cluster analysis using the dissimilarity index was used to generate a dendrogram. The dendrogram was constructed using dendextend and circlize packages in R. A Q matrix was generated and used to design the admixture plot for the inbred lines.

3.3 Results

3.3.1 Phenotypic variability and trait association

3.3.1.1 Variance in FAW damage and agronomic traits

There were significant differences (< 0.001) in FAW leaf damage score at 7(LD1), 14 (LD2), 21 (LD3) days after artificial FAW infestation and in cob damage (CD) among the 140 genotypes (Table 3.5). In addition, there were highly significant differences (< 0.001) between season A and B in FAW leaf damage score at 7th (LD1) and 14th (LD2) days after artificial FAW infestation and in cob damage (CD). There was no significant difference in seasons on LD3. There was significant variation (< 0.01) in genotype by season interaction in LD1, and a significant difference (< 0.05) in FAW cob damage (CD)

Table 3.5. Mean squares for FAW damage traits of 140 tropical maize inbred lines under artificial FAW infestation in Kiboko

Source of Variation	Df	LD1	LD2	LD3	CD
Genotype	139	0.09***	0.32***	0.34***	1.21***
Season	1	6.57***	7.03***	0.052	168.92***
Block x Replicate	26	0.12***	0.49***	0.73***	0.65.
Genotype x Season	139	0.07**	0.19	0.18	0.65*
Residuals	224	0.05	0.17	0.2	0.47

Significant difference codes: 0 '***' 0.001 '**' 0.01 '*' 0.05, Df - degrees of freedom; Leaf damage score at 7 days (LD1), 14 days (LD2), and 21 days (LD3) after artificial infestation; CD-FAW cob damage.
Explanation for the degrees of freedom highlighted. The experimental design is a 14x10 alpha lattice; number of genotypes =140. The number of blocks per replication is 14, and the number of replications is 2, and the seasons are 2: Block x Replicate = r(k-1) = replicate (block-1) 2(14-1) =26: Genotype x season = (genotypes-1) (season-1) = (140-1) (2-1) =139

Highly significant differences (< 0.001) were observed among the genotypes for plant height, ear height, number of ears, ear aspect, and grain yield (t ha^{-1}), and significant differences (< 0.01) in root lodging and plant aspect (Table 3.6). There were highly significant differences (< 0.001) in the performance of genotypes within the two seasons on plant height, ear height, number of ears, ear aspect, and grain yield (t ha^{-1}), and significant differences (< 0.05) in plant aspect. There were highly significant differences (< 0.01) in genotype by season interaction among the genotypes (< 0.001) for ear height and number of ears, and significant differences (< 0.05) in grain yield (t ha^{-1}).

Table 3.6. Mean squares for agronomic traits of 140 tropical maize inbred lines under artificial FAW infestation in Kiboko

Source of Variation	Df	Plant height	Ear height	Number of Ears	Ear Aspect	Grain yield
Genotype	139	2447***	764.5***	29.64***	1.00***	0.14***
Season	1	56708***	5658.3***	1687.23***	12.21***	0.14***
Block x Replicate	26	342***	114.9**	20.72***	0.87***	0.15***
Genotype x Season	139	264***	113.9***	15.03***	0.59.	0.07**
Residuals	224	182.00	67.9	8.24	0.4653	0.05

Significant difference codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.', Df - degrees of freedom; Explanation for the degrees of freedom highlighted. The experimental design is a 14x10 alpha lattice; number of genotypes =140. The number of blocks per replication is 14, and the number of replications is 2, and the seasons are 2: Block x Replicate = r(k-1) = replicate (block-1) 2(14-1) =26: Genotype x season = (genotypes-1) (season-1) = (140-1) (2-1) =139

There was variation in FAW damage scores across the seasons, with different genotypes identified as best performing in seasons A and B. In season A, CML 24 was the most resistant genotype and CKL201368 was the worst, while in season B, CKL21619 was the best and CKL21683 was the worst in terms of resistance to FAW. Only CKL21613 and CKL21608 were amongst the 10 best performers in the two seasons (Table 3.7; Table 3.8). Combined analysis for FAW leaf damage during the year 2023 seasons A and B in Kiboko showed that the mean

performance for FAW leaf damage scores taken at 7th day after artificial infestation (LD1) ranged from 3.20 to 3.33, with CML24 scoring the least damage while CKL201409 scoring the highest. LD2 taken 14 days after artificial infestation had a mean range of between 5.45 and 5.97, with the most tolerant genotype being CML 24 and the most susceptible being CKL201409. The lowest mean score for LD3 taken 21 days after artificial infestation was 5.35 for genotype CKL21624, while the highest was 5.89 for CKL201366. CKL21613 genotype produces the greatest number of ears per plot (18 ears) while CKL21633 produces the least number (6 ears). The genotypes with the best ear aspect had a score of 2 while those with the worst ear aspect scored 4. CKL21624 produced the highest grain yield of 3.59 t ha^{-1} while CKL21633 produced the least amount of yield (0.56 t ha^{-1}). The tallest genotype was CKL21624 with a plant height of 210.11 cm, whereas the shortest was CML24 with a plant height of 121.67 cm. Similarly, CKL21625 had the tallest ear height of 88.48 cm, and CML24 had the shortest ear height of 59.30 cm. Most of the genotypes had a FAW cob damage score of below 3, with the most resistant genotype scoring 1.84 (Table 3.9). The mean values for LD1, LD2, LD3, number of ears, ear aspect, grain yield, plant height, ear height, and cob damage were 3.27, 5.78, 5.56, 12, 2.77, 0.55, 156.90, 73.8 and 1.97, respectively (Table 3.9). The coefficient of variation of the combined mean performance for all the traits evaluated ranged from 5.56 to 30.26. Broad sense heritability was estimated as follows:

$$H = \frac{\sigma^2_G}{\sigma^2_G + (\sigma^2_{GE}/e) + (\sigma^2_e/re)}$$

σ^2G - genotype variance; σ^2GE – Genotype x environmental variance; σ^2e- residual (error) variance; e-environment and re-replicate

The heritability estimates varied across the seasons for FAW leaf and cob damage. Moderate heritability values were recorded for FAW LD1 (0.55), LD2 (0.46), LD3 (0.41) and a high heritability for Cob damage (0.68) in season A while low estimates were observed in LD2 (0.04) and moderate values for LD1 (0.32), LD3 (0.31) and CD (0.37) in season B. Moderate Heritability was also observed for ear aspect, grain yield for both season A and B, and number of ears for season B. High estimates (above 60%) were reported for plant and ear height for both seasons (Table 3.7; Table 3.8). Results from combined analysis indicated that heritability (H^2) estimates were high for plant height (0.91) and ear height (0.86), moderate for leaf damage score 2 (0.40), leaf damage score 3 (0.43), number of ears (0.51), ear aspect (0.39), grain yield (0.52), and cob damage (0.40). Leaf damage score 1 recorded the lowest heritability of 0.16 (Table 3.9).

Table 3.7. Mean performance for agronomic and FAW damage traits of the top 10 and bottom 5 maize inbred lines based on average FAW leaf damage during the 2023 season A in Kiboko

Genotype	A_LSD	LDS1	LDS2	LDS3	NE	EA	GY	PH	EH	CD
					Top ten					
CML24	4.56	3.07	5.31	5.28	14.26	2.41	0.66	137.70	63.45	1.61
CKL21668	4.67	3.13	5.49	5.38	16.25	3.22	0.78	196.49	82.15	2.83
CKL21631	4.72	3.33	5.54	5.30	15.26	2.51	0.75	167.67	64.39	1.91
CKL21624	4.73	3.15	5.58	5.46	15.34	2.88	0.77	220.44	91.33	2.69
CKL21627	4.73	3.12	5.61	5.46	18.76	3.54	0.66	189.81	76.24	3.54
CKL21613	4.74	3.35	5.51	5.34	17.16	2.95	0.78	192.09	77.13	2.82
CKL21641	4.77	3.30	5.56	5.44	14.82	2.94	0.63	186.72	78.35	2.89
CKL21657	4.77	3.26	5.66	5.40	12.35	2.72	0.65	214.05	93.64	2.50
CKL21634	4.77	3.28	5.63	5.41	14.77	2.40	0.84	211.07	126.59	2.15
CKL21608	4.78	3.35	5.56	5.43	13.73	3.02	0.61	163.74	78.29	2.66
CKSBL1008 Res.Check	4.73	3.12	5.62	5.46	19.13	2.37	0.99	192.65	91.33	2.17
					Bottom five					
CML610A Sus. Check	5.03	3.39	6.00	5.71	13.89	2.38	0.66	149.35	77.73	1.82
CKL201385	5.11	3.46	6.10	5.78	12.08	2.75	0.83	159.13	83.54	2.64
CKL201409	5.13	3.57	6.09	5.73	11.04	2.97	0.63	176.66	70.38	2.86
CKL201594	5.14	3.50	6.11	5.81	11.20	2.64	0.62	177.66	95.35	2.21
CKL201366	5.15	3.49	6.12	5.84	13.37	3.34	0.65	202.55	86.79	2.05
CKL201368	5.18	3.63	6.12	5.80	16.45	2.95	0.63	129.93	75.33	2.49
Heritability	0.47	0.55	0.46	0.41	0.75	0.59	0.58	0.93	0.93	0.68
Genotype Variance	0.04	0.02	0.05	0.04	8.72	0.19	0.03	647.73	234.57	0.40
Residual Variance	0.09	0.04	0.11	0.11	5.74	0.28	0.04	95.36	32.73	0.38
Grand Mean	4.94	3.38	5.83	5.61	13.76	2.90	0.67	167.03	77.13	2.52
LSD 5%	0.41	0.31	0.48	0.44	4.46	0.83	0.32	20.82	11.94	1.07
CV	5.84	5.86	5.80	5.87	17.41	18.08	30.12	5.85	7.42	24.48
n Replicates	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Genotype significance	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00

CV- Coefficient of variation; LSD- Least significant difference; LD1-FAW Leaf damage score at 7 days, LD2-14 days and LD3-21 days after artificial infestation; NE-Number of ears; EA-Ear aspect; GY - Grain yield (t ha^{-1}); PH- Plant height; EH- Ear Height; CD- FAW

Cob damage

Table 3.8. Mean performance for agronomic and FAW damage traits of the top 10 and bottom 5 maize inbred lines based on FAW leaf damage during the 2023 season B in Kiboko

Genotype	A_LDS	LDS1	LDS2	LDS3	NE	EA	GY	PH	EH	CD
				Top ten						
CKL21619	4.59	2.98	5.44	5.36	11.52	2.72	0.38	138.76	70.53	1.25
CKL21584	4.60	3.04	5.46	5.29	12.15	2.22	0.44	145.49	72.74	1.62
CKL201328	4.63	3.02	5.45	5.41	9.53	3.05	0.34	132.05	68.80	1.25
CKL21610	4.63	3.08	5.46	5.34	10.15	2.35	0.38	154.11	69.20	1.44
CKL21578	4.63	3.02	5.44	5.43	10.77	2.26	0.60	173.82	77.18	1.62
CKL21613	4.63	3.00	5.44	5.47	14.10	2.09	0.63	177.41	76.04	1.62
CKL21611	4.64	3.06	5.45	5.41	9.71	2.70	0.39	138.46	60.50	1.44
CKL21663	4.64	3.02	5.45	5.46	11.42	2.50	0.60	153.64	88.50	1.62
CKL21608	4.64	3.01	5.45	5.48	14.50	2.50	0.63	160.75	78.62	1.25
CKSBL1008 Res. Check	4.67	3.07	5.44	5.49	9.67	3.07	0.35	171.57	78.84	1.44
				Bottom 5 genotypes						
CML610A Sus. Check	4.77	3.05	5.47	5.77	10.68	2.06	0.58	150.84	73.41	1.44
CKL201389	4.82	3.28	5.46	5.73	9.87	2.70	0.48	143.59	77.08	1.25
CKL201409	4.83	3.29	5.47	5.72	10.32	2.84	0.48	134.08	63.05	1.62
CKL201512	4.83	3.18	5.47	5.84	8.96	2.73	0.36	133.46	59.54	1.81
CKL21597	4.84	3.31	5.46	5.73	8.67	2.77	0.34	155.75	68.16	1.44
CKL21683	4.90	3.29	5.46	5.96	6.45	3.18	0.27	150.48	77.02	1.36
Heritability	0.22	0.32	0.04	0.31	0.55	0.55	0.56	0.93	0.93	0.37
Genotype Variance	0.03	0.02	0.01	0.06	4.94	0.22	0.02	647.73	234.57	0.10
Residual Variance	0.20	0.10	0.24	0.26	8.09	0.37	0.03	95.36	32.73	0.35
Grand Mean	4.72	3.14	5.46	5.56	10.25	2.61	0.43	167.03	77.13	1.40
LSD 5%	0.96	0.67	1.10	1.10	6.02	0.93	0.40	20.82	11.94	0.71
CV	9.39	10.05	9.01	9.12	27.77	23.19	40.75	5.85	7.42	41.86
Replicates	2	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Genotype significance	0.31	0.04	0.84	0.05	0.00	0.00	0.00	0.00	0.00	0.01

CV- Coefficient of variation; LSD- Least significant difference; LD1-FAW Leaf damage score at 7 days, LD2-14 days and LD3-21 days after artificial infestation; NE-Number of ears; EA-Ear aspect; GY - Grain yield (t ha^{-1}); PH- Plant height; EH- Ear Height; CD-FAW Cob damage

Table 3.9. Combined mean performance for agronomic and FAW damage traits of the top 10 and bottom 5 maize inbred lines based on FAW leaf damage during the 2023 seasons A and B in Kiboko

Genotype	LD1	LD2	LD3	NE	EA	GY	PH (cm)	EH	CD
				Top ten					
CML24	3.20	5.45	5.38	13	2	1.67	121.67	59.30	0.99
CKL21613	3.22	5.56	5.36	18	2	3.16	190.06	77.88	2.56
CKL21619	3.22	5.64	5.41	16	3	2.75	142.70	67.55	2.32
CKL21696	3.25	5.70	5.35	14	3	3.17	185.63	75.73	3.05
CKL21624	3.24	5.61	5.39	14	2	3.59	210.62	88.48	2.20
CKL21608	3.23	5.62	5.46	16	3	2.73	162.11	78.07	1.87
CKL21584	3.22	5.75	5.39	15	2	2.07	156.75	76.62	2.32
CKL21578	3.24	5.65	5.46	13	3	2.87	190.74	82.31	2.39
CKL21631	3.25	5.66	5.36	12	3	2.43	155.35	63.04	1.27
CKL21625	3.25	5.69	5.44	14	3	3.06	148.43	62.15	1.84
CKSBL1008 Res. Check	3.21	5.70	5.49	15	3	3.19	186.04	87.25	1.70
				Bottom 5 genotypes					
CKL201366	3.27	5.92	5.89	13	3	3.06	193.43	80.48	1.83
CKL201343	3.28	5.87	5.84	10	3	2.16	153.66	64.32	1.72
CKL201368	3.38	5.89	5.58	15	3	2.08	117.99	71.44	1.90
CKL21633	3.32	5.92	5.73	6	4	0.56	173.94	78.62	2.28
CKL201409	3.33	5.97	5.73	10	3	2.42	154.93	64.86	2.17
CML610A Sus. Check	3.25	5.86	5.75	12	2	2.71	148.71	75.15	1.77
Heritability	0.16	0.40	0.43	0.51	0.39	0.52	0.91	0.86	0.40
Genotype Variance	0.01	0.02	0.03	3.52	0.09	0.01	558.95	161.11	0.11

GenxLoc Variance	0.02	0.01	0.00	3.39	0.12	0.01	42.62	23.71	0.14
Residual Variance	0.07	0.1	0.19	6.81	0.32	0.04	141.77	56.83	0.38
Grand Mean	3.27	5.78	5.58	12.00	2.76	0.55	156.90	73.88	1.97
LSD 5%	0.19	0.32	0.41	3.77	0.66	0.24	21.47	13.89	0.72
CV	8.05	5.56	7.76	21.75	20.55	34.26	7.59	10.20	30.87

CV- Coefficient of variation; LSD- Least significant difference; LD1-FAW Leaf damage score at 7 days, LD2-14 days and LD3-21 days after artificial infestation; NE-Number of ears; EA-Ear aspect; GY - Grain yield (t ha^{-1}); PH- Plant height; EH- Ear Height; CD-FAW Cob damage

3.0.1.1 Correlation among agronomic and FAW damage traits

Genotypic correlation among agronomic and FAW damage traits (Figure 3.3) showed that there was significant (< 0.001) positive correlation between LD1 and LD2 with the two-trait having a perfect positive correlation of 1, LD1 and LD3 (0.80), LD1 and ear aspect (0.77), LD1 and cob damage (0.58), LD2 and LD3 (0.83), plant height and ear height (0.62), number of ears and grain yield (0.62), and ear aspect and cob damage (0.52). Additionally, there was a significant (< 0.01) positive correlation between LD2 and cob damage (0.26) and ear height and grain yield (0.22). A significant (< 0.05) positive correlation coefficient was recorded between LD3 and ear height (0.19), and grain yield and cob damage (0.21). High significant (< 0.001) negative correlation coefficient was observed between LD1 and plant height (-0.58), LD1 and ear height (-0.61), LD1 and number of ears (-0.71), LD1 and grain yield (-0.75), LD2 and number of ears (-5.1), number of ears and ear aspect (-0.41) and ear aspect and grain yield (-0.60). There was significant (< 0.01) negative correlation between LD2 and plant height (-0.27), LD2 and grain yield (-0.28), and ear height and ear aspect (-0.26) and lastly, there was significant (< 0.05) negative correlation coefficient observed between LD3 and ear height (- 0.19) and between grain yield and cob damage (-0.21) (Figure 3.3).

	LD1							
LD2	1.00***	LD2						
LD3	0.80***	0.83***	LD3					
PH	-0.58***	-0.27**	-0.16	PH				
EH	-0.61***	-0.02	0.19*	0.62***	EH			
NE	-0.71***	-0.68***	-0.51***	0.15	0	NE		
EA	0.77***	0.16	0.06	-0.1	-0.26**	-0.41***	EA	
GY	-0.75***	-0.28**	-0.07	0.16	0.22**	0.62***	-0.60***	GY
CD	0.58***	0.26**	-0.02	0.08	0.02	0.16	0.52***	-0.21*

Significant difference codes: 0 '***' 0.001 '**' 0.01 '*' 0.05; LD1, FAW Leaf damage score at 7 days, LD2-14 days and LD3-21 days after artificial infestation; NE-Number of ears; EA-Ear aspect; GY- Grain yield; PH- Plant height; EH- Ear Height; CD- FAW Cob damage

Figure 3.3. Pairwise Pearson correlation for agronomic and FAW damage traits

3.0.1.2 Relative trait contributions to total variation

Principal component analysis (PCA) showed first principal component (PC1) and PC2 described 85% of the phenotypic variation observed among the 140 genotypes. The first principal component (PC1) accounted for 66.47% while PC2 accounted for 18.48% of the total variation (Figure 3.4). Eight out of the nine traits contributed highly to the total variation observed in PC1 with the traits having values of over 0.70. However, cob damage trait had the least contribution to the variation observed (- 0.47) in PC1. In PC2, cob damage (1.00), LD3 (-0.75), and ear height (-0.70) had the highest loading score, while LD1 had the lowest (0.01) (Table 3.10). The genetic correlation biplot demonstrated that the phenotypic traits clustered into four sub-groups according to their relationships (Figure 3.4). Plant height was observed to be closely correlated with ear height,

number of ears with grain yield, ear aspect with cob damage, and the last cluster consisted of the fall armyworm leaf damage traits LD1, LD2, and LD3 (Figure 3.4).

Table 3.10. Contributions of agronomic and FAW damage traits to principal component1 *(*PC1) and principal component 2 (PC2)

Phenotypic trait	PC1	PC2
Leaf damage score 1 (LD1)	-1.00	0.01
Leaf damage score 2 (LD2)	-0.93	-0.45
Leaf damage score 3 (LD3)	-0.80	-0.75
Plant height	0.82	-0.24
Ear height	0.72	-0.70
Number of ears	0.91	0.52
Ear aspect	-0.85	0.45
Grain yield	0.90	0.10
Cob damage	-0.47	1.00
Explained variation (%)	66.50	18.50

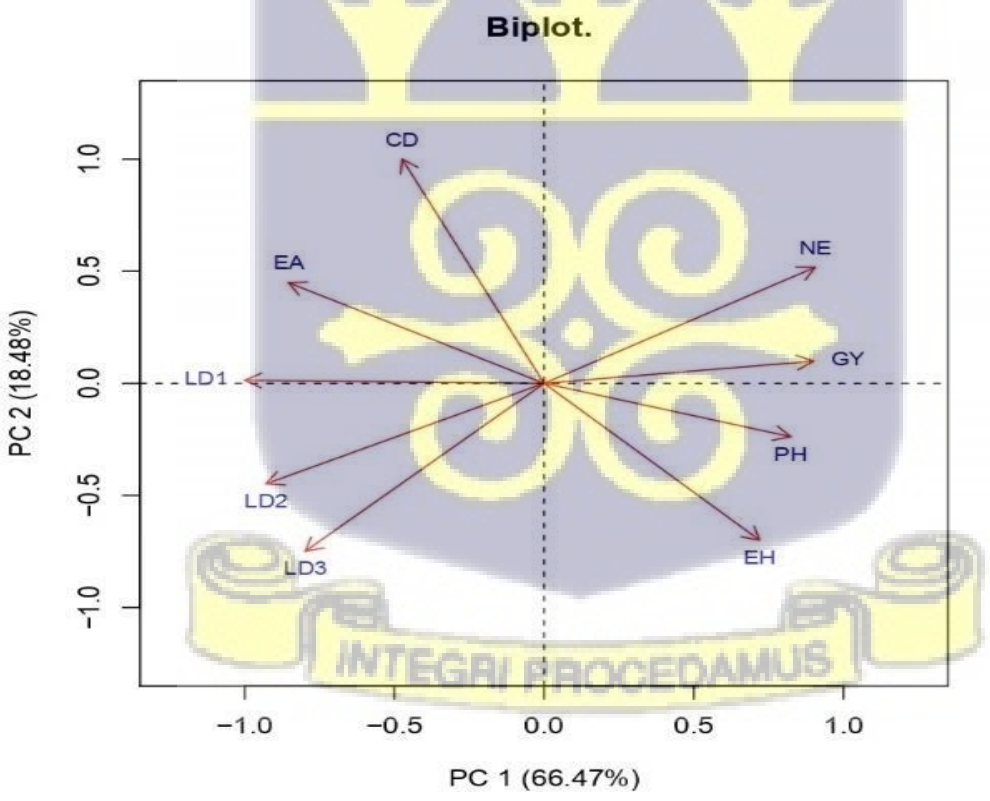


Figure 3.4. Biplot showing the relationship between the agronomic and FAW damage traits

3.0.2 Diversity Array Technology Sequence-based SNP markers

3.0.2.1 Characterization of single-nucleotide polymorphisms (SNP) markers

A total of 24,741 SNP markers were used for genetic diversity studies. The gene diversity (GD) within the population of 140 tropical inbred lines had a mean of 0.35 with a range of between 0.1 and 0.5. The Polymorphic Information Content (PIC) value across the chromosomes ranged from

0.09 to 0.38 with a mean of 0.28. The highest number of markers (12,622) had a PIC of above 0.3. SNP markers with PIC of values less than 0.1 were 708, those with PIC values between 0.1 to 0.2 were 4984, and markers with PIC value between 0.2 to 0.3 were 6427 (Figure 3.5). Minor allele frequencies (MAF) had a range of 0.05 to 0.5 with an average value of 0.26. The SNPs with MAF of below 0.1 were 3393, while those with MAF of above 0.4 were 4387 (Figure 3.6). The mean value for observed heterozygosity (Ho) was 0.25 with a range of between 0.18 and 0.49. The inbreeding coefficient (F) ranged from -0.41 to 0.48, with a range of 0.32. The effective population size (Ne) had a mean of 235.33. The additive variance estimate was 8691, while the dominant variance was 3444.23 (Table 3.11).

Table 3.11. Genetic diversity of tropical inbred lines using DArTseq SNP markers

Genetic parameters	mean	lower	upper		Estimate
GD	0.35	0.1	0.5	Ne	235.33
PIC	0.28	0.09	0.38	Va	8691.28
MAF	0.26	0.05	0.5	Vd	3444.23
Ho	0.24	0.18	0.49	Number of genotypes	140
F	0.32	-0.41	0.48	Number of markers	24741

GD-gene diversity; PIC- polymorphism information content; MAF-minor allele frequency; Ho-Observed heterozygosity; F- fixation index; Va = additive variance; Vd = dominance variance; Ne = effective population size

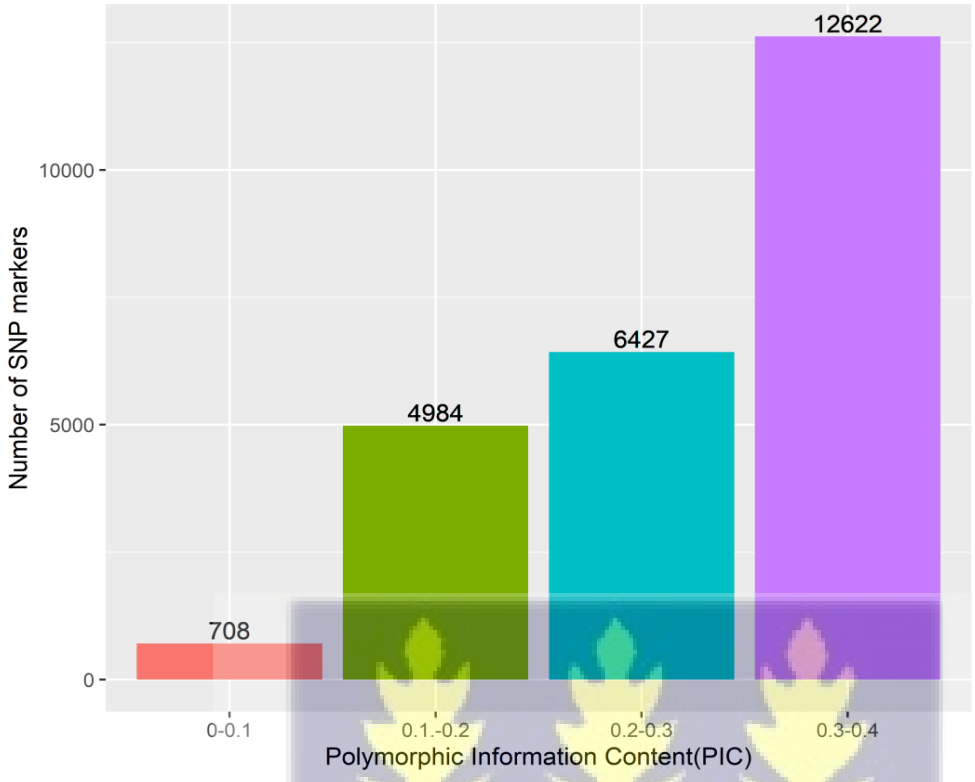


Figure 3.5. Polymorphic information content (PIC) of SNP markers

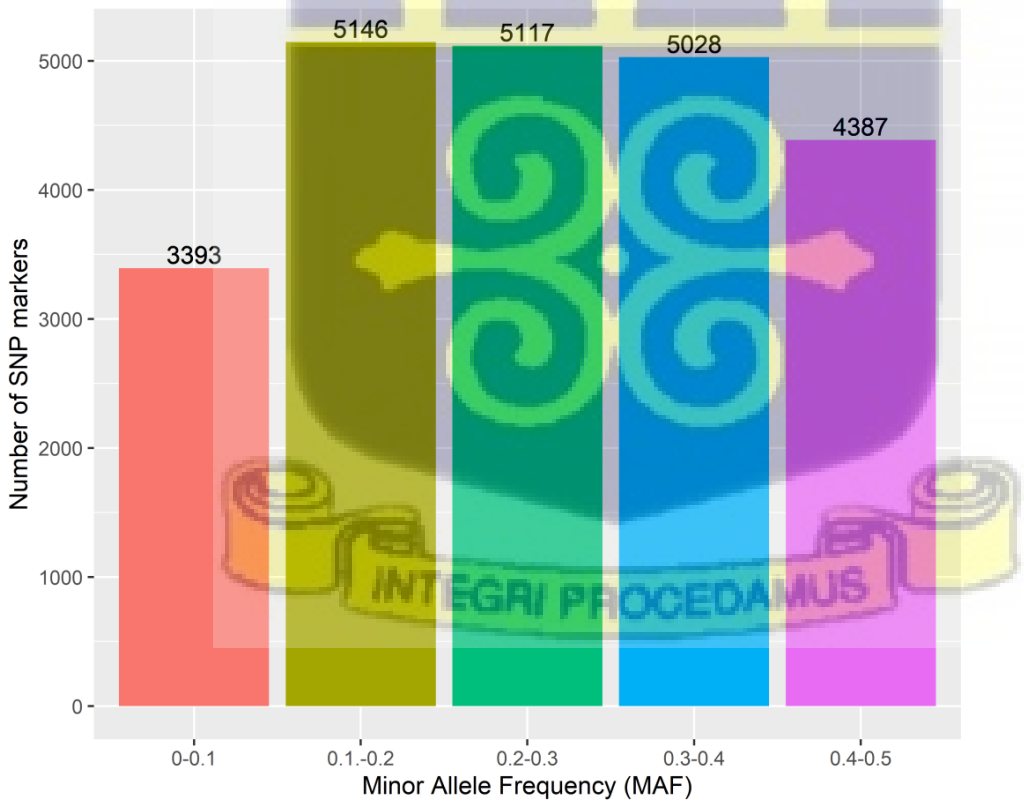


Figure 3.6. Minor Allele Frequencies of SNP makers

3.2.2.2 Population structure of the 140 tropical inbred lines

Population structure analysis based on cross-entropy criteria using 24741 high-density SNP markers showed that the 140 inbred lines grouped into nine subpopulations (K=9) (Figure 3.7). Analysis of the structure by the admixture model revealed that there was genetic admixture amongst the nine subpopulations (Figure 3.8). The fixation index (F), which indicates the population differentiation due to genetic structure, ranged from 0.02 to 0.15. The highest fixation index of 0.15 was observed between sup populations 1 and 8, 1 and 9, 3 and 8, and 8 and 9, while the lowest (0.02) was observed between subpopulation 4 and 8 (Figure 3.9).

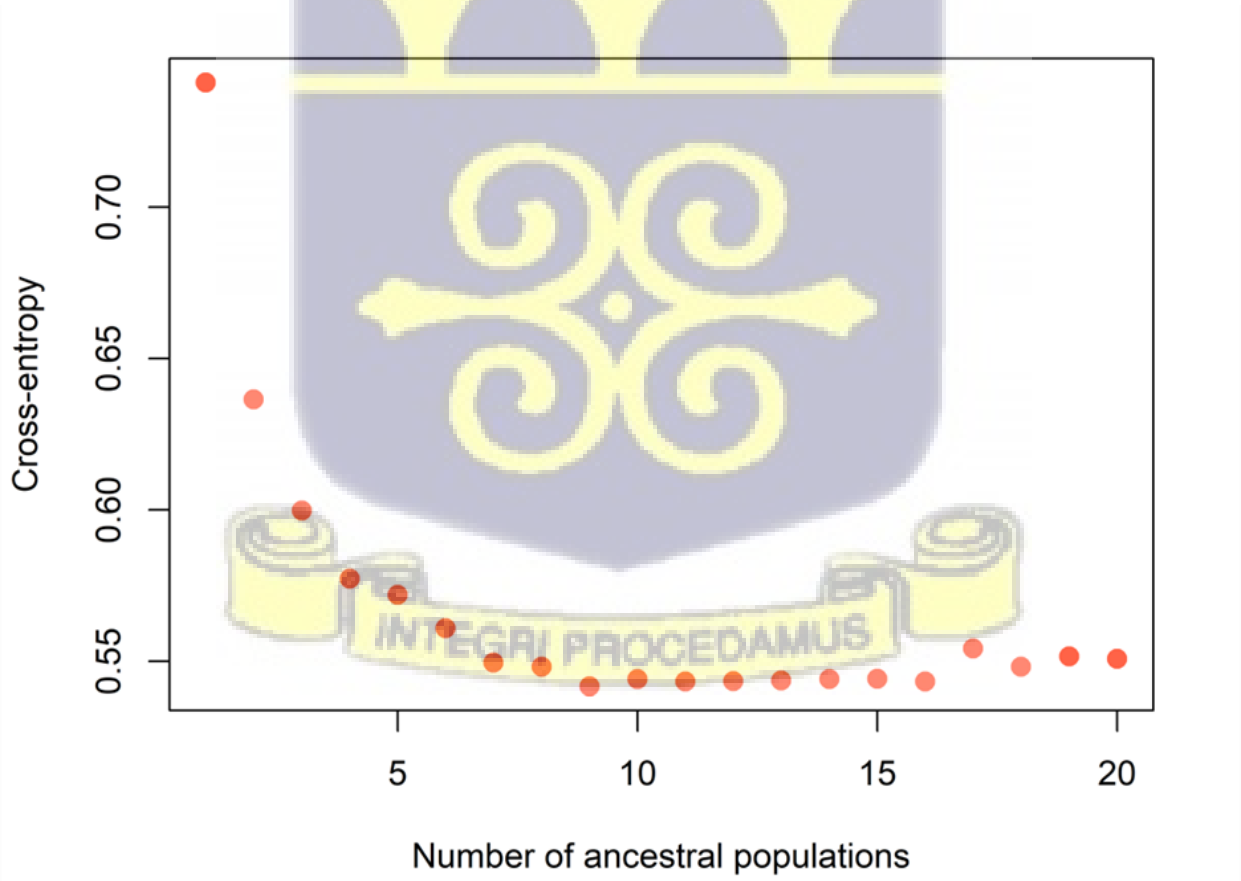


Figure 3.7. Population structure of 140 maize inbred lines based on cross-entropy using 24741 high-density SNP markers

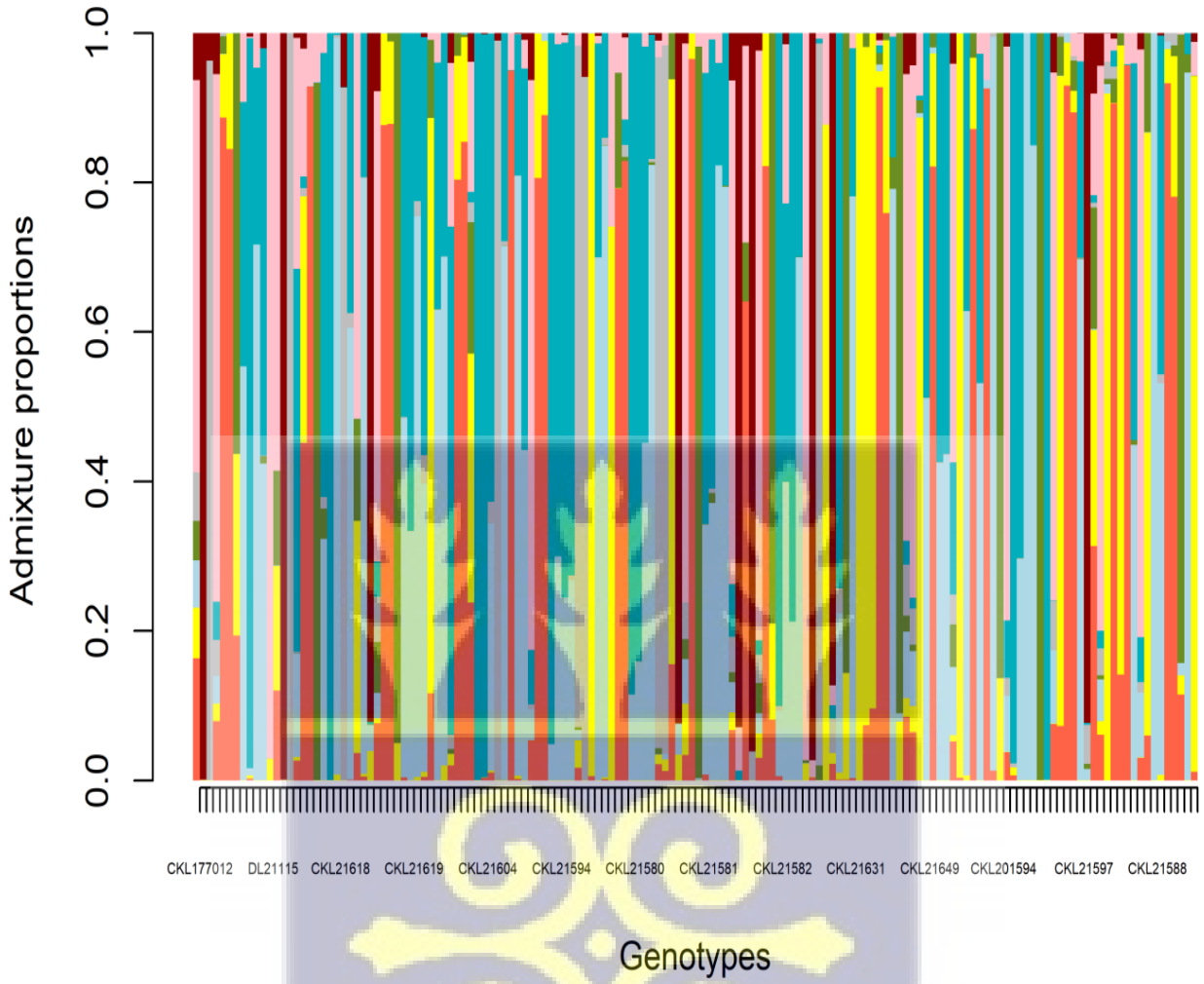


Figure 3.8. Population admixtures of 140 maize inbred lines based on cross-entropy using 24741 high-density SNP markers

Sub pop	I	II	III	1V	V	VI	VII	VIII	IX
I									
II	0.07								
III	0.08	0.12							
IV	0.06	0.12	0.13						
V	0.07	0.14	0.14	0.07					
VI	0.07	0.14	0.14	0.13	0.13				
VII	0.05	0.13	0.14	0.10	0.10	0.12			
VIII	0.08	0.15	0.15	0.13	0.14	0.02	0.14		
IX	0.08	0.15	0.14	0.06	0.08	0.14	0.10	0.15	

Figure 3.9. Population pair-wise fixation index of 140 maize inbred lines using 24741 SNP markers.

3.2.2.3. Analysis of molecular variance

The analysis of molecular variance confirmed that there was genetic diversity among the 140 inbred lines. There was higher variation (65%) within the subpopulation compared to variation among the subpopulation (35%) (Table 3.12).

Table 3.12. Analysis of molecular variance among 140 maize inbred lines based on 24741 SNP markers.

Source of variation	Df	Sum of Squares	Mean Squares	Estimate of variation	Proportion of Variance (%)
Between population	8	499197.2	62399.64	3799.304	35.37005
Within population	131	888612	6942.281	6942.281	64.62995
Total	139	136 1387809.2	10204.48	10741.586	100

Df: degrees of freedom

3.2.2.4 Genetic grouping of 140 inbred lines

Neighbor joining hierarchical cluster analysis using the dissimilarity index grouped the genotypes into 9 clusters. Cluster 1 had 30 genotypes, cluster 2 (32 genotypes), cluster 3 (9 genotypes), cluster 4 (9 genotypes), cluster 5 (7 genotypes), cluster 6 (20 genotypes), cluster 7 (14 genotypes), cluster (10 genotypes) and cluster 9 (8 genotypes) (Figure 3.10).

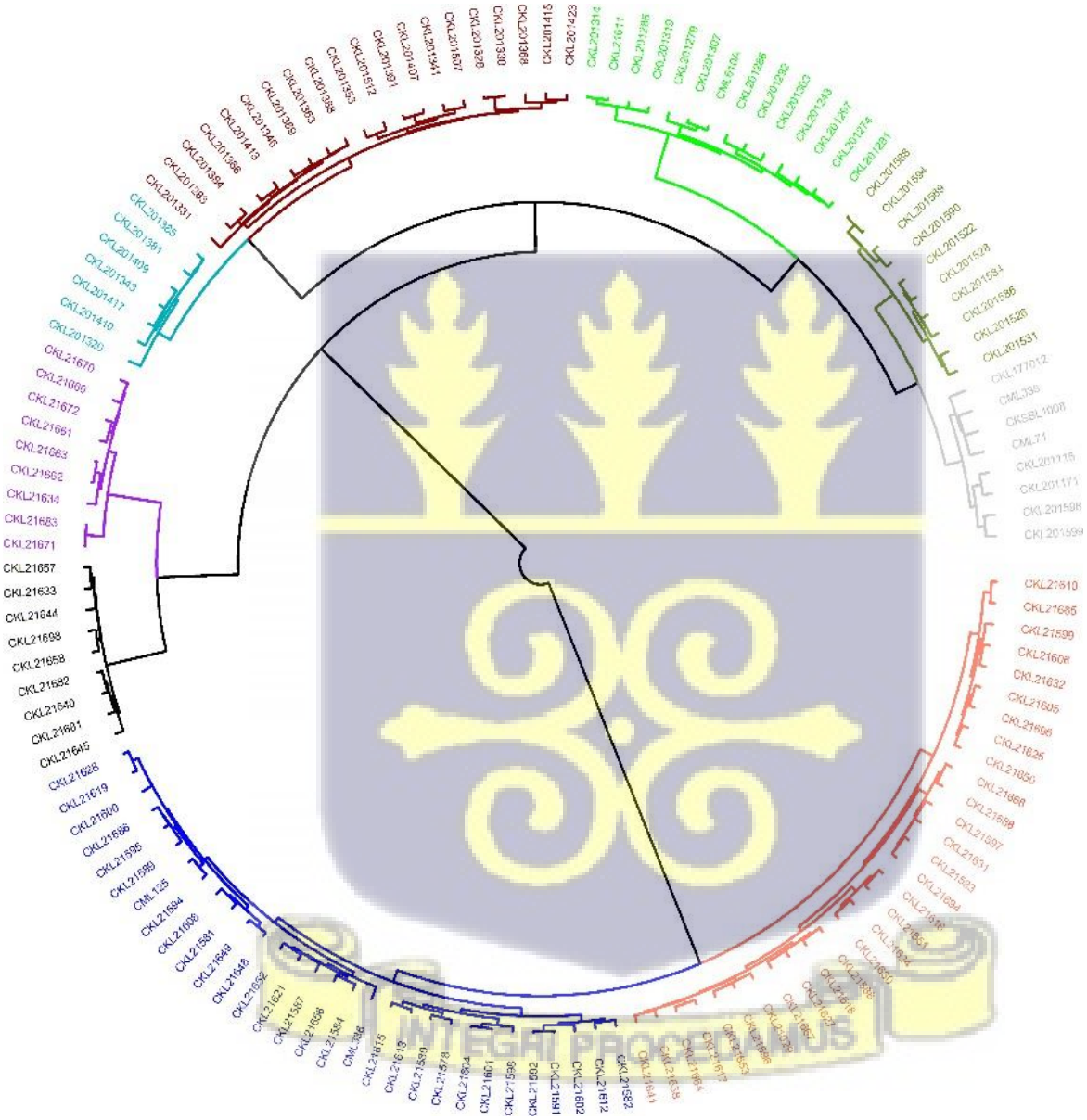


Figure 3.10. Genetic relationships among 140 maize inbred lines based on 24741 high-density SNP markers

3.1 Discussion

Determination of the genetic diversity existing amongst germplasm is a prerequisite for any maize crop improvement program because breeders use the information to develop the best breeding strategies to maximize genetic gains. The variation in performance of the genotypes for FAW damage and agronomic traits signified that the tropical inbred lines were genetically diverse and may be used in maize breeding programs for crop improvement. Highly significant differences among genotypes on the level of FAW leaf and cob damage score show the existence of diversity in the trait that could be exploited when breeding for resistance to FAW in maize. Breeding mainly depends on genetic diversity for the selection of parental lines to be used for genetic improvements of specific traits (Kosgei *et al*., 2025). The highest FAW damage mean scores recorded on the 14th day after infestation, followed by a decline in the scores documented on the 21st day, indicate that some plants had recovered from the pest attack due to HPR. The level of FAW leaf damage at the 7th and 14th day after infestation may determine the plant's ability to recover from the attack. Resistant genotypes are more likely to recover fast due to less damage compared to the susceptible ones (Abang *et al*., 2024). Kamweru *et al*. (2022) further stated that the extensive damage recorded on the 14th day after infestation was because the most destructive FAW developmental stage (5th and 6th instar) coincides with the most susceptible maize growth phase . The best performing FAW-resistant tropical inbred lines selected based on the damage profile when compared to the resistant check can be recommended as sources of FAW-resistant genes in breeding programs. Similar observations were made by Kasoma *et al*. (2022). Significant variation in genotypes by season interaction for FAW leaf damage score 1 (LD1) and cob damage shows the difference in genotypic performance for the traits across the seasons (A and B). The variation in the top-most FAW-resistant inbred lines within the two seasons further confirms that the performance of the genotypes was greatly influenced by the environmental factors, implying that breeding efforts

should focus on developing FAW-resistant genotypes adapted to specific environments. Subsequently, the significant genotype by environment interaction for LD1, cob damage, plant and ear height, number of ears, and grain yield requires more multi-locational trials of the inbred lines to evaluate their stability across different locations for useful breeding decisions to be made (Badji *et al*., 2020). These results corroborate the findings of Job et al. (2022). However, contrary to the above findings, there was no significant genotype by season interaction for leaf damage score 2 (LD2) and leaf damage score 3 (LD3), showing that the traits were regulated by the genotype and had no environmental influence.

Estimation of heritability is important in selection methods to use for trait improvement in maize, predicts the genetic gain, and shows the importance of genetic gain (Ochigbo *et al*., 2021). Moderate heritability estimates for FAW leaf damage traits and high estimates for cob damage show that the trait is heritable and that the phenotypic variance observed is also attributed to the average effects of genes (both additive and non-additive effects) (Schmidt *et al*., 2019). This indicates that selection of the traits based on the damage scores could result in the achievement of genetic gain since the resistance traits could be transmitted to the offspring during crossing (Ayesiga *et al*., 2025). Similarly, high heritability values have been reported for cob damage, and moderate estimates obtained for FAW leaf damage (LD1, LD2, and LD3) (Kamweru *et al*., 2023).

Additionally, a high heritability estimate for plant and ear height and a moderate estimate for the number of ears and grain yield indicate that these traits can be improved through breeding.

Correlation studies showed the relationship between traits and enabled breeders to carry out either direct or indirect selection based on whether the correlation effect is positive or negative. Significant negative correlation between FAW leaf damage LD1, LD2, and cob damage parameters and grain yield and yield-related parameters, such as number of ears and ear height,

means that an increase in the damage scores will decrease grain yield. The highly significant negative correlation between FAW damage parameters and grain yield and yield-related traits, like the number of ears per plant, implies that the FAW damage can be used for indirect selection and yield improvement in maize. The results suggest that reduced FAW damage activities may contribute towards increased yield by reducing losses attributed to pest infestations; therefore, the selection will be indirect. Fall armyworm damage on the leaves directly affects grain yield. The yield losses result from the leaves' destruction, which reduces the efficiency of photosynthesis, thereby interfering with the translocation of nutrients required for plant growth during both vegetative and reproductive phases (Job *et al.*, 2022). Cob damage caused by the mature FAW larvae burrowing into the ears reduces the yield by destroying the maize kernel and exposing the cobs to secondary infection after burrowing into them (Job *et al.*, 2022). There was a high positive correlation between FAW leaf damage and cob damage parameters, which agrees with the findings of Kamweru *et al.* (2022). Maize yield losses of up to 20.6 million tons annually were predicted at the beginning of the FAW invasion in 12 maize-producing countries in Africa (Yaméogo *et al.*, 2024). Recent reports show that without intervention, FAW damage could cause between 4.1 to 17.7 million tons of maize yearly in 12 maize-producing countries (Sisay *et al.*, 2019). Plant height has been reported to be highly correlated with FAW tolerance in maize (Matova *et al.*, 2022).. This implies that plant height is an important trait to be considered when breeding for FAW resistance in maize.

The presence of genetic diversity shown by variation in phenotypic traits among tropical inbred lines was confirmed using Diversity Array Technology Sequence-based SNP markers. The use of a high number of SNP markers (24,741) enables a more accurate estimation of the population structure (Dube *et al.*, 2023). Mean gene diversity (GD) of 0.35 observed indicated the presence of moderate diversity. These findings were similar to those of Dube *et al.* (2023). The PIC of a

marker determines the ability of the marker to detect polymorphism between individuals in a population (Serrote *et al.*, 2020). Despite SNPs being codominant markers, they are biallelic and therefore their PIC is calculated as that of dominant markers, with the maximum PIC value being 0.5. Markers with a PIC value of between 0.40 to 0.5 are considered to be highly informative, 0.3 to 0.4 as high, 0.10 to 0.25 as medium, and those between 0 to 0.10 as low (Nantongo *et al.*, 2022). In this study, the mean PIC value (0.28) of SNP markers across the chromosomes showed that the markers had medium informativeness. However, more than half of the total number of markers (12622) had a PIC of above 0.3 and therefore exhibited high. This showed that the SNP markers used for the diversity studies exhibited between medium to high informativeness, and could effectively discriminate among the genotypes (Dube *et al.*, 2023). The genetic purity of inbred lines is assessed by the percentage of heterozygosity of the SNP markers; the inbred lines used in the study are expected to be homozygous and therefore fixed with SNP heterozygosity of below 5% (Josia *et al.*, 2021). The mean observed heterozygosity of 0.25 (25%) indicates the presence of genetic diversity. However, since the observed heterozygosity exceeded the maximum accepted limit of 5% for inbred lines, it shows low genetic purity within the study population (Gunundu *et al.*, 2025). Therefore, some of the inbred lines within the population may need to undergo selfing and extensive selection to increase their level of homozygosity (Gunundu *et al.*, 2025). Heterozygosity level may also increase due to pollen contamination during maintenance breeding (Josia *et al.*, 2021). High heterozygosity among maize inbred lines has been reported in similar studies (Abu *et al.*, 2021). The moderate level of inbreeding coefficient (F) (mean of 0.32) observed from the study further confirms that the inbred lines were not genetically pure. Maintaining the genetic purity in inbred lines is key to quality seed production. The threshold value attained by a breeding program may vary depending on the objective and level of inbreeding (Wegary *et al.*, 2019). The demand for developing hybrids that

are uniform by using genetically pure parental lines has increased due to advantages such as better heterosis, ease of maintaining parental lines, and quality control implementation during hybrid seed production (Wegary *et al*., 2019).

Population structure analysis based on cross-entropy criteria using 24741 high-density SNP markers classified 140 inbred lines into nine subpopulations (K=9), indicating high genetic diversity among the inbred lines. Analysis of the structure by the admixture model revealed genetic admixture amongst the nine sub-populations. Genetic admixture among clusters of maize inbred lines has also been reported by Patel *et al*. (2024) and Dube *et al*. (2023). Admixture of genes occurs when genomically divergent populations mix as a result of the imprecise nature of inheritance and recombination to introduce new lineages. The progenies from the same admixing ancestors have mosaics of genetic identity unique to each individual (Brugger and Davis, 2023). The knowledge of population structure and ancestry contributes towards the selection of parental lines for enhanced heterosis in a maize breeding program (Patel *et al*., 2024). Population differentiation due to genetic structure is determined by the fixation index (F). Fixation indices illustrate the level of genetic similarity or divergence within the evaluated population and are classified as high (> 0.15), moderate (0.05–0.15), and low (< 0.05) (Mwale *et al*., 2023). The population pair-wise fixation index of between 0.02 and 0.15 observed in the study indicates a moderate divergence of selected alleles across the subpopulations, which could be used to maximize the heterotic effect during breeding. Genetic divergence analysis is vital in maize breeding to enhance selection efficiency, broaden the genetic base of the breeding population, and enhance the formation of high-yielding stress-tolerant hybrids adapted for different agroecological zones (Ivy *et al*., 2007). Inbred lines from diverse subpopulations possess unique alleles that could be useful for crop improvement of different economically important traits (Ayesiga *et al*., 2023). Kasoma *et al*. (2020) stated that genetic variations between and within

populations offer breeders more opportunities to enhance genetic gains during breeding.

Analysis of molecular variance further confirmed that there was higher genetic variation within the subpopulation (65%) compared to variation among the subpopulation (35%). The high variation showed high allelic variability within the inbred lines, resulting in high genetic differentiations (Elec *et al*., 2022).

The 9 clusters generated by neighbor-joining hierarchical cluster analysis using the dissimilarity based on the Euclidean distance between the inbred lines revealed their uniqueness. These results further indicate that the 140 inbred lines were genetically diverse and could be exploited in maize breeding programs. Establishing the genetic relationship between inbred lines is important for maximizing heterosis during hybrid development (Josia *et al*., 2021).

3.5. Conclusion

The study revealed significant phenotypic and genetic diversity among the tropical inbred lines that may be used for maize improvement. Phenotypic diversity observed for FAW leaf and cob damage among the inbred lines can be exploited for trait improvement. The PIC value of SNP markers across the chromosomes showed that the SNP markers used for the diversity studies exhibited between medium to high informativeness and could effectively discriminate among the genotypes. Gene diversity (GD) of 0.35 recorded indicated the presence of moderate diversity. The high level of observed heterozygosity (Ho) further confirmed the presence of genetic diversity within the subpopulation. The population pair-wise fixation index revealed moderate divergence of selected alleles across the subpopulations, which could be used to enhance selection efficiency, broaden the genetic base of the breeding population, and enhance the formation of high-yielding stress-tolerant hybrids adapted for different agroecological zones. Analysis of molecular variance further confirmed the presence of higher genetic diversity within

the subpopulation (65%), resulting in high genetic differentiations that can be equally exploited in breeding. Furthermore, Population structure analysis based on cross-entropy criteria and neighbor-joining hierarchical cluster analysis emphasized that the 140 inbred lines, which were grouped into 9 subclusters, were genetically diverse and could be used in maize breeding programs for improvement.

CHAPTER FOUR

4.0 ESTIMATION OF GENE ACTION FOR RESISTANCE TO FALL ARMYWORM IN TROPICAL MAIZE INBRED LINES

4.1 Introduction

Maize is globally cultivated in over 202 million hectares with a production of about 1.1 billion tons of grain (Al-kahtani *et al*., 2023). FAW is among the most damaging insects affecting maize production in sub-Saharan Africa (Matova *et al*., 2020). The use of host plant resistance (HPR) as an integral part of the FAW management strategy is important in maize farming and offers a cost-effective, safe, and most sustainable method of controlling the pest (Asare *et al*., 2023). However, very few varieties of maize have been identified to be both high-yielding and resistant to FAW in SSA, therefore limiting the use of the HPR management method (Kasoma *et al*., 2021). Rigorous screening of available maize germplasm needs to be carried out to identify sources of resistance genes for developing FAW-resistant maize varieties (Asare *et al*., 2023).

For the successful development of superior hybrids in a maize breeding program, it is important to know the combining ability of parental inbred lines (Tavarini *et al*., 2023). Combining ability analysis is one of the most effective ways of determining the best combiners to use when making crosses for maximum exploitation of heterosis or for the accumulation of productive genes (Temesgen, 2021). Breeders should determine the gene action mode governing a particular trait. There are two types of combining ability, namely the general combining ability (GCA) and the specific combining ability (SCA) (Matongera *et al*., 2023). General combining ability is the average performance of inbred lines in different cross-combinations and is associated with additive genes effects while SCA which is associated with non-additive genes effects (epistasis and dominance) refers to the performance of a specific cross (Engida *et al*., 2024). Inbred lines

showing high GCA means that the additive gene effect is predominant and may be used for the improvement of allele frequencies for the trait of interest in a population and to maximize the genetic gain (Matongera *et al*., 2023). Domination of the non-additive gene action allows selection of the best hybrid combination for maximum heterosis to be achieved (Osuman *et al.*, 2022). North Carolina Design II (NCDII) is one of the most commonly used mating designs for combining ability studies. This is a factorial design involving the grouping of parents into a set of males and females and crossing every member of a male group to every member of the female group, thereby generating a cross-classification design (Ngaboyisonga *et a*l., 2019). NCDII allows for analysis where the sources of variations are divided into males, females, and males x females interaction (Awata *et al*., 2018).

Previous studies on temperate environments documented that FAW resistance in maize was mainly controlled by additive gene effects (Job *et al*., 2022). Equally, non-additive gene effects have also been reported to control FAW resistance (Kasoma *et al.*, 2021). FAW resistance is a quantitative trait that is influenced by the environment; however, there's limited information on the mode of inheritance, stability, and gene action governing FAW resistance in tropical maize inbred (Job *et al*., 2022). The objective of the current study was to evaluate the performance of hybrids and their parents for FAW resistance in different environments to determine their heritability and estimate their combining ability.

4.2 Materials and methods

4.2.1 Plant materials and generation of the hybrids

Forty maize inbred lines with diverse levels of multiple borer resistance and multiple insect-resistant traits were sourced from the International Maize and Wheat Improvement Center (CIMMYT) (Table 4.1). The selected lines were grouped into eight sets of breeding nurseries and randomized; each set had five males and 5 females. The inbred lines were intercrossed using the NCD II mating design. Before pollination, the tassels of the male plants were covered with pollination bags for pollen collection and to avoid contamination. Similarly, shoots of all the female plants were covered with shoot bags before shoot emergence to prevent unwanted pollination from stray pollen. Pollen from each male was collected and used to manually pollinate the silks of a set of 5 females. The process was repeated such that all the males pollinated all the females in a breeding nursery. Each breeding nursery generated 25 single-cross hybrids. A total of 200 hybrids were obtained from the 8 breeding nurseries. One hundred and twenty-three hybrids with enough seed for multi-locational evaluation trials in 3 locations across 2 seasons were selected for evaluation.

Table 4.1. List of inbred lines used as parents for hybrid formation

Parental codes	Genotypes	Parental codes	Genotypes
1	CKL177012	21	CKL21625
2	CKL187008	22	CKL21630
3	CKL187009	23	CKL21653
4	CKL187010	24	CKL21661
5	CKL187011	25	CKL21698
6	CKL187012	26	CKSBL10025
7	CKL187019	27	CKSBL1008
8	CKL201115	28	CML125
9	CKL201171	29	CML24
10	CKL201243	30	CML336
11	CKL201285	31	CML338
12	CKL201297	32	CML444
13	CKL201331	33	CML610A

14	CKL201346	34	DL21113
15	CKL201415	35	DL21115
16	CKL201586	36	CKL21584
17	CKL201598	37	CKL21682
18	CKL201599	38	CML566
19	CKL21611	39	CKL201590
20	CKL21618	40	CKL201281

4.2.2 Experimental design and crop establishment

Evaluation of 123 hybrids and 5 checks was carried out in a 16 x 8 alpha lattice design with 2 replications in 3 sites (Kiboko, Kakamega, and Kirinyaga) for 2 seasons (season A and B) (Table 4.2).

Table 4.2. Geographical and Climatic conditions for Kiboko, Kakamega, and Kirinyaga during 2024 season A (March – May) and B (October – December)

Name of Location	Geographic location			Mean seasonal Rainfall (mm)	Seasonal Temperature (˚C)		Soil type
	Longitude	Latitude	Altitude		Min	Max	
Kiboko	37˚75˚E	2˚15˚S	993m				Semi-arid with ferrasols to Ferric Luvisol soils
Season A				2.87	18.13	32.19	
Season B				12.62	18.54	29.99	
Kirinyaga	37.38° E	0.66° S	1159m				Andisols soils
Season A				318.63	16.77	27.17	
Season B				127.43	15.50	26.87	
Kakamega	34˚45˚E	0˚16˚N	1585m				Sub-humid with basaltic loam soils
Season A				236.5	15.56	26.85	
Season B				232.17	15.00	26.48	

Source: Kenya Meteorological Department, 2024

Two seeds per hill of each inbred line were sown in a 5m single row plot with an inter-row spacing of 0.75m and inter-hills spacing of 0.25m, and later thinned to one. Fertilizer was applied at recommended rates (60kg N and 60kg P_2O_5 ha^{-1}) and the field kept free from weeds. Supplementary irrigation was applied on a need basis. In Kiboko, the hybrids were evaluated

under artificial FAW infestation. The FAW larvae were reared and infestation carried out as described in Chapter 3. In Kirinyaga and Kakamega, the hybrids were evaluated under natural infestation.

4.2.3 Data collection

Morphological data on FAW leaf damage scores taken 7 days (LD1), 14 days (LD2) and 21 days (LD3) after FAW infestation, plant and ear height, ear aspect, grain yield (t ha^{-1}), cob damage and ear rot parameters were collected are as described in Chapter 3.2.3.2.

4.2.4 Data Analysis

Analysis of combining ability was carried out using *plantbreeding* and *lmer* packages in R statistical software (R). GCA and SCA averaged across locations and seasons were computed using *carolina2* function of *plantbreeding* package in R. Combining ability on each location and ANOVA was computed using mixed linear model implemented on *lmer* package in R.

$$y_{ijklmn} = u + L_i + Sn_j + S_k + r_l + M_m + F_n + L{:}S{:}M_{ijm} + L{:}S{:}F_{ijn} + L{:}S{:}M{:}F_{ijmn} + \varepsilon_{ijklmn}$$

Where: μ is general mean, L= Effect of location, Sn=Random effect of season, S = effect of set, r= random effect of replication, M =fixed effect of males, F = fixed effects of females, and ε= residue terms

Combined Analysis of variance for NCDII was carried out across the 3 locations for each trait being evaluated using R software. The analysis was to determine the effects of location, season, replication, males, females and their interaction. The variations within the hybrid component were partitioned into males, females and male x female interaction.

General combining ability (GCA) was defined as the main effect male sets and female set while the specific combining ability (SCA) was the female × male interaction effect. The ratio of GCA to SCA was determined using Baker's ratio formula (Osuman *et al.*, 2022).

Bakers' ratio = $\frac{GCA}{SCA} = \frac{2\sigma 2GCA}{2\sigma 2GCA + \sigma 2SCA}$ $2\sigma^2GCA$= GCA male +GCA female

Using *ranef* function, GCA and SCA effects were generated. The variance components for GCA, SCA, and residual were extracted and used to narrow-sense (h^2) heritability. Check formula

$$h^2 = \frac{\delta_A^2}{\delta_A^2 + \delta_D^2 + \delta_r^2}$$

$$\delta_A^2 = \delta_M^2 + \delta_F^2$$

Where δ_M^2=Variance component for GCA males, δ_F^2=Variance components for GCA females, δ_D^2= Dominance (SCA) variance components and δ_r^2 =residual term variance component

4.3 Results

The combined analysis of variance across the three locations (Kiboko, Kirinyaga, and Kakamega) showed that highly significant mean squares (< 0.001) were observed for location, seasons, male, female, and location by season interaction for all the FAW leaf damage traits (LD1, LD2, LD3) and cob damage (Table 4.3). In addition, significant mean squares at P < 0.001, P < 0.01, and P

< 0.05 were observed in all the sources of variation in cob damage except for replication. Significant differences (< 0.01) were observed for the interaction of location x male in LD1, LD3 at P < 0.05, and cob diameter at P < 0.001. The location x female interaction was significant for LD3 and cob diameter. The season x location x male x female interaction was significant for cob diameter (Table 4.3).

Table 4.3. Combined mean squares for FAW damage of hybrids evaluated in Kiboko, Kakamega and Kirinyaga during the 2024 seasons A and B

Source of Variation	DF	LD1	LD2	LD3	Cob Damage
Location	2	80.94***	1285.05***	397.64***	286.73***
Season	1	181.51***	182.76***	329.64***	24.31***
Rep	1	0.02	0.85	0.90	0.05
Male	34	1.14***	1.28***	1.35***	1.2***
Female	33	1.03***	1.02**	1.19***	1.36***
Male x Female	122	0.46	0.46	0.46	0.53
Location:Male	68	0.68**	0.58	0.74*	0.86***
Location:Female	66	0.43	0.70	0.85**	0.87***
Season:Male	34	0.53	0.43*	0.31	0.54**
Season:Female	33	0.41	0.54	0.56	0.74***
Location:Season	2	19.450***	58.52***	401.2***	153.64***
Location:Male:Female	244	0.40	0.42	0.46	0.51***
Season:Male:Female	122	0.53	0.53	0.40	0.39*
Location:Season:Male	68	0.49	0.49	0.56	0.52***
Location:Season:Female	66	0.38	0.59	0.61	0.74***
Location:Season:Male:Female	244	0.55	0.60	0.45	0.40**
Residuals	736	0.45	0.49	0.53	0.29

Significant difference codes: 0 '***' 0.001 '**' 0.01 '*' 0.05
Df - degrees of freedom; Leaf damage score at 7 days (LD1), 14 days (LD2) and 21 days (LD3) after FAW infestation

Highly significant mean squares (p< 0.001) were observed in location for plant height, ear height, ear aspect, grain yield, and ear rot (Table 4.4). Similar observations were shown on male, female, and location x male for all traits except grain yield. Highly significant mean squares (< 0.001) were also seen in season for plant height, grain yield, and ear rot. Significant mean squares (< 0.01) were observed in the male x female interaction for plant height, ear height, and ear rot. Additionally, location x female interaction significantly varied (p< 0.05) for ear height and P < 0.001 for ear aspect and ear rot. Location x season interaction was significant at P < 0.001 for plant height, ear height, and ear rot, and at P < 0.05 for ear aspect. Location x season x male interactions for ear height, ear aspect, and ear rot were significant, as well as location x season x female interactions for ear aspect and ear rot. The season x location x male x female interaction was significant for ear rot (Table 4.4)

Table 4.4. Combined mean squares for agronomic traits of hybrids evaluated in Kiboko, Kakamega and Kirinyaga during the 2024 seasons A and B

Source of Variation	DF	Plant Height	Ear height	Ear Aspect	Grain yield (t ha^{-1})	Ear rot
Location	2	218198***	80239***	24.7***	1471.17***	23239.6***
Season	1	56684***	365	0.04	1151.92***	1590.2***
Rep	1	1048	262	2.99**	90.87	0.0242
Male	34	7308***	3458***	1.64***	143.79	199.1***
Female	33	5766***	4405***	1.26***	87.72	257.8***
Male: Female	123	902***	290***	0.47	147.36	92.1**
Location: Male	68	598***	278***	1.01***	155.28	134.5***
Location: Female	66	337	210*	1.04***	82.59	139.1***
Season: Male	34	330	272**	0.74**	153.01	86.7*
Season: Female	33	274	179	0.61	92.61	83.8.
Location: Season	2	17634***	18851***	1.9*	113.26	10917.8***
Location:Male: Female	246	293	132	0.48	142.34	81.3**
Season:Male: Female	123	224	156	0.32	143.57	44.3
Location:Season: Male	68	358	195*	0.64*	151.8	82.8**
Location:Season: Female	66	316	143	0.71**	88.13	92.9**
Location:Season:Male: Female	246	287	118	0.41	140.19	72.5*
Residuals	736	297	140	0.44	132.88	56.1

Significant difference codes: 0 '***' 0.001 '**' 0.01 '*' 0.05, Df - degrees of freedom

Male entries 8,9,13,15,17,18,20,32,33,34 in LD1, 6,8,9,12,23,32,33,34 in LD2, 1,13,32,33,34 in LD3, 1,2,3,12,14,24,29,30,33,35 in cob damage, 1,3,14,29,30,34,35 in ear rot 2,3,5,6,10,19,20,22,24,25,26,27,28,29,31,32 in plant height, 2,3,6,10,11,12,14,19,20,25,26,27,32,34 in ear height and 2,6,11,12,15,18,28,29,30,34,35 in ear aspect exhibited significant positive GCA effects. Significant negative GCA effects were observed in entries 4,7,11,19,21,24,25,26,27,28,29 in LD1, 4,7,19,24,25,26,27,28,29 in LD2, 3,7,11,19,27,28 in LD3, 6,7,8,15,17,18,20,21,23,25,26,27,31 in cob damage, 7,8,15,20,23,24,25,26,27,31 in ear rot, 1,4,7,8,9,12,13,16,17,18,21,23,30,33,34 in plant height, 1,4,7,8,9,13,16,17,18,23,29,30,33,35 in ear height and 3,5,7,8,9,16,17,20,21,22,23,25,26,27,30,31,32 in ear aspect. No significant GCA male effects were recorded for grain yield (Table 4.5)

Table 4.5. Combined male GCA effect for FAW damage and agronomic traits of hybrids evaluated in Kiboko, Kakamega and Kirinyaga during the 2024 seasons A and B

M	LD1	LD2	LD3	CD	Ear rot	PH	EH	EA	GY
1	0.01	-0.02	0.03**	0.04*	1.53***	-7.94***	-15.01***	-0.02	0
2	0.03	0.02	-0.01	0.05**	0.17	8.35***	5.73***	0.18***	0
3	-0.02	-0.03	-0.03*	0.07***	0.79***	5.84***	7.77***	-0.11***	0
4	-0.04*	-0.07**	-0.01	-0.01	0.26	-19.18***	-11.76***	0.05	0
5	0	0.03	-0.01	0	-0.18	2.56	2.04	-0.08**	0
6	0.01	0.17***	0.02	-0.08***	-0.31	18.2***	11.71***	0.11***	0
7	-0.06**	-0.07***	-0.03*	-0.02	-0.66***	-15.45***	-7.6***	-0.13***	0
8	0.08***	0.05*	0.02	-0.03*	-0.43*	-6.41***	-4.68***	-0.17***	0
9	0.06***	0.07***	0.01	0.02	-0.08	-4.92***	-5.57***	-0.09***	0
10	-0.01	-0.02	0	0	-0.3	6.02***	11.97***	-0.03	0
11	-0.04*	0.01	-0.03*	-0.01	-0.18	2.55	5.1***	0.14***	0
12	0.02	0.05*	0.02	0.03*	0.33	-3.87*	2.7*	0.12***	0
13	0.06**	0.03	0.02*	-0.01	0.16	-5.1**	-14.65***	-0.05	0
14	0	0	-0.02	0.05***	0.54***	1.75	5.46***	0.01	0
15	0.09***	-0.01	0	-0.04**	-0.28*	1.43	-0.18	0.05**	0
16	-0.02	-0.01	0.01	0	0.16	-9.77***	-2.08**	-0.2***	0
17	0.08***	0.01	0	-0.02*	-0.19	-15.08***	-7.46***	-0.03*	0
18	0.04**	-0.01	0	-0.02	-0.14	-9.97***	-1.11	0.13***	0
19	-0.07***	-0.07***	-0.04***	0	-0.05	8.93***	2.86***	-0.01	0
20	0.03*	0	0	-0.02*	-0.68***	21.51***	13.07***	-0.06**	0

21	-0.04*	-0.02	-0.01	-0.02**	-0.03	-2.68**	1.04	-0.14***	0
22	-0.03	0.02	0	-0.01	-0.05	5.54**	-1.76	-0.11***	0
23	-0.01	0.09***	0.01	-0.04**	-0.5**	-3.68*	-10.09***	-0.1***	0
24	-0.09***	-0.07***	0	0.07***	0.64***	4.78**	1.23	0.04	0
25	-0.08***	-0.03*	-0.01	-0.04***	-0.55**	9.5***	4.62***	-0.06**	0
26	-0.05*	-0.05*	0	-0.07***	-0.67**	9.72***	8.73***	-0.07*	0
27	-0.08***	-0.13***	-0.04**	-0.07***	-0.85***	8.51***	6.11***	-0.07*	0
28	-0.19***	-0.17***	-0.06***	0.01	-0.21	14.31***	0.36	0.28***	0
29	-0.04**	-0.05**	0.01	0.03*	0.51**	4.05***	-6.08***	0.07***	0
30	-0.02	-0.03	-0.01	0.1***	0.81***	-16.96***	-15.32***	0.14***	0
31	0.01	-0.03	-0.02	-0.05***	-0.98***	9.85***	1.97	-0.13***	0
32	0.21***	0.19***	0.05***	0	0.06	6.71***	4.5**	-0.04	0
33	0.09***	0.08***	0.03**	0.04**	0.2	-18.45***	-5.16***	0.03	0
34	0.11***	0.11***	0.02*	0.02	0.76***	-6.28***	8.55***	0.21***	0
35	-0.01	-0.04	0.02	0.04**	0.54**	-3.68*	-3.66**	0.18***	0
SE	0.02	0.02	0.01	0.01	0.14	1.5	1.31	0.01	0

Significant difference codes: ***' 0.001 '**' 0.01 '*' 0.05, SE_ standard error, M_ male parent, LD1_Leaf damage score at 7 days, LD2_14 days and LD3_21 days after FAW infestation, CD_ cob damage, PH_ plant height, EH_ ear height, EA_ ear aspect, GY_ grain yield (t ha^{-1}), ER_ ear rot

Significant positive GCA effect was detected on female entries 1,2,8,9,12,13,17,20,21,30,32,33,34,34 in LD1, 6,8,10,12,13,17,21,32,33,35 in LD2, 1,2,3,9,12,13,24,25,32,33,34,35 in cob damage, 1,2,3,12,22,24,32,34,35 in ear rot, 3,5,6,10,11,20,22,24,25,26,31,32,34 in plant height, 2,3,5,6,10,11,14,20,24,25,26,32,34 in ear height, 3,12,20,23,27,32,33,34,35,36 in ear aspect, and 27 in grain yield. Similarly, significant negative GCA effects were identified on entries 5,7,11,14,15,18,19,22,23,24,25,27,31,36 in LD1, 11,19,23,24,27,31,35 in LD2, 5,6,7,8,10,11,16,17,19,23,26,31,36 in cob damage, 5,8,10,11,13,16,17,18,19,20,21,23,30,31,33 in ear rot, 1,7,9,12,13,16,17,18,23,30,33,35,36 in plant height, 1,7,8,13,16,17,18,23,30,31,33,35,36 in ear height, 5,7,10,11,16,21,24,25,31 in ear aspect and entry 27 in grain yield. There were no significant female GCA effects identified in LD3 (Table 4.6)

Table 4.6. Combined female GCA effect for FAW damage and agronomic traits of hybrids evaluated in Kiboko, Kakamega and Kirinyaga during the 2024 seasons A and B

F	LD1	LD2	LD3	CD	E_rot	PH	EH	EA	GY
1	0.05**	0.03	0	0.21***	4.16***	-14.82***	-15.17***	-0.01	-0.01
2	0.04*	-0.02	0	0.09***	1.67***	1.99	4.56**	0.01	-0.03
3	0	-0.01	0	0.15***	2.56***	9.75***	10.55***	0.26***	-0.03
5	-0.01	-0.02	0	-0.16***	-2.77***	5.5***	6.6***	-0.09***	-0.06
6	0.01	0.04*	0	-0.07**	-0.28	12.02***	9.88***	-0.02	0.01
7	-0.05	-0.02	0	-0.1***	-0.67	-15.25***	-11.78***	-0.09***	-0.03
8	0.05***	0.04**	0	-0.08***	-1.86***	-1.71	-2.37**	-0.02	0.02
9	0.03*	0.01	0	0.12***	2.03***	-2.6**	0.4	0.02	0.06
10	0	0.05***	0	-0.2***	-2.84***	6.4***	16.83***	-0.06***	0.03
11	-0.04**	-0.09***	0	-0.05***	-1.3***	15.99***	13.18***	-0.07***	0.05
12	0.06***	0.03*	0	0.14***	2.09***	-6.69***	0.02	0.11***	-0.03
13	0.05***	0.03*	0	0.05**	-0.79***	-9***	-15.89***	-0.01	-0.06
14	-0.04**	0	0	0.04	0.6	0.17	3.08*	0.03	0.01
15	-0.05**	0.02	0	-0.03	-0.28	1.87	1.3	0.02	0.01
16	0.01	0.02	0	-0.11***	-1.6***	-8.82***	-7.18***	-0.11***	-0.02
17	0.04**	0.03**	0	-0.09***	-1.06**	-10.64***	-3.77***	-0.01	-0.01
18	-0.03	0.02	0	-0.02	-1.28**	-11.16***	-5.08***	-0.04	0.05
19	-0.07***	-0.06***	0	-0.12***	-1.96***	1.38	1.95	-0.14	-0.02
20	0.03*	0.01	0	-0.03	-1.06*	12.7***	4.96**	0.06**	-0.03
21	0.11****	0.04**	0	-0.05	-0.93*	-0.15	-1.88	-0.16***	0.05
22	-0.02	0.01	0	0.03	2.01***	9.06***	2.68	0	0.01
23	-0.04**	-0.03*	0	-0.07**	-1.44***	-7.85***	-14.46***	0.06**	-0.07
24	-0.04*	-0.06**	0	0.13***	1.32***	9.87***	5.97***	-0.06**	-0.03
25	-0.06***	-0.02	0	0.08**	-0.67	8.42***	6.01***	-0.09***	-0.01
26	-0.01	-0.01	0	-0.05*	0.66	6.74***	9.39***	-0.02	-0.05
27	-0.12***	-0.09***	0	-0.03	-0.57	1.45	1.14	0.06**	0.44***
29	-0.02	0.01	0	0.01	0.43	11.23	-1.37	0.02	0
30	0.03*	0	0	0	-0.71**	-9.85***	-11.74***	0.02	-0.03
31	-0.08***	-0.08***	0	-0.05*	-1.48***	3.66***	-2.74**	-0.18***	0
32	0.03**	0.06***	0	0.07**	1.04**	2.81**	2.25**	0.1***	-0.03
33	0.03*	0.03*	0	0.06**	-0.59*	-21.25***	-9.94***	0.06***	-0.02
34	0.04**	0.01	0	0.15***	4.23***	13.54***	17.03***	0.08***	0.01
35	0.07***	0.06***	0	0.12***	1.13***	-10.27***	-8.99***	0.24***	-0.04
36	-0.03**	-0.04**	0	-0.02	0.05	-4.01***	-5.48***	0.06***	-0.06
SE	0.01	0.01	0	0.02	0.29	0.89	1.5	0.02	0.06

Significant difference codes: ***' 0.001 '**' 0.01 '*' 0.05, SE_ standard error, F_ female parent, LD1_Leaf damage score at 7 days, LD2_14 days and LD3_21 days after FAW infestation, CD_ cob damage, PH_ plant height, EH_ ear height, EA_ ear aspect, GY_ grain yield (t ha^{-1}), ER_ ear rot

Specific combining ability estimates for plant height, ear height and ear rot showed significant variation among the 123 hybrids evaluated in Kiboko, Kirinyaga and Kakamega. Significant positive SCA effect was observed for plant height in hybrids 1x20, 2x34, 3x16, 4x11, 5x14, 6x11, 6x16, 6x17, 7x15, 8x21, 8x25, 9x19, 9x21,10x19, 10x35, 11x05, 11x26, 12x32, 13x2,13x27, 14x2, 14x32, 15x33, 16x33, 17x26, 18x32, 19x6, 19x7, 19x31, 20x8, 20x9, 20x10, 21x6, 21x31, 22x29, 26x12, 26x24, 28x20, 28x22, 29x35, 31x11, 31x16, 32x34, 32x36, 33x14, 33x24, 34x10, 35x3. In addition, there were significant negative SCA effect observed in crosses 1x22, 1x34, 1x36, 3x17, 5x24, 6x15, 7x16, 7x17, 8x35, 9x25, 9:35, 10x21, 10x25, 11x33, 12x27, 13x1, 13x32, 14x27, 15x5, 16x26, 16x30, 17x05, 17x30, 18x02, 18x27, 19x3, 20x29, 21x7, 22x10, 23x10, 23x29, 24x2, 26x13, 26x18, 27x22, 28x23, 28x36, 29x19, 30x14, 32x22, 32x23, 33x12, 33x18, 34x8, 34x9, 35x6, 35x7, 35x31 for plant height.

Estimates of SCA for ear height exhibited significant positive SCA effect in 42 hybrids (1x20, 2x34, 3x11, 6x16, 6x17, 7x15, 7x17, 8x21, 8x25, 9x19, 9x21, 9x25, 10x35, 11x05, 11x30, 12x27, 15x33, 16x26, 16x33, 18x32, 19x6, 19x31, 20x8, 21x3, 21x31, 22x10, 23x29, 24x2, 25x3, 26x12, 26x14, 28x20, 28x34, 29x35, 30x14, 32x23, 32x36, 33x14, 33x24, 34x10, 34x29, 35x3). Likewise, 24 hybrids had significant negative SCA effect for ear height, these included 1x23, 1x34, 1x36, 3x17, 4x11, 6x11, 7x16, 8x35, 9X35, 10x21, 11x33, 12x2, 13x1, 13x32, 15x5, 16x30, 17x05, 17x30, 18x02, 19x3, 21x7, 22x29, 23x10, 24x27, 24x32, 25x7, 25x31, 26x13, 26x18, 27x20, 27x23, 27x36, 28x22, 28x23, 28x36, 29x19, 29x21, 31x11, 32x22, 33x12, 33x18, 35x31.

Twenty-two crosses (1x34, 1x36, 2x22, 3x17, 9x25, 11x33, 13x1, 14x27, 16x26, 18x27, 19x3, 19x7, 21x3, 24x32, 28x34, 28x36, 32x22, 33x12, 33x24, 34x9, 34x29, 35x6) had significant positive SCA effect for ear rot. In addition, significant negative SCA effect for ear rot were observed in twenty-three hybrids (2x34, 7x11, 7x15, 8x25, 9x21, 10x19, 13x2, 14x32, 15x33,

16x30, 19x6, 20x29, 24x27, 25x3, 26x12, 26x13, 27x22, 28x22, 31x11, 31x16, 32x23, 32x36, 33x14). Non-significant SCA effects were recorded in FAW leaf and cob damage, ear aspect and grain yield (Table 4.7).

Table 4.7. Combined SCA effect for FAW damage and agronomic traits of hybrids evaluated in Kiboko, Kakamega and Kirinyaga during the 2024 seasons A and B

H	PH	EH	ER	H	PH	EH	ER
1x20	3.05***	0.47*	-0.05	19x7	1.03**	-0.06	0.07**
1x22	-1.73**	0.48	0.02	19x31	1.13***	1.46***	-0.08
1x23	-0.47	-0.73**	0	20x8	3.8***	1.24***	0.03
1x34	-1.75***	-1.07***	0.23***	20x9	2.19***	0.31	0
1x36	-2.19***	-1.54***	0.18***	20x10	1.99***	0.16	0
2x22	-0.48	0.13	0.17***	20x29	-2.42***	0.11	-0.08*
2x23	0.36	-0.05	0.05	21x3	0.07	0.88***	0.09*
2x34	3.37***	0.7**	-0.21***	21x6	1.9***	-0.11	-0.03
3x11	0.65	1.35***	0.05	21x7	-4.84***	-1.18***	-0.01
3x16	2.36***	-0.07	0.03	21x31	1.9***	0.71**	-0.04
3x17	-2.06***	-0.61***	0.13***	22x10	-1.76***	1.9***	-0.02
4x11	0.85*	-1.07***	0.04	22x29	3.78***	-1.73***	0
4x16	-7.51***	-0.35	0.01	23x10	-4.37***	-2.21***	0.02
5x13	-0.78	-0.24	0	23x29	2.88***	0.82**	-0.05
5x14	1.78***	0.41	-0.04	24x1	0.86	-0.27	0.03
5x24	-1.24***	0.04	-0.03	24x2	-1.17*	2.26***	0.08
6x11	2.58***	-0.89***	-0.04	24x27	2.95	-1.03***	-0.12**
6x15	-1.89***	0.54***	0.01	24x32	-1.04*	-0.73**	0.13**
6x16	2.29***	1.78***	-0.02	25x3	4.06***	1.7***	-0.07*
6x17	2.76***	-0.06	-0.08	25x7	-1.58***	-0.76***	-0.06
7x11	-0.69	0.47	-0.12**	25x31	0.59	-0.45***	-0.02
7x15	2.5***	-0.5*	-0.11*	26x12	3.24***	2.37***	-0.21***
7x16	-3.09***	-2.14***	0.08	26x13	-2.65***	-1.87***	-0.07**
7x17	-3.8***	0.77**	-0.08	26x14	0.49	0.83***	0.04
8x21	1.53**	1.09***	-0.04	26x18	-0.98**	-0.26*	0.03
8x25	4.91***	0.41**	-0.07*	26x24	1.8***	-0.3	-0.01
8x35	-8.89***	-1.71***	0	27x20	-1.38**	-1.32***	0.05
9x19	2.79***	0.58*	0	27x22	5.9***	3.41***	-0.12**
9x21	1.64***	0.8**	-0.1*	27x23	-0.66	-0.73**	-0.02
9x25	-1.24***	0.35**	0.08**	27x36	-1.76	-0.93***	-0.02
9X35	-5.86***	-2.46***	0.06	28x20	1.86***	1.15***	0
10x19	1.42**	-0.16	-0.12**	28x22	5.2***	-1.19***	-0.13**
10x21	-1.07*	1.02***	0.05	28x23	-1.18*	-1.31***	-0.03
10x25	-0.99	-0.22	0.01	28x34	0.66	1.84***	0.1*
10x35	2.37***	0.74***	0	28x36	-1.68***	-0.82**	0.11*

11x05	14.63***	4.36***	-0.05	29x19	-3.69***	-0.51***	0.05
11x26	2.83***	0.21	-0.02	29x21	-2.75	-3.1***	0.06
11x30	0.92	1.09***	-0.03	29x35	7.98***	2.72***	0.06
11x33	-19.35***	-5.01***	0.07**	30x12	0.68	-0.19	0.06
12x2	-0.53	-0.6***	0.01	30x13	-0.51	0.33	0.09
12x27	-0.69*	0.87***	-0.03	30x14	-6.02***	-2.28***	0.04
12x32	0.67*	-0.14	0.01	31x11	1.75***	1.01***	-0.1*
13x1	-6.48***	-0.97***	0.16***	31x16	2.43***	0.15	-0.14**
13x2	1.98***	0.06	-0.09*	31x17	-0.74	-0.44	-0.02
13x27	3.9***	0.38	-0.07	32x20	0.32	0.2	0.02
13x32	-1.2*	-1***	0.02	32x22	-3.88***	-2.71***	0.12**
14x2	2.21***	0.01	0.08	32x23	-1.1***	0.77***	-0.11**
14x27	-3.82***	0.14	0.2***	32x34	2.86***	0	0.03
14x32	1.79***	0.26	-0.14**	32x36	3.32***	2.33***	-0.12***
15x5	-6.37***	-2.99***	0.02	33x12	-7.78***	-2.1***	0.24***
15x33	7.12***	3.16***	-0.1*	33x14	3.64***	1.17***	-0.11*
16x26	-3.61***	0.54***	0.09*	33x18	-3.58***	-0.66***	-0.05
16x30	-4.15***	-1.73***	-0.09*	33x24	1.75***	1.07***	0.14**
16x33	4.23***	1.03***	-0.01	34x8	-6.59***	-1.32***	-0.07
17x05	-5.53***	-0.56*	-0.06	34x9	-2.93***	0.18	0.17***
17x26	1.92***	0.42	0.05	34x10	5.88***	1.65***	-0.06
17x30	-1.09**	-0.39**	0	34x29	0.91	0.83***	0.18***
18x02	-1.47**	-1.38***	0.05	35x3	1.66***	0.45*	-0.04
18x27	-2.19***	-0.27	0.09*	35x6	-1.34**	0.2	0.16***
18x32	1.04**	1.79***	-0.06	35x7	-0.24	0.31	-0.07
19x3	-3.56***	-2.02***	0.14***	35x31	-2.88***	-1.35***	0.05
19x6	3.73***	1.02***	-0.13***				
SE	0.33	0.23	0.05		0.33	0.23	0.05

Significant difference codes: ***' 0.001 '**' 0.01 '*' 0.05 H_ hybrid, PH_ plant height, EH_ ear height, ER_ ear rot, SE_ standard error

There were highly significant ($p < 0.001$) mean squares observed in male and female GCA effects for all traits evaluated except for grain yield (Table 4.8). Highly significant ($p < 0.001$) mean squares for SCA effects were only observed in plant height and ear height, as well as significant ($p < 0.01$) for ear rot. GCA/SCA ratio recorded was 83% in LD1 and LD2, 85% in LD3, 94% in plant height, 96% in ear height, 86% in ear aspect, 61% in grain yield, and 83% in ear rot (Table 4.8).

Table 4.8. Mean squares for GCA and SCA effects for FAW resistance and agronomic traits

Source of Variation	DF	LD1	LD2	LD3	C D	Plant Height	Ear height	Ear Aspect	Grain yield	Ear rot
GCA Male	34	1.14 ***	1.28 ***	1.35 ***	1.2 ***	7308 ***	3458 ***	1.64 ***	143.79	199.1 ***
GCA Female	26	1.03 ***	1.02 **	1.19 ***	1.36 ***	5766 ***	4405 ***	1.26 ***	87.72	257.8 ***
Total GCA	62	2.17	2.3	2.54	2.56	13074	7863	2.9	231.51	456.9
SCA(MXF)	34	0.46	0.46	0.46	0.53	902 ***	290 ***	0.47	147.36	92.1 **
GCA/SCA ratio		0.83	0.83	0.85	0.83	0.94	0.96	0.86	0.61	0.83

Significant difference codes: ***' 0.001 '**' 0.01 '*' 0.05, Df - degrees of freedom; Leaf damage score at 7 days (LD1), 14 days (LD2) and 21 days (LD3) after FAW infestation, CD_FAW cob damage scores

Additive variance was greater than dominance variance for all the traits examined. FAW damage traits and ear rot percentage recorded higher phenotypic variance compared to genotypic (additive + dominance) variance, resulting in low heritability values of below 30%. In addition, these traits had high residual variance. Moderate heritability was observed for ear aspect (0.43) and grain yield (58%), while high heritability values were observed for ear height (78%) and plant height (71%). Similarly, traits with high heritability values recorded higher genotypic variance than phenotypic variance. (Table 4.9).

Table 4.9. Variance components and heritability of maize hybrids evaluated under artificial FAW infestation in Kiboko, and natural FAW infestation in Kakamega and Kirinyaga during 2024 season A and B

Trait	Additive Variance	Dominance Variance	Residual Variance	Phenotypic Variance	h^2
Leaf damage 1 (LD1)	0.07	0	0.49	0.56	0.13
Leaf damage 2 (LD2)	0.04	0	0.6	0.64	0.06
Leaf damage 3 (LD3)	0.02	0	0.56	0.58	0.03
Cob damage	0.12	0	0.64	0.76	0.15
Plant height	973.17	62.02	328.21	1363.4	0.71
Ear height	663.93	6.33	181.06	851.32	0.78
Ear aspect	0.36	0	0.46	0.83	0.43
Grain yield	0.22	0.01	0.15	0.38	0.58
Ear rot	27.25	3.34	82.07	112.66	0.24

4.4 Discussion

Determination of the pattern of inheritance of FAW resistance and important agronomic traits in tropical maize by estimating the gene action governing the traits is key during breeding. From the study, the significant mean squares observed in males and females for FAW LD1, LD2, LD3, cob damage, plant and ear height, ear aspect, and ear rot percentage indicated the presence of GCA effect, which implies that additive gene action would be effective for selection of the traits. Based on the additive gene action observed, the traits could be improved through recurrent selection (Agbowuro and Salami, 2021). A similar finding was reported on FAW leaf damage in maize (Asare *et al*., 2023). Significant mean squares for crosses (male x female) plant and ear height and ear rot percentage confirmed the presence of SCA effects, showing that non-additive genes were also involved in their regulation and therefore breeding heterosis could be exploited (Fulgence *et al*., 2019).In the analysis of variance for NCDII, the mean squares of male and female parents indicate the GCA effects, while the mean squares of crosses show SCA effects (Ngaboyisonga *et al*., 2019).

Significant mean squares for male, female, and their interactions with location and season for the traits evaluated showed that the inbred lines and hybrids displayed a broad range of variation in performance across the evaluation sites (Osuman *et al*., 2022). Particularly, variation due to location was highly significant for cob damage, plant and ear height, grain yield, and ear rot, and variation due to season was highly significant for plant height and ear rot.

Selection of parental lines for FAW resistance in maize is based on significant negative combining ability estimates for the damage scores. Negative GCA and SCA indicate that the genotypes have alleles associated with resistance and can be used as sources of resistant genes when improving the trait (Kamweru *et al*., 2023). From the current study, entries 4,7,11,19,21,24,25,26,27,28,29 in LD1, 4,7,19,24,25,26,27,28,29 in LD2, 3,7,11,19,27,28 in LD3

and 6,7,8,15,17,18,20,21,23,25,26,27,31 in cob damage exhibited significant negative GCA male effects and entries 5,7,11,14,15,18,19,22,23,24,25,27,31,36 in LD1, 11,19,23,24,27,31,35 in LD2 and 5,6,7,8,10,11,16,17,19,23,26,31,36 in cob damage had significant negative female GCA effects for fall armyworm damage traits. These lines can be used parental lines for improvement of tropical maize germplasm for resistance to FAW in breeding programs. Lines with significant negative GCA effect are reported to have the potential of transmitting the FAW-resistant genes to their progenies (Asare *et al*., 2023). Entries with negative GCAs for insect resistance are considered as good combiners and can be used for developing resistant hybrids or open-pollinated varieties (Karaya *et al*., 2009).

Negative GCA effect is also an important indicator of good combiners when selecting parental lines for improvement of maize for resistance to ear rot disease. Hybrids with high levels of resistance to ear rot are formed from inbred lines with a negative GCA effect (Ayesiga *et al*., 2025). Male entries 3,4,6,7,31 and females 7,9,24,28 with a significant negative GCA effect for ear rot from the study can be used for improvement of resistance to ear rot in maize. Inbred lines with positive GCA effects for pest and disease resistance are considered to be susceptible and therefore selected against (Ayesiga *et al*., 2025). That is because the lines are deemed to be susceptible to FAW infestation and ear rot, respectively, and should therefore be excluded when breeding for FAW and ear rot disease in maize (Tembo *et al*., 2022).

Significant positive GCA effects in plant and ear height in inbred lines are important for height improvement in maize (Tilahun *et al*., 2019). Male entries 2,3,5,6,10,19, 20,22,24,25,26,27,28,29,31,32, and female lines 3,5,6,10,11,20,22,24,25,26,31,32,34, with significant positive GCA effect for plant height as well as male lines 2,3,6,10,11,12,14,19,20,25,26,27,32,34, and female entries 2,3,5,6,10,11,14,20,24,25,26,32,34 with significant positive GCA effect for ear height identified from the study could be used as

parents when breeding for increased plant height in maize. However, shorter hybrids are preferred in environments prone to lodging (Osuman *et al*., 2022). Therefore, male entries 1,4,7,8,9,12,13,16,17,18,21,23,30,33,34 and female entries 1,7,9,12,13,16,17,18,23,30,33,35,36 with significant negative GCA effect for plant height from the study can be used for the reduction of plant height in maize. Entry 27 has a significant positive GCA effect for grain yield and can contribute favorable alleles for improvement of grain yield potential when used as parents in the formation of a hybrid (Engida *et al*., 2024). Similar findings have been reported (Bedassa *et al*., 2021).

Based on the SCA estimates, hybrids 1x20, 2x34, 3x16, 4x11, 5x14, 6x11, 6x16, 6x17, 7x15, 8x21, 8x25, 9x19, 9x21,10x19, 10x35, 11x05, 11x26, 12x32, 13x2,13x27, 14x2, 14x32, 15x33, 16x33, 17x26, 18x32, 19x6, 19x7, 19x31, 20x8, 20x9, 20x10, 21x6, 21x31, 22x29, 26x12, 26x24, 28x20, 28x22, 29x35, 31x11, 31x16, 32x34, 32x36, 33x14, 33x24, 34x10, 35x3 with positive significant SCA effect for plant height and those with positive significant SCA effect for ear height (1x20, 2x34, 3x11, 6x16, 6x17, 7x15, 7x17, 8x21, 8x25, 9x19, 9x21, 9x25, 10x35, 11x05, 11x30, 12x27, 15x33, 16x26, 16x33, 18x32, 19x6, 19x31, 20x8, 21x3, 21x31, 22x10, 23x29, 24x2, 25x3, 26x12, 26x14, 28x20, 28x34, 29x35, 30x14, 32x23, 32x36, 33x14, 33x24, 34x10, 34x29, 35x3) are suitable for improving the traits in maize. Hybrids with significant negative SCA effect for ear rot (2x34, 7x11, 7x15, 8x25, 9x21, 10x19, 13x2, 14x32, 15x33, 16x30, 19x6, 20x29, 24x27, 25x3,

26x12, 26x13, 27x22, 28x22, 31x11, 31x16, 32x23, 32x36, 33x14) indicates that the crosses have a low incidence of the disease and are therefore desirable when breeding for resistance to maize ear rot (Tembo *et al*., 2022). Non-significant SCA effects observed in FAW leaf and cob damage parameters suggested that the traits were controlled by additive gene action. Equally, non-significant negative SCA have been reported for the FAW leaf damage trait (Asare *et al*., 2023).

However, contrary to these findings, maize hybrids with significant negative SCA effects for FAW damage have been identified (Asare *et al*., 2023; Kamweru *et al*., 2023).

The comparison of GCA to SCA mean squares through Baker's displayed that GCA effects were higher (above 0.61) for all the traits, indicative of the high contribution of GCA effects in regulating the traits. GCA/SCA (Baker's) ratio observed in the study was above 0.80 for fall armyworm leaf and cob damage traits, implying that additive gene action was predominant in controlling FAW resistance in the genotypes (Mukaro *et al*., 2023). A high GCA/SCA ratio means that the additive genes' action contributes largely to regulating the trait of interest (Asare *et al*., 2023). Baker's ratio of above 0.5 suggests that the GCA effect is more important than SCA in the inheritance of the trait, while ratios of below 0.5 imply that the SCA effect is key in the trait's inheritance (Mukaro *et al*., 2023). Contrary to the present findings of the study, Baker's ratio of below 50% has been documented for FAW leaf damage in maize (Asare *et al*., 2023). Significant GCA and SCA effects observed for cob damage, plant and ear height, and ear rot indicated that the traits are governed by both additive and non-additive gene actions. However, the GCA/SCA ratio of above 83% for all the traits except grain y emphasizes the greater extent of involvement of additive gene action in controlling the traits (Feyzian *et al*., 2009). Lower Bakers ratios of 0.22 and 0.34 for plant height and ear height, respectively have been reported, meaning that the traits were predominantly controlled by non-additive gene effects (Huz *et al*., 2021). While a GCA/SCA ratio of 0.61 was observed for grain yield from the study, lower ratios of 0.40 have also been documented (Muthoni and Shimelis, 2020). NCDII mating design allows the estimation of variance components using information from half-sib families (Muthoni and Shimelis, 2020). The observed significant SCA variances from the study for plant height, ear height, and ear rot in maize under FAW infestation suggest that non-additive gene actions were also vital in the regulation of these traits (Makinde *et al*., 2023). Combining ability analysis is used in the

selection of inbred lines based on their breeding values. Lines with high breeding values are selected for use in the breeding programs, while those with low or negative breeding values are discarded (Al-Naggar *et al*., 2016). Heritability of a trait is the proportion of the breeding value (additive genetic effect) of the phenotypic variance and depends on the frequency of the alleles in a population (*Makinde et al*., 2023).

Low genetic and high residual variance observed in FAW damage traits and ear rot resulted in low heritability estimates being recorded. This implies that the traits were highly influenced by the environment, which could interfere with the direct selection of resistant genotypes. (Magar *et al*., 2021). Similar results with low heritability estimated for FAW damage parameters were observed across different environments due to highly significant environmental effect and genotype by environment interaction (Badji *et al*., 2020). Residual variance as a result of variation in environmental conditions of the trial sites may result in differences in genotypic responses to the various environments (seasons and location). High residual variance observed for FAW damage traits may be due to the pest population depending on the environmental conditions during natural infestation. Moisture, temperature, and relative humidity are major determinants of the rate of FAW population growth (Adunola *et al*., 2021). FAW population growth rate is enhanced by the availability of high moisture content and an average temperature of around 29^0C °C (Adunola *et al*., 2021). Moderate narrow-sense heritability and moderate and high broad-sense heritability have been reported for FAW damage traits (Kamweru *et al*., 2022). Despite the present study showing that the maize ear rot trait had high residual variance and low genetic variance, resulting in low heritability values (24%), moderate heritability estimates for ear rot (37.41%) have been reported (Agbowuro and Salami, 2021). Additionally, high heritability estimates of above 70 % were documented for resistance to ear rot in maize, indicating that the trait was controlled mainly by additive gene effects (Wen *et al*., 2021).

High heritability values for ear height (78%) and plant height (71%) resulted from high additive and low residual variances. These results corroborate observations from previous studies (Ochigbo *et al*., 2021). Moderate heritability estimates were reported for grain yield (58%) in the current study, implying that the trait could be improved through selection. High heritability values of 93% for grain yield have been documented (Magar *et al*., 2021). In addition, low heritability estimates for maize yield have also been reported (Amissah *et al*., 2019). Heritability is important because it's used by breeders for the calculation of the response to selection ($R = h^2S$) for a particular phenotypic trait, where R is the response to selection, S is the selection differential, and h^2 is the narrow-sense heritability (Schmidt *et al*., 2019). Traits with higher heritability values are highly heritable and likely to pass the desired alleles for the specific traits to the progeny, and therefore their response to selection is higher compared to those with low heritability estimates, in which variation among genotypes is mainly due to non-heritable factors (residual variance) (Orton, 2020).

4.5 Conclusion

The evaluation of hybrids under FAW artificial and natural infestation for damage parameters and agronomic traits enabled the identification of gene action governing the traits. Fall armyworm leaf and cob damage, and ear aspect were controlled by additive gene effects, while plant height, ear height, and ear rot were regulated by both additive and non-additive gene effects. Additive gene was the predominant gene action in all the traits evaluated, implying that GCA effects mainly accounted for the variation among the parental inbred lines. Twenty inbred lines with a significant negative GCA effect in fall armyworm damage parameters and eight for ear rot were selected to be used for future improvement of the traits. Lines with significant GCA effect can contribute favorable alleles for improving grain yield in maize. Lines with significant positive GCA and SCA effects for plant and ear height are suitable for improving the height in maize.

CHAPTER 5

5.0 GENOME-WIDE ASSOCIATION STUDIES AND GENE ONTOLOGY FUNCTIONAL ENRICHMENT ANALYSIS FOR RESISTANCE TO FALL ARMYWORM IN TROPICAL MAIZE INBRED LINES

5.1 Introduction

Maize is a global stable food crop and plays a key role in food security. There is an urgent need to increase maize production to ensure food security due to the continuous increase in population growth, climate change, and a decrease in arable land (Qu *et al*., 2022). Fall armyworm is a devastating pest for maize farmers. FAW destroys the plants mainly by feeding on the leaves' whorl, thereby interfering with photosynthesis and retarding maize growth and development (Kamweru *et al*., 2022). Host plant insect resistance (HPR) is a safe and economical way of managing FAW (Warburton *et al*., 2023). Genetic mechanisms causing resistance to FAW in maize may be due to phenotypic features like structural barriers, antibiosis, or synthesis of toxic metabolites that repel the pest, or secretion of hormones that attract the insect predators (Warburton *et al*., 2023).

Genome-wide association studies are a modern biotechnological tool used to analyze interspecies association between genotype and phenotype based on the development of next-generation sequencing technology (Zheng *et al*., 2021). The method analyzes the high genetic diversity and recombination history existing across the genomes in a population (Narkhede *et al*., 2023). Marker-trait association in GWAS is based on linkage disequilibrium resulting from the relationship of a specific trait with an adjacent marker (Okunlola *et al*., 2023). The power of association studies is determined by the size of the population being evaluated, the rate of linkage

disequilibrium decay between target allele and molecular marker, the degree of the allele effect of the trait of interest, and data accuracy (minimum errors) (Kumar *et al*., 2017). Non-random association in GWAS can identify candidate genes for breeding programs (Gangurde *et al*., 2022). GWAS is a powerful method for detecting the genes governing quantitative traits affected by the environment, such as FAW resistance in maize and their functions (Okunlola *et al*., 2023). GWAS and metabolic pathway analysis have been used to identify mechanisms of maize resistance to Corn Earworm (Warburton *et al*., 2018), to map complex traits such as resistance to diseases, and response to insect pests like Mediterranean corn borer (MCB) and maize weevils (MW) (Badji *et al*., 2020). GWAS has also been utilized in the evaluation of maize grain quality (Zheng *et al*., 2021) and traits related to yield (Zeng *et al*., 2022), candidate genes contributing to kernel size (Qu *et al*., 2022), as well as striga tolerance (Okunlola *et al*., 2023). Limited GWAS studies have been carried out on the genetic architecture for FAW resistance in tropical maize germplasm (Kamweru et al., 2022). Prasanna et al. (2021) and Warburton *et al*. (2023) identified SNPs associated with FAW damage. Similar GWAS studies have been reported on tolerance to FAW in maize inbred lines (Badji *et al*., 2020).

During GWAS, a precise scan of the genomic regions is carried out to reveal the genes linked with the trait being evaluated. However, the method rarely identifies genes with large effects for highly quantitative traits, and many genes may be missed out (Warburton *et al*., 2023). This problem may be resolved by carrying out further investigations on GWAS results to identify the candidate genes (Qu *et al*., 2022). When insects feed on the host plants, they stimulate various immune responses as a defense mechanism by the plant. The HPR defense response involves complex signaling pathways initiated upon recognition of specific effector molecules found in insect saliva (Zhang *et al*., 2024). Using gene ontology and pathway analyses, more information about the genes

regulating the mechanisms of resistance can be identified (Warburton *et al.*, 2023). Gene ontology enrichment analysis can be used to identify biological annotations and interpret the gene interactions associated with the trait of interest (Garcia-Moreno *et al.*, 2022).

However, there are limited studies on GWAS and candidate gene functional analysis for FAW resistance in African-adapted tropical maize inbred lines. Therefore, further GWAS studies need to be carried out on African-adapted tropical maize germplasm to identify the genetic basis of FAW resistance (Uffelmann *et al.*, 2021). The objective of this study was to identify the genomic regions and candidate genes associated with resistance to FAW in tropical maize germplasm by i) identifying single-nucleotide polymorphisms (SNPs) associated with FAW resistance using GWAS and ii) identifying candidate genes regulating FAW resistance and their functions by gene ontology functional enrichment analysis.

5.2 Materials and methods

5.2.1 Germplasm

Inbred lines evaluated in the study are described in Chapter 3 (Table 3.1). However, GWAS studies were conducted on 137 lines excluding CKL201294, CKL21603, and CKL21646. These lines were eliminated when the SNP data were subjected to further stringent quality control using Sliding Window Extraction of Explicit Polymorphisms (SWEEP) analysis. The SWEEP tool filters SNP markers using sub-genome polymorphism haplotypes as a contrast, and allows for exclusion of false positives from a set of SNP calls and detection of true SNPs (Clevenger and Ozias-Akins, 2015).

5.2.3. Phenotypic characterization

5.2.3.1 Experimental design

Experimental design and crop establishment used in this study are described in 3.2.3.1

5.2.3.2 Data collection

Data was collected as described in 3.2.3.2.

5.2.3.3 Genotyping by sequencing

Genotyping by sequencing was conducted as stated in Chapter 3.2.4.1

5.2.4 Data Analysis

The Genome Association and Prediction Integrated Tool (GAPIT version 3.1.0) package in the R statistical software was used to carry out genome-wide association studies. Fixed and random model Circulating Probability Unification (FarmCPU), Blink, and multi-loci mixed model (MLMM) models in GAPIT were used to identify SNPs linked with the trait. These three multi-locus GWAS models are a modification of the mixed linear model (MLM) to integrate both the population structure (Q), having nine subpopulations, and the kinship matrix (K) to account for variation in population stratification and relatedness among the maize inbred lines. This is because the use of MLM encounters the problem of false negatives and false positives. The Q and K matrices were computed using the Landscape and Ecological Association Studies (LEA) of the R version 4.3.3 package and fitted into GWAS models as covariates and consequently used to reduce the number of false positives. The population structure of the traits in the association mapping panel was controlled by gradually increasing the number of principal components (PCs) for each trait within the GWAS model until the false negative and false positive rates were adequately controlled through the scrutiny of the Quantile–quantile (Q-Q) plots. The Q-Q plots

in R was used to evaluate the fitness of the GWAS model used by comparing the association between the expected and observed theoretic p-values distribution across all the SNPs evaluated. GWAS findings per chromosome were displayed using Manhattan plots. These scatter plots were derived by plotting the positions of the SNPs on the genome against the negative logarithms of the association p-value (log10(P)) generated by the model. The Bonferroni genome-wide significance level (BGSL) of P < 1.92308E-06 was used to identify SNPs significantly associated with resistance traits based on the Bonferroni test of –log10 (p-value). Both the Manhattan and Q-Q plots were generated using the CMplot package in R. Candidate genes located within a window of 100 kb upstream and downstream of significant SNPs associated with FAW resistance were screened using the *Zea mays* PHJ40v1.1 reference genome retrieved via the *Phytozome* version 13 database using the *biomaRt* package in R and recorded. The genetic information about these candidate genes, including the name of the gene, its description, coordinates on the reference genome, and the consensus sequence, was retrieved from the maize genome database (**https://www.maizegdb.org/**) and used for functional analysis.

The *ClusterProfiler* package in R was used to conduct gene ontology analysis based on biological processes involving the candidate genes. Background genes for *Zea mays* were obtained from the *OrgDb* annotation package using the *AnnotationHub* tool in R. The Top 20 highly enriched genes were visualized through dot plots and gene interaction plots generated using the *enrichplot* package in R.

5.3 Results

5.3.1 Genome-wide association studies (GWAS)

Genome-wide association (GWAS) results for leaf and cob damage, grain yield, and ear rot percentage were presented on Manhattan plots. Q-Q plots shown alongside the Manhattan plots

evaluated the fitness of the GWAS model. Seven SNPs were highly significantly associated with leaf damage (Table 5.1). Three SNPs located on chromosomes 1, 5, and 8 were found to have a significant association with leaf damage score taken on the 7th day after artificially infesting the plants with FAW (LD1) (Figure 5.1). Leaf damage score taken on the 14th day after artificial FAW infestation (LD2) was significantly associated with 3 SNPs, two of which were located on chromosome 2, while the third SNP was on chromosome 3 (Figure 5.2). A single SNP significantly associated with leaf damage scores taken on the 21st day after artificial FAW infestation (LD3) was located on chromosome 9 (Figure 5.3). One SNP found on chromosome 1 was highly significantly associated with cob damage (Figure 5.4). There was a highly significant association between grain yield and two SNPs found in chromosomes 2 and 4 (Figure 5.5). Ear rot percentage was significantly associated with three SNPs located on chromosomes 2, 7, and 8 (Figure 5.6).

Table 5.1. Characteristics of significant SNP makers identified in GWAS of 137 maize inbred lines

SNP Identity	SNP	Chr	Pos	P.value	MAF	Traits
2383848|F|0-58:T>C-58:T>C	58:T>C	1	184280787	1.42E-08	0.314815	LD1
5590349|F|0-34:A>G-34:A>G	34:A>G	5	134134981	1.23E-08	0.318519	LD1
2464808|F|0-61:G>C-61:G>C	61:G>C	8	21391014	7.05E-08	0.37037	LD1
5588012|F|0-19:T>C-19:T>C	19:T>C	2	6867718	4.50E-10	0.103704	LD2
9714249|F|0-26:T>C-26:T>C	26:T>C	2	153784054	5.24E-07	0.251852	LD2
100075893|F|0-66:C>T-66:C>T	66:C>T	3	8051091	1.18E-08	0.177778	LD2
9703383|F|0-12:C>T-2:C>T	12:C>T	9	104398520	2.02E-06	0.385185	LD3
4774028|F|0-64:C>A-64:C>A	64:CA	1	245855107	2.80E-06	0.107407	Cob damage
4582632|F|0-31:A>G-31:A>G	31:A>G	2	244350135	1.81E-08	0.425926	Grain Yield
2418713|F|0-16:A>G-16:A>G	16:A>G	4	32082507	1.58E-08	0.318519	Grain Yield
2427823|F|0-15:C>T-15:C>T	15:C>T	2	241928907	1.31E-08	0.051852	Ear rot percentage
4774038|F|0-23:A>G-23:A>G	23:A>G	7	2680303	2.75E-07	0.092593	Ear rot percentage
2490205|F|0-27:G>T-27:G>T	27:G>T	8	61547117	8.15E-07	0.414815	Ear rot percentage

Chr-chromosome, Pos- Position, MAF-minor allele frequency

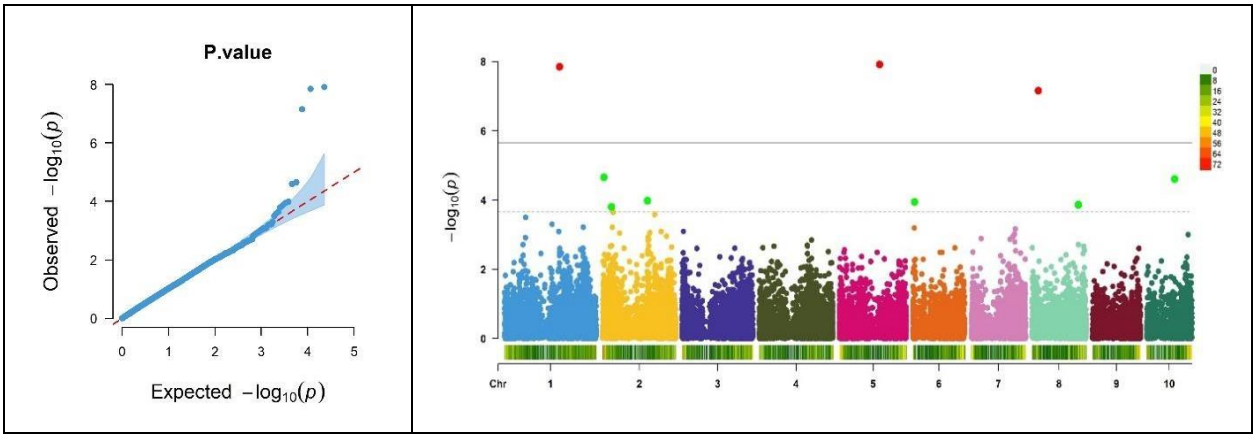


Figure 5.1. Q–Q plots and Manhattan plots resulting from the GWAS analysis for Leaf damage score 1 (LD1)

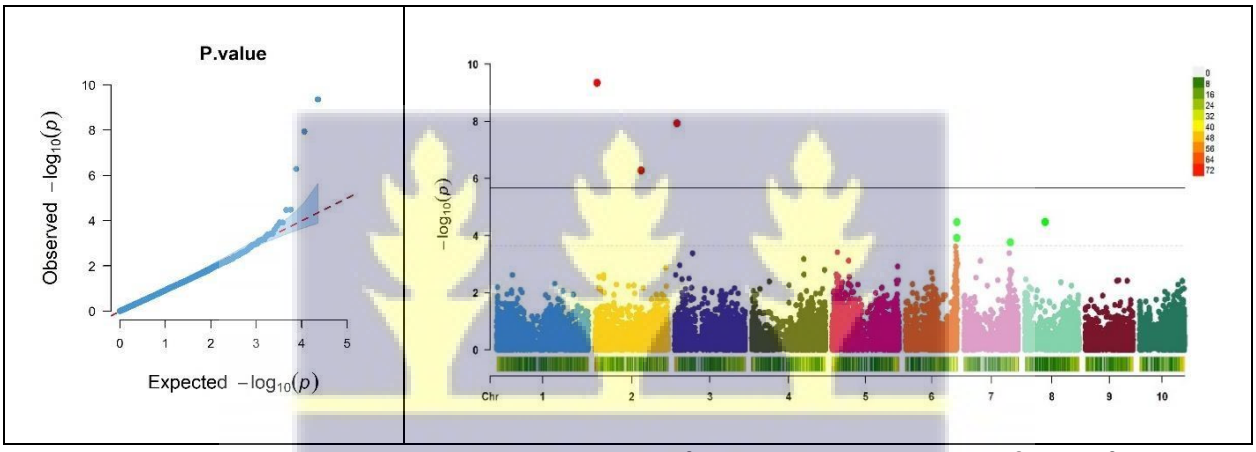


Figure 5.2. Q–Q plots and Manhattan plots resulting from the GWAS analysis for Leaf damage score 2 (LD2)

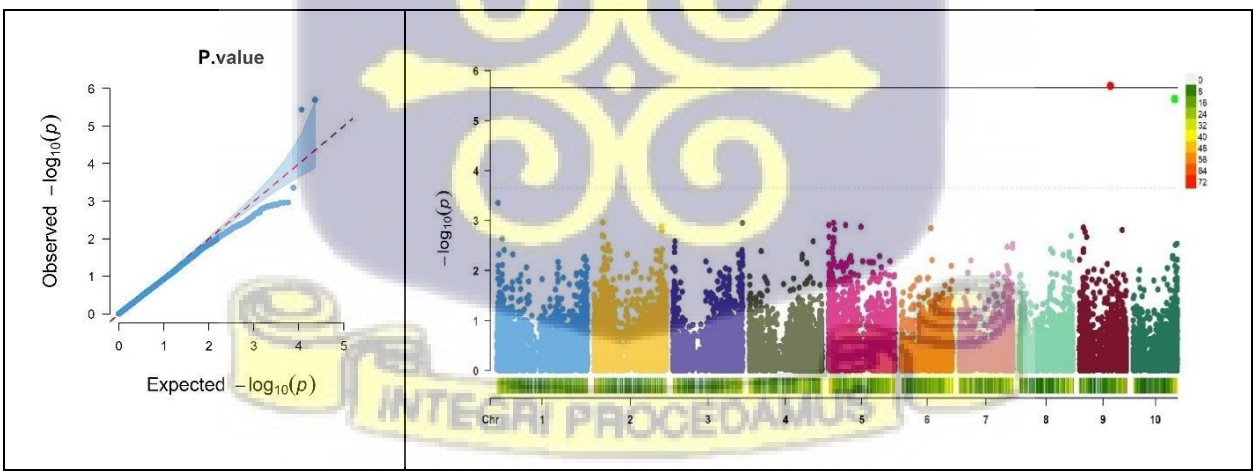


Figure 5.3. Q–Q plots and Manhattan plots resulting from the GWAS analysis for Leaf damage score 3 (LD3)

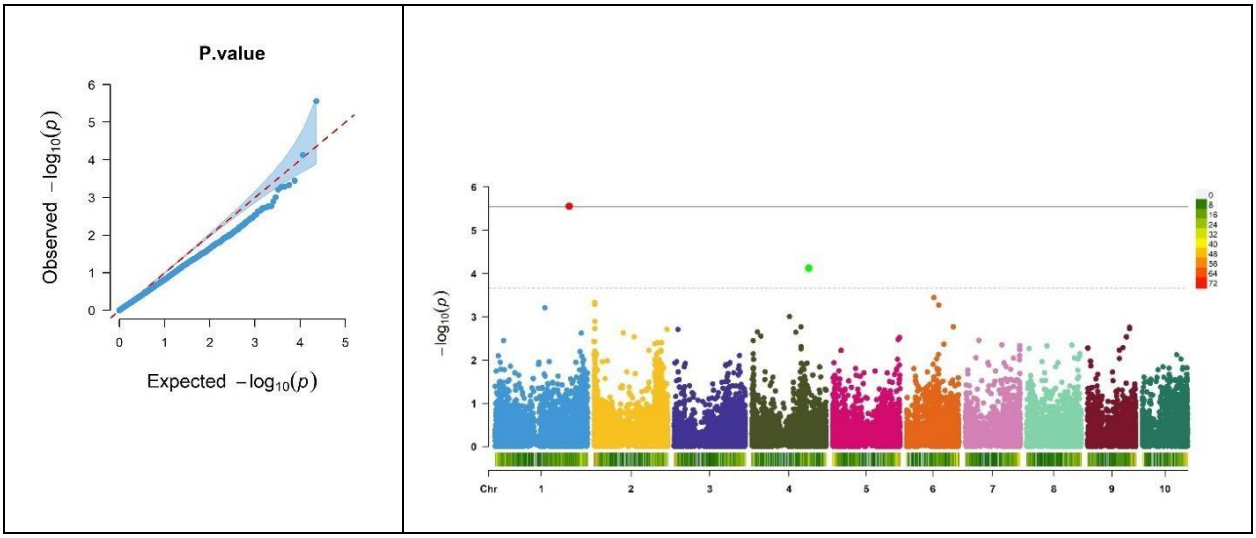


Figure 5.4. GWAS analysis Q–Q and Manhattan plots for Cob damage

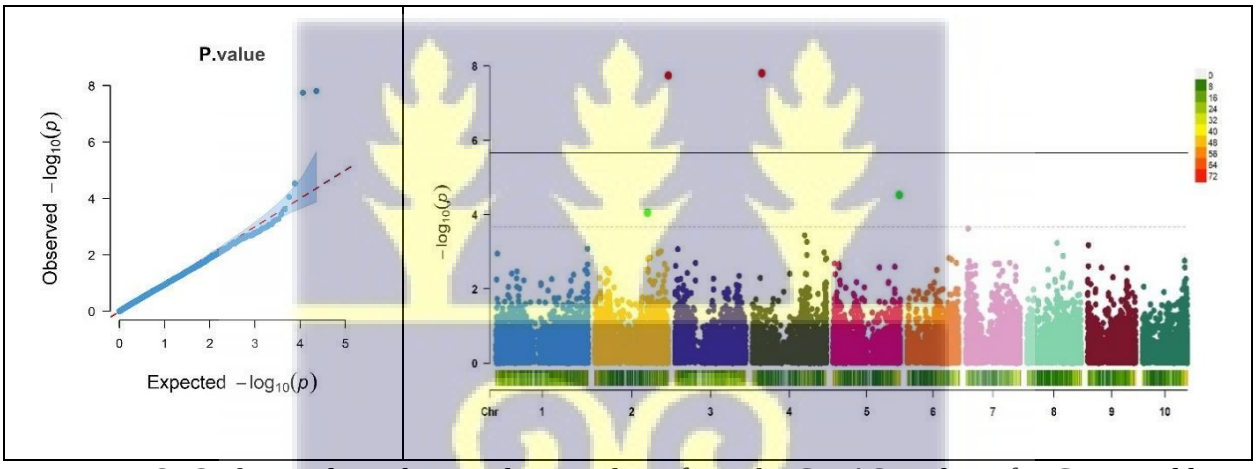


Figure 5.5. Q–Q plots and Manhattan plots resulting from the GWAS analysis for Grain yield

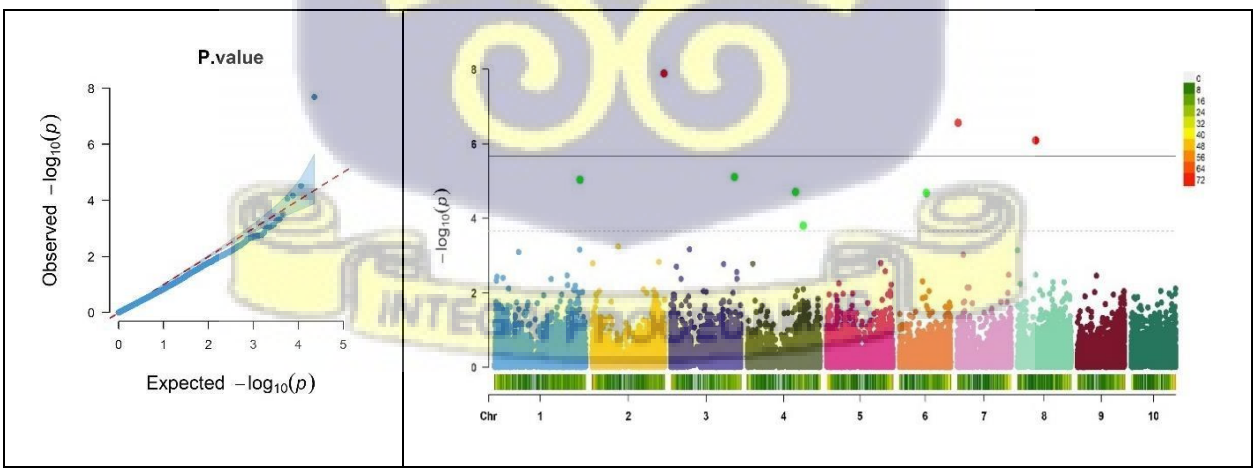


Figure 5.6. Q–Q plots and Manhattan plots resulting from the GWAS for Ear rot percentage

5.3.2 Candidate Genes

The number of candidate genes situated within a window of a hundred kilobase pairs up and downstream of significant SNPs associated with resistance to FAW leaf damage trait was a total of 10,950; these included 7100 candidate genes associated with LD1, 3847 candidate genes associated with LD2, and only 3 candidate genes associated with LD3 (Table 5.2). Four thousand, four hundred and ninety-five candidate genes were associated with cob damage trait, 5540 genes related to grain yield, and 8754 candidate genes associated with ear rot % (Table 5.2). From 10950 candidate genes associated with FAW leaf damage, 32 genes governing various metabolic pathways were identified (Table 5.3). These candidate genes were involved in regulating pathways for benzoxazinoid glucosides biosynthesis, geranylgeranyl diphosphate biosynthesis, acetyl-CoA biosynthesis, 2,4-dihydroxy-1,4-benoxazin-3-one (DIBOA)-glucoside biosynthesis, ethylene biosynthesis I, flavanol biosynthesis, jasmonic acid biosynthesis, nicotinamide adenine dinucleotide (NAD) biosynthesis, sporopollenin biosynthesis, and wax esters biosynthesis II (Table 5.3).

Table 5.2. Number and location of candidate genes identified for various traits evaluated

Trait	No of Candidate genes	Chromosome Location
LD1	2392	1
	2300	5
	2408	8
LD2	2017	2
	1830	3
LD3	3	9
Cob damage	4192	1
Grain yield	2676	2
	2864	4
Ears Rot %	3525	2
	2449	7
	2780	8

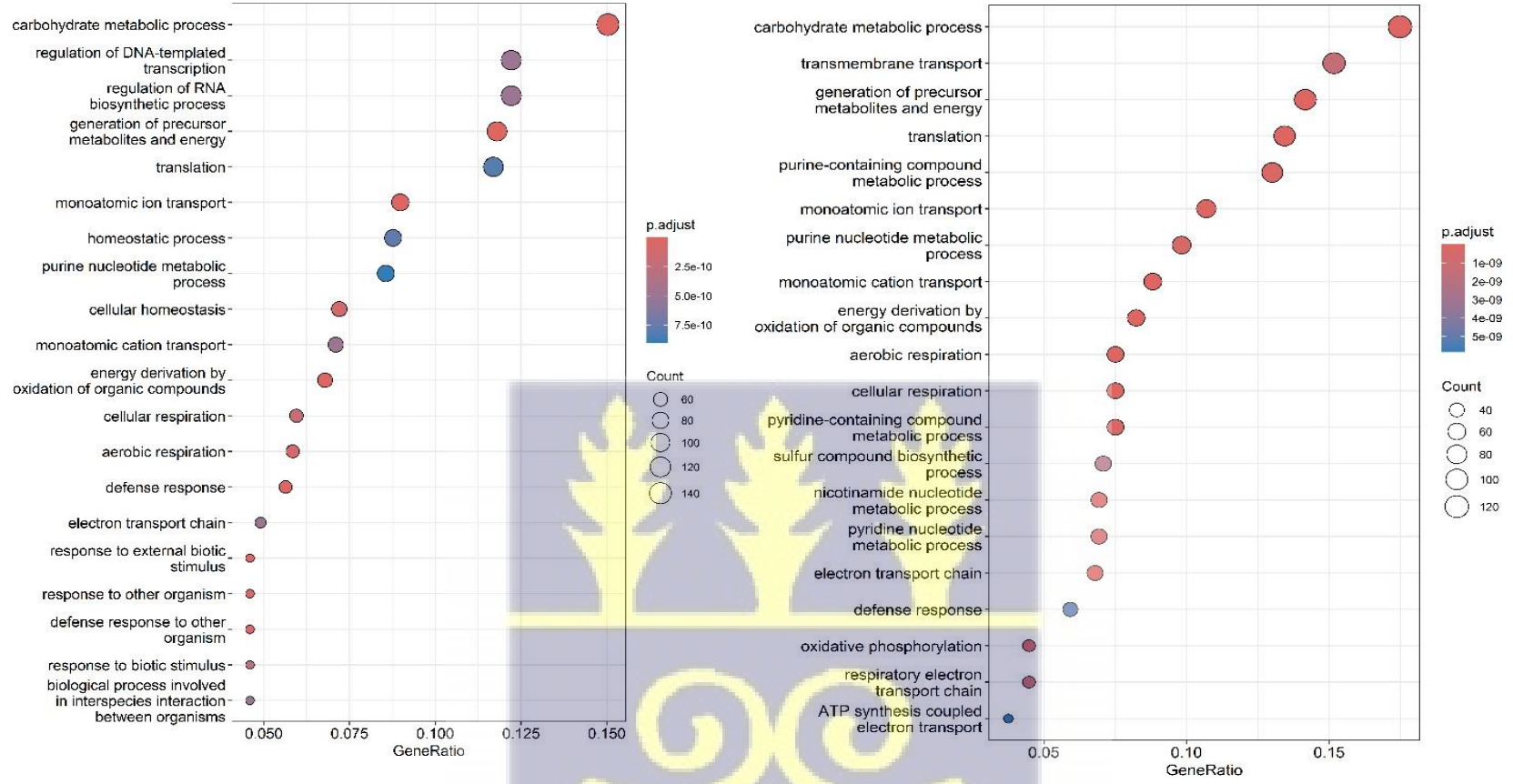
Table 5.3. Selected genes and Pathways associated with Fall armyworm resistance in tropical maize germplasm

gene name	pathway description	chromosome Number
Leaf damage score 1 (7th day rating)		
ZmPHJ40.01G185900	ethylene biosynthesis I	1
ZmPHJ40.08G161500	ethylene biosynthesis I	8
ZmPHJ40.05G121000	ethylene biosynthesis I	5
ZmPHJ40.05G207200	ethylene biosynthesis I	5
ZmPHJ40.08G052100	ethylene biosynthesis I	8
ZmPHJ40.08G139000	flavonol biosynthesis	8
ZmPHJ40.08G075800	NAD/NADH phosphorylation and dephosphorylation	8
ZmPHJ40.01G177500	sporopollenin precursors biosynthesis	1
ZmPHJ40.01G109000	sporopollenin precursors biosynthesis	1
ZmPHJ40.01G271100	sporopollenin precursors biosynthesis	1
ZmPHJ40.05G254200	sporopollenin precursors biosynthesis	5
ZmPHJ40.05G241200	acetyl-CoA biosynthesis	5
ZmPHJ40.01G087600	geranylgeranyl diphosphate biosynthesis	1
ZmPHJ40.05G232800	glyoxylate cycle and fatty acid degradation	5
ZmPHJ40.05G086100	glyoxylate cycle and fatty acid degradation	5
Leaf damage score 2 (14th day rating)		
ZmPHJ40.02G203100	DIBOA-glucoside biosynthesis	2
ZmPHJ40.02G087500	DIBOA-glucoside biosynthesis	2
ZmPHJ40.02G053300	ethylene biosynthesis I	2
ZmPHJ40.02G109000	flavonoid biosynthesis	2
ZmPHJ40.02G113500	jasmonic acid biosynthesis	2
ZmPHJ40.02G109300	jasmonic acid biosynthesis	2
ZmPHJ40.02G200300	NAD biosynthesis	2
ZmPHJ40.02G133100	NAD/NADH phosphorylation and dephosphorylation	2
ZmPHJ40.02G200300	pyridine nucleotide cycling	2
ZmPHJ40.02G160400	pyruvate decarboxylation to acetyl CoA	2
ZmPHJ40.02G113500	sporopollenin precursors biosynthesis	2
ZmPHJ40.02G113500	suberin monomers biosynthesis	2
ZmPHJ40.02G203100	benzoxazinoid glucosides biosynthesis	2
ZmPHJ40.02G087500	benzoxazinoid glucosides biosynthesis	2
ZmPHJ40.02G096200	geranylgeranyl diphosphate biosynthesis	2
ZmPHJ40.02G088600	pyrimidine deoxyribonucleotides	2
ZmPHJ40.02G143900	wax esters biosynthesis II	2
Leaf damage score 3 (21st day rating)		
ZmPHJ40.09G134300	Not annotated	9
ZmPHJ40.09G134100	Not annotated	9
ZmPHJ40.09G134200	Not annotated	9

5.3.3 Gene ontology

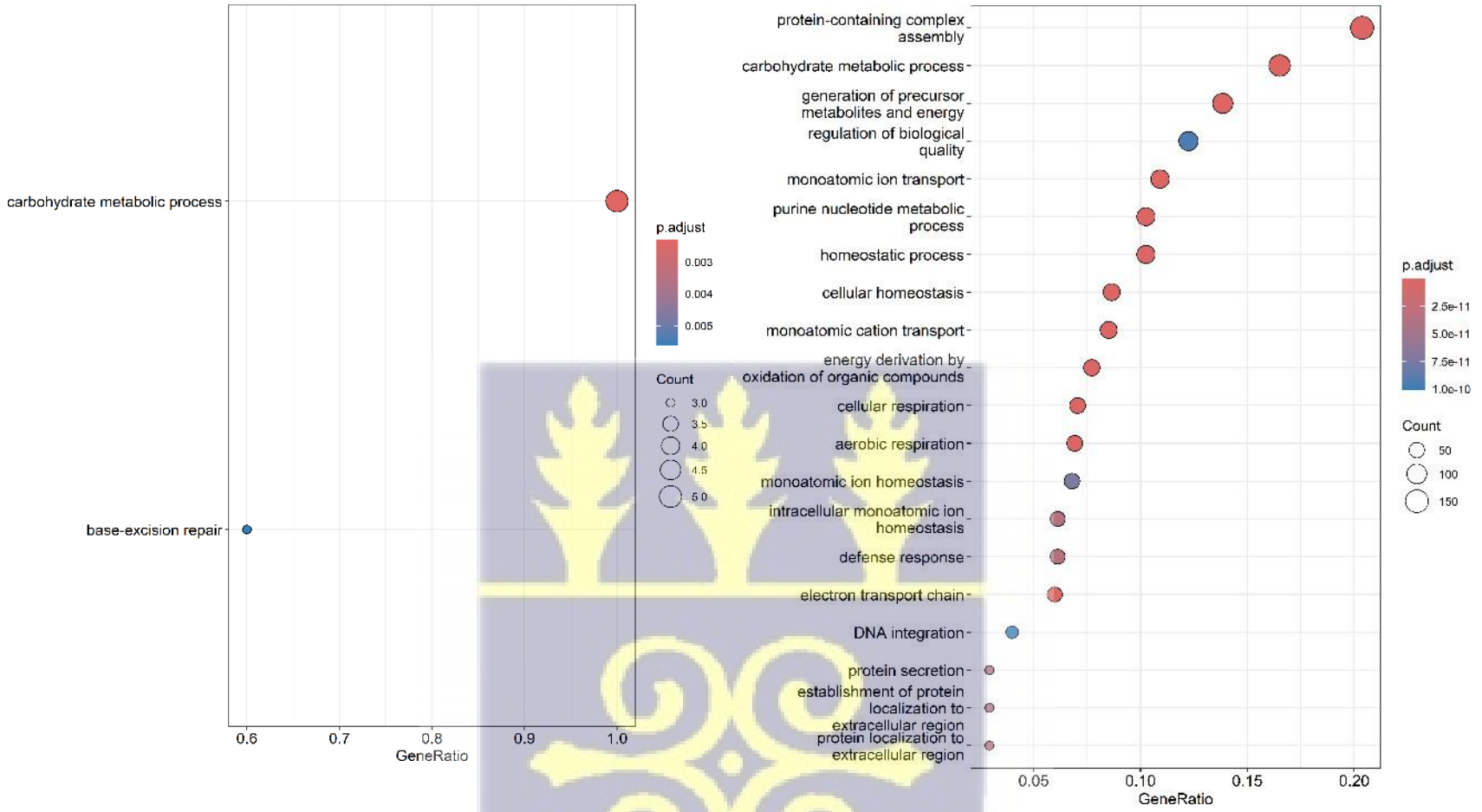
Gene Ontology (GO) enrichment analysis identified processes and molecular functions of genes associated with the FAW damage, grain yield, and ear rot. Dot plots of 20 highly enriched genes associated with FAW larval leaf feeding (LD1, LD2, and LD3) showed that there was highly significant enrichment in Gene Ontology (GO) biological process, mostly involved in regulation of plant defense response, DNA transcription, RNA biosynthesis, and secondary metabolite biosynthesis (Figures 5.7 and 5.8). Gene interaction plots for LD1 further confirmed that the interaction of enriched genes associated with the trait resulted in plant defense processes, aerobic respiration, and ion transportation. Gene interactions for LD2 lead to nicotinamide metabolic process, aerobic respiration, and cation transport, and those for LD3 resulted in cellular response to DNA damage, leading to base excision DNA repair (Figures 5.10 and 5.11).

Enrichment was observed in biological processes associated with resistance to cob damage, comprising defense response, homeostasis, respiration, and generation of metabolites (Figure 5.8). Most of the top 20 highly enriched genes associated with grain yield regulated metabolic processes, the electron transport chain, and respiration, while those associated with ear rot percentage were mainly involved in metabolic processes (Figure 5.9). Nucleotide metabolic processes, aerobic respiration, protein assembly, and cellular respiration were associated with fall armyworm cob damage (Figure 5.11). The interactions between genes associated with grain yield resulted in purine nucleotide metabolic process, aerobic respiration, and cation transportation, and the interaction for ear rot resulted in the metabolism of secondary metabolites such as nicotinamide nucleotide metabolism (Figure 5.12).



Leaf damage score 1 (LD1) **Leaf damage score 2 (LD2)**

Figure 5.7. Gene ontology (GO) revealing the 20 most significantly enriched genes associated with LD1 and LD2 traits



Leaf damage score 3 (LD3)

Cob damage score

Figure 5.8. Gene ontology (GO) revealing the 20 most significantly enriched genes associated with LD3 and cob damage

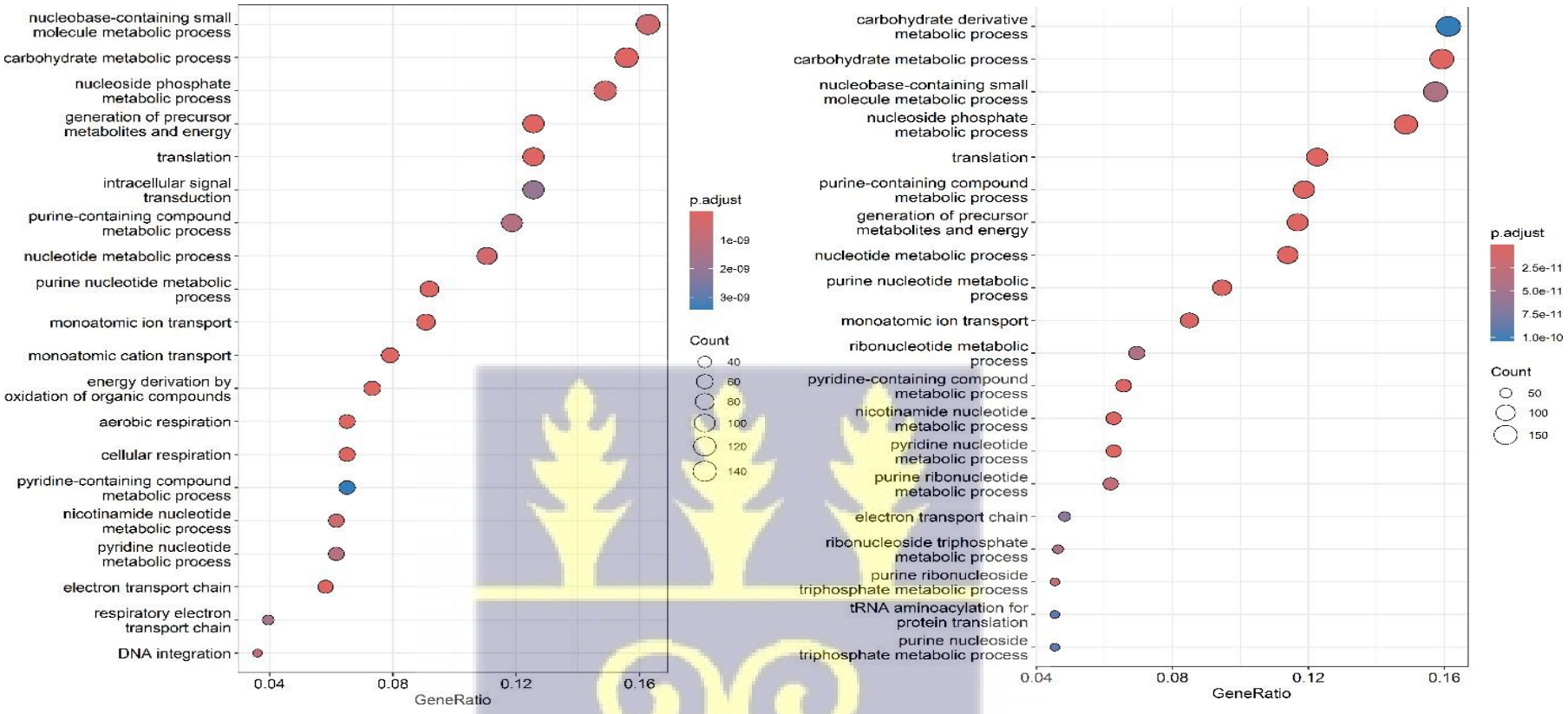
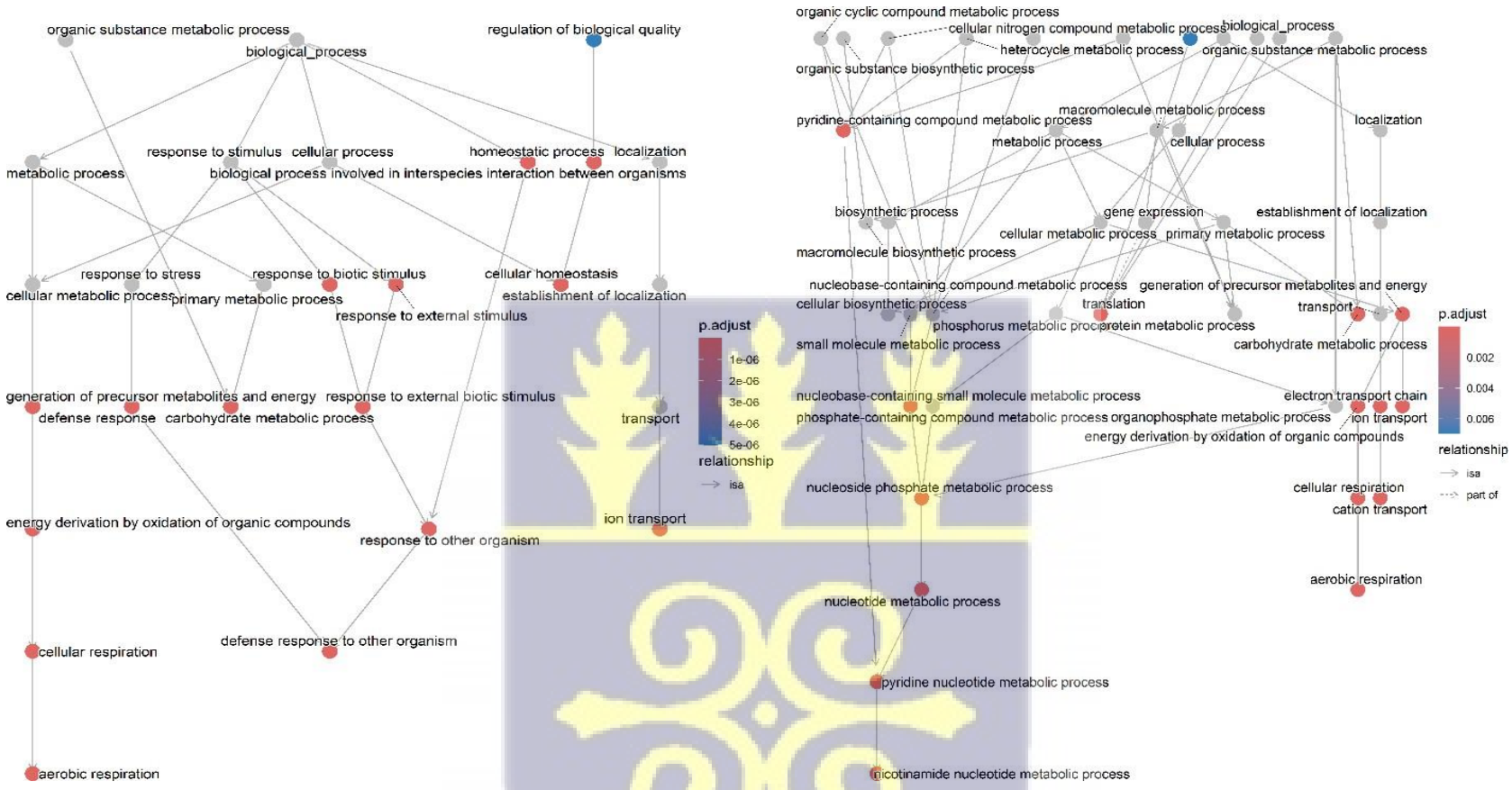
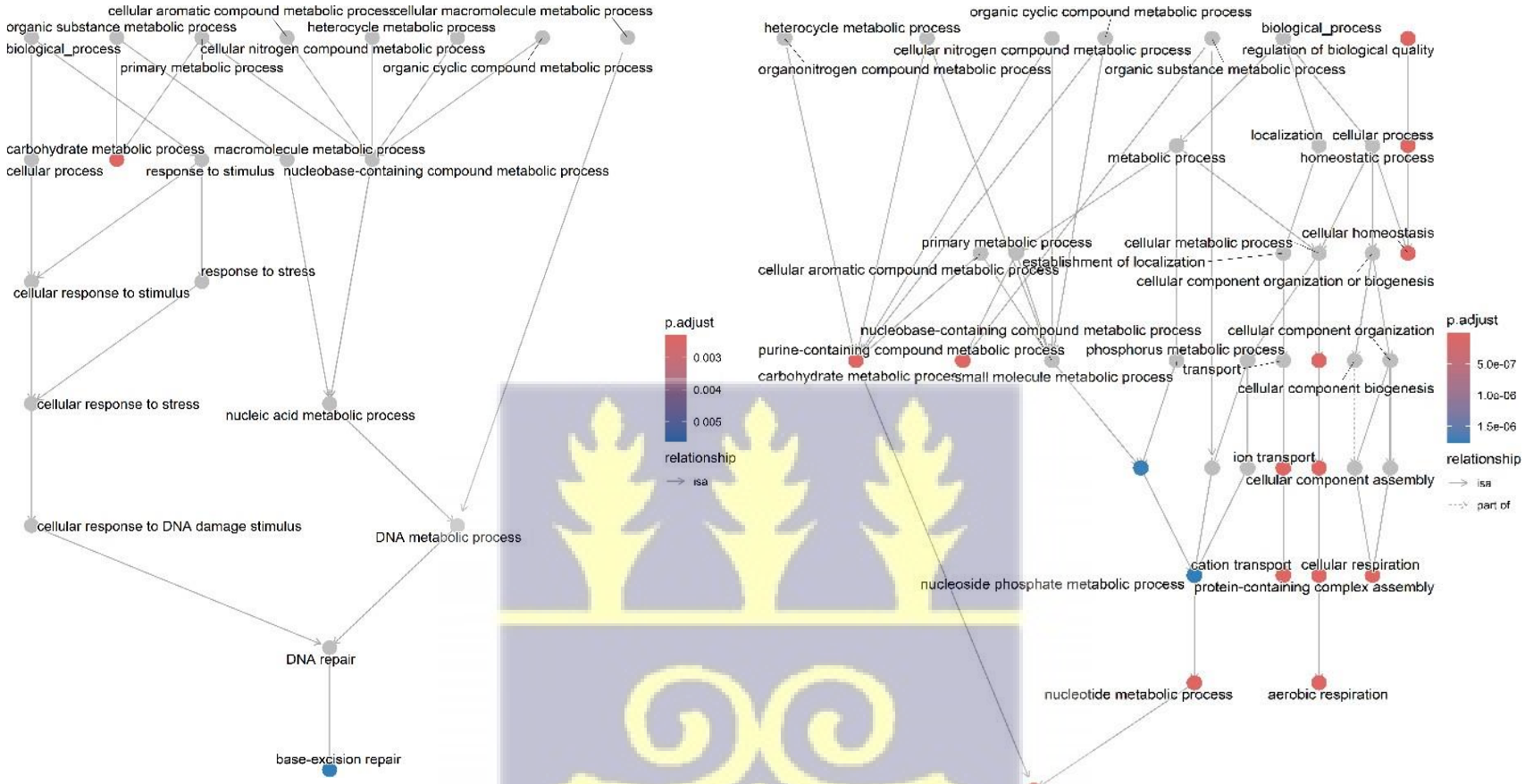


Figure 5.9. Gene ontology (GO) revealing the 20 most significantly enriched genes associated with grain yield and ear rot percentage



Leaf damage score 1 (LD1) **Leaf damage score 2 (LD2)**

Figure 5.10. Interactions of Gene Ontology Enrichment Processes for LD1 and LD2



Leaf damage score 3 (LD3)

Cob damage score

Figure 5.11. Interactions of Gene ontology enrichment processes for LD2 and cob damage

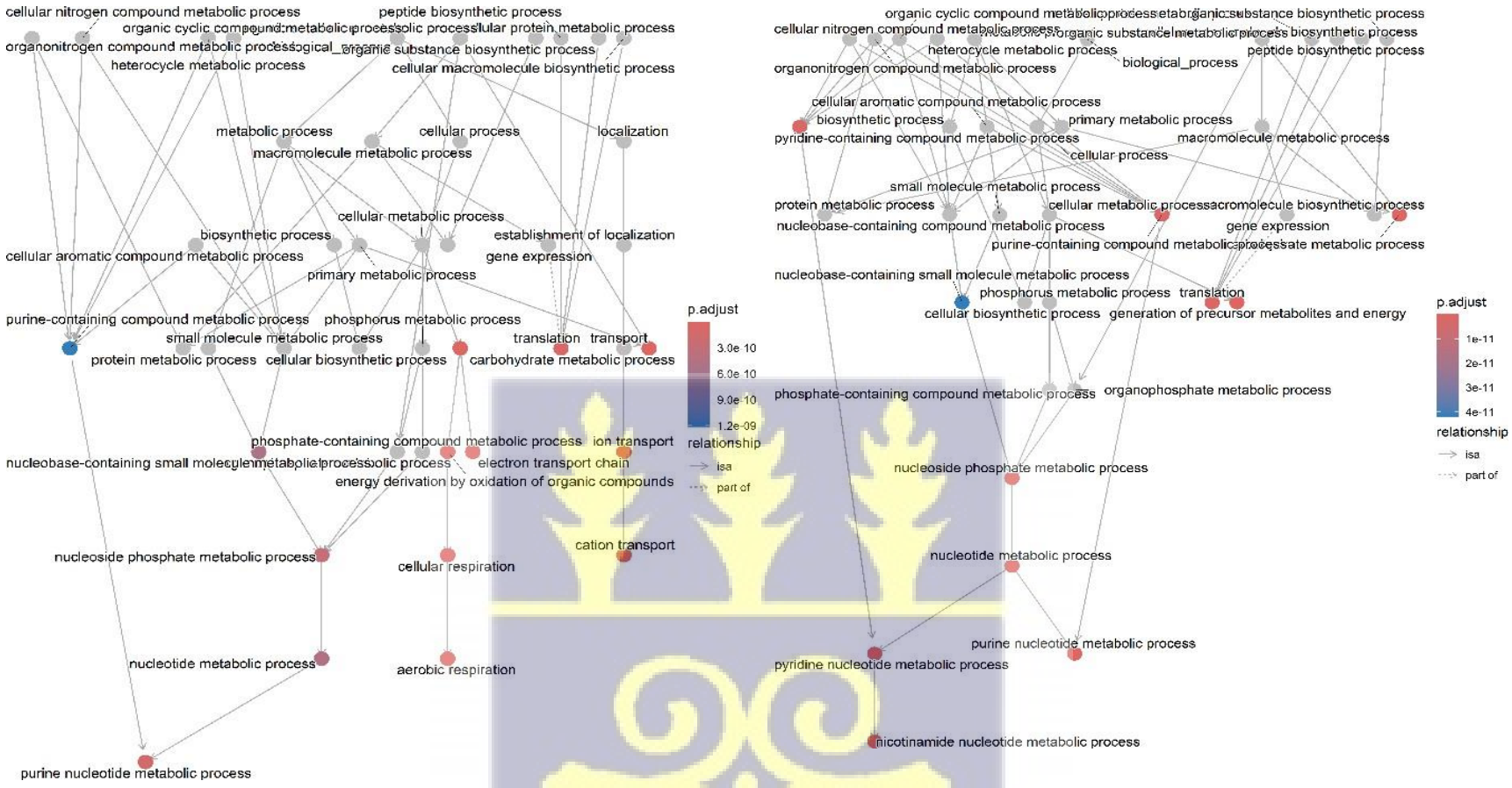


Figure 5.12. Interactions of Gene Ontology enrichment processes for grain yield and ear rot percentage

5.4 Discussion

Genome-wide association studies are a high-throughput biotechnological tool that allows for the detection of SNPs and candidate genes associated with quantitative traits like FAW resistance in maize (Qu *et al*., 2022). Even distribution of SNPs within the Manhattan density plots for all the traits across the entire maize genome in the study indicates that the results obtained were free from biased estimations and false positives (Narkhede *et al*., 2023). This shows that the FarmCPU model used in GWAS analysis effectively controlled false positives that might have arisen from population and kinship structure (Kaler and Purcell, 2019). All the Q-Q plots observed further verified that the GWAS model used in the study was fit, and the kinship and population structure were effectively controlled during the analysis. A Q-Q plot is used in the evaluation of the extent of observed association between SNPs and traits being studied in relation to the expected association statistics (Wang, 2023). As projected, only highly significantly associated SNPs were observed to have deviated from the identity line (y = x) when the observed –log10 (p-value) was plotted against the expected SNP distribution across the genome.

Single-nucleotide polymorphism markers linked to resistance to leaf damage in maize were located on chromosomes 1, 2, 3, 5, 8, and 9. These results corroborate the findings of Kamweru *et al*. (2022). Similarly, Badji *et al*. (2020) reported non-significant SNPs associated with FAW damage resistance identified on chromosomes 6 and 7. However, additional SNP markers associated with FAW resistance in maize were identified on chromosomes 4, 6, and 7 (Kamweru *et al*., 2022). In the present study, 3 SNPs located on chromosomes 1, 5, and 8 were found to have a significant association with LD1. These results agreed with the findings by Warburton et al. (2023). However, the chromosomal location of the significant SNPs differed, with a single SNP located on chromosome 1, while the remaining two were on chromosome 6. In addition to 1 significant SNP on the first chromosome associated with cob damage, 13 SNPs have been

detected on chromosomes 1, 2, 3, 4, 5, 6, and 10 (Kamweru *et al*., 2022). The study confirmed the presence of native host plant resistance for FAW within the population and disclosed that FAW resistance is a quantitative trait regulated by polygenes with minor additive effects. These imply that alleles for resistance to FAW could be accumulated within the population through recurrent selection.

The current study identified SNPs linked to grain yield on chromosomes 2 and 4. Eleven SNPs associated with grain yield have been observed on chromosomes 1, 2, 3, 7, 8, 9, and 10, while 29 SNPs associated with grain weight were discovered on all the chromosomes (Zeng *et al., 2022)*. SNPs associated with yield-related traits such as kernel-related traits (Qu *et al*., 2022), grain yield per plant, tassel branch number, kernel number per row, grain width, grain length, and 100 kernel weights (Zeng *et al*., 2022), and grain qualitative traits have been identified (Patel *et al*., 2024). Knowledge of SNPs and genes associated with yield is important when breeding for high-yielding maize varieties.

A significant association between ear rot percentage and 3 SNPs located on chromosomes 2, 7, and 8 was observed in the study. In addition, 13 SNPs distributed across 9 maize chromosomes, excluding chromosome 10, have been reported to be associated with ear rot resistance in maize (Kamweru *et al*., 2022). These studies indicate that SNPs associated with maize ear rot resistance have been found in all the chromosomes of the maize genome.

GWAS allows for candidate gene identification and functional prediction and can be used for gene expression studies (Narkhede *et al*., 2023). Functional prioritization of candidate genes found in 100 kb upstream and downstream of significant SNPs revealed 29436 putative candidate genes associated with resistance to leaf and cob damage, grain yield, and ear rot percentage among the 137 inbred lines that were evaluated. The prioritization of candidate genes located within a specified window helps avoid possible expenses incurred from the validation of potentially unfit

genes, since not all genes adjacent to the SNPs regulated the trait of interest (Zhang *et al*., 2024). However, annotation has been carried out on just one percent of the maize genome, thus posing a challenge in prioritization of candidate genes based on the genetic information documented. Consequently, an integrated approach involving gene ontology-based functional gene enrichment analysis offers a promising alternative (Zhang *et al*., 2024).

Thirty-two candidate genes associated with FAW leaf damage in the study were identified based on their role in regulating various metabolic pathways involved in stress (biotic and abiotic) control. The identified genes and pathways leading to the biosynthesis of geranylgeranyl diphosphate, acetyl-CoA, ethylene, flavanol, jasmonic acid, sporopollenin, and wax esters are highly associated with resistance to FAW leaf damage in maize. These results are in line with those of Warburton *et al*. (2023). Wax ester is a key component in the biosynthesis of Epicuticular wax, which creates a physical barrier to insect infestation in maize. Reports indicate that biosynthesis of Suberin monomers, which was associated with FAW resistance in the study, is normally activated when the plant is exposed to various environmental stresses (Chen et al., 2022). When suberin is deposited on the endodermis, it forms a hydrophobic barrier, hence protecting the plants from pests and pathogens through antixenosis (Chen et al., 2022). Genes involved in the regulation of Benzoxazinoids and DIMBOA were found to be closely associated with resistance to FAW leaf damage. Benzoxazinoids have been reported to be associated with biochemical defense against various biotic stresses in maize (Zhou *et al*., 2018). DIMBOA and benzoxazinoids have been reported to confer resistance to leaf feeding in Asian corn borer, corn leaf aphid (Niu *et al*., 2023). Genes responsible for the glyoxylate cycle and fatty acid degradation, shown to be associated with resistance to FAW, have been reported to be activated during plant defense reactions against pathogens (Cots *et al*., 2002).

The geranylgeranyl diphosphate (GGPP) biosynthesis pathway is important in plants' resistance

to larval insect pests (Warburton *et al*., 2023). The levels of jasmonic acid have been documented to increase before FAW infestation in maize, indicating that the compound contributes significantly to resistance to the pest (Prasanna *et al*., 2022). Plant signaling molecules like jasmonic acid, ethylene, salicylic acid, and abscisic acid regulate the production of secondary metabolites required for plant response to insect infestation (Badji *et al*., 2020). Manipulation of biosynthesis and recycling of Nicotinamide adenine dinucleotide (NAD) leads to variation in the level of metabolites produced and changes in resistance to various pathogens. This indicates that NAD should be further evaluated as a potential substance for improvement of resistance to biotic stresses such as FAW resistance in maize (Pétriacq *et al*., 2013). Plants' resistance to pests like FAW is based on their immune system mechanism, which alerts and initiates plant response to the pest attack. Maize plants exhibit host plant resistance through antibiosis, antixenosis, and tolerance. Antibiosis and antixenosis affect pest populations (Purnomo, 2021). Plants' defense mechanisms may be activated in response to pest infestation or mechanical damage. The plants might respond by producing secondary metabolites that are toxic to the pest, thus interfering with their feeding behavior (antibiosis) or through attraction of the insect's natural enemies (Zhang *et al*., 2024). Gene ontology and functional enrichment studies enable the understanding of biological processes and pathways affected by FAW infestation in maize (Zhang *et al*., 2024).

Gene Ontology (GO) enrichment analysis revealed that the biological processes regulating FAW resistance in maize comprised plant defense response, DNA transcription, RNA biosynthesis, secondary metabolite biosynthesis, homeostasis, and respiration. The pathways were highly significantly enriched in maize inbred lines infested with FAW. Similar results were reported after infestation of maize with FAW larvae (Zhang *et al*., 2024). In addition, there was significant enrichment in pathways associated with the regulation of defense response resulting from insect

damage, jasmonic acid-mediated signaling pathways, and biosynthesis of secondary metabolites.

Homeostatic regulation of hormones like jasmonic acid, abscisic acid, and ethylene is key in plant resistance to insects like FAW (Niu *et al.*, 2023). FAW Resistance is mostly regulated by jasmonic acid-mediated signaling (Huang *et al.*, 2023). FAW infestation in maize induces the production and accumulation of jasmonic acid. High levels of wound-induced jasmonic acid have been reported to be associated with FAW leaf damage resistance in maize, while lower levels increased susceptibility to the pest (Huang *et al.*, 2023).

In the current study, the high enrichment of biological processes involved in transcription of DNA, RNA biosynthesis may be because metabolic pathways that synthesize secondary metabolites are controlled by posttranscriptional and posttranslational mechanisms (Patra et al., 2013). Secondary metabolites are allelochemicals produced by plants that repel insect pests or make the plant unpalatable (Purnomo, 2021). High enrichment of processes regulating biosynthesis of secondary metabolites, such as total flavonoid and phenolic content, was shown to be higher in maize infested with FAW as compared to those without (Zhang *et al.*, 2024). Furthermore, after FAW infestation of maize plants, the changes in the total phenolic and flavonoid contents were greater (Zhang *et al.*, 2024). The enriched biological processes may lead to the biosynthesis of hormones like jasmonic acid, abscisic acid, and ethylene, salicylic acid, and secondary metabolites such as phenolic and flavonoids for plant defense (Yang *et al.*, 2024). These observations should be investigated further for HPR to FAW in maize.

Biotic stresses induced by pathogens are a major concern to global food production *(van Esse et al.*, 2020). *Fusarium verticillioides, Aspergillus niger, Rhizoctonia* spp, *Penicillium* spp, and *A. flavus* are fungi causing ear rot disease in maize and have been reported to be predominantly associated with FAW cob infestation (Akinbode *et al.*, 2022). FAW infestation causes maize ear

damage and may predispose maize to infections by fungi, resulting in reduced quantity and quality (Akinbode *et al*., 2022). Grain yield has been reported to be highly negatively correlated to FAW leaf and cob damage and ear rot (Kamweru *et al*., 2022). This implies that an increase in the percentage of ear rots will decrease yield and lower the quality of maize grains produced.

Plant surface receptors recognize microbe-associated molecular patterns (MAMPs) and initiate immune responses, which produce regulatory proteins, protein kinases, and transcription factors involved in host-pathogen genetic interactions and initiation of defense-related gene regulation (Nazir *et al*., 2019).

Highly enriched genes associated with ear rot in maize were involved in the regulation of metabolic processes for the biosynthesis of pyridine, nicotinamide, and the regeneration of precursors of metabolites and energy. Similarly, extracellular nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD) in plants have been shown to control diseases caused by phytopathogens such as fungi (Ueda *et al*., 2021). Enrichment of genes regulating the biosynthesis of nicotinamide adenine dinucleotide in plants affects redox signaling by production of pyridine alkaloids and reactive oxygen species, resulting in activation of defense response through the salicylic acid-dependent signaling pathway (Miwa *et al*., 2017). Invasion by fungi causing ear rot in maize stimulates reactive oxygen species production, which may activate the hypersensitive response, thereby killing the fungi and/or preventing the spread of the fungal infection by thickening the adjacent cell walls (Huang *et al*., 2019). Some of the candidate genes identified by the SNP markers associated with ear rot in the study may be involved in the synthesis of NADP and NAD compounds, which are important when breeding for resistance to maize ear rot and should be further investigated.

Maize grain size and weight contribute directly to yield (Long *et al*., 2024). These traits are

highly affected by the efficiency of the starch biosynthesis process and storage, which involves transportation of sucrose to the storage sites, such as the kernel, and its conversion to uridine diphosphate-glucose and fructose by sucrose synthase (Long *et al*., 2024). These complex processes are also regulated by several phytohormones such as auxin, ethylene, and abscisic acid *(He et al., 2023).*

The biological processes associated with grain yield that were significantly enriched were purine nucleotide metabolism, carbohydrate metabolism, peptide synthesis and aerobic respiration, and cation transportation processes. Previous studies indicated that carbohydrate metabolic processes, such as starch synthesis, were high during biosynthesis in maize endosperm (Finegan *et al*., 2022). In addition, purine nucleotide metabolic processes have been identified to be among the most highly enriched biological processes induced by the presence of abscisic acid in maize during the grain filling stage (He *et al*., 2023).

5.5 Conclusion

The study identified genomic regions and candidate genes associated with resistance to FAW in tropical maize germplasm. Eight highly significant SNPs associated with FAW leaf and cob damage traits confirmed the presence of native host plant resistance for FAW within the population that could be exploited when improving the trait. Thirty-two candidate genes associated with FAW leaf damage in the study were identified based on their role in regulating various metabolic pathways involved in stress (biotic and abiotic) control. Candidate gene and pathways analysis provided a more detailed view of FAW resistance in maize by uncovering new genes and potential metabolic pathways associated with resistance to the pest; these included genes governing pathways regulating the biosynthesis of NAD/NADH, DIBOA-glucoside, benzoxazinoids, phenolic, and flavonoid pyrimidine deoxyribonucleotides, suberin monomers

biosynthesis, glyoxylate cycle, and fatty acid degradation. Gene Ontology (GO) enrichment analysis further revealed the biological processes regulating plant defense response during FAW infestation in maize. The SNPs and candidate genes, pathways, and biological processes identified can be used in FAW-resistant breeding programs and, therefore, should be further investigated.

CHAPTER SIX

6.0 General conclusions and recommendations

6.1 Genetic diversity and population structure of tropical maize inbred lines for resistance to fall armyworm using phenotypic characterization and Diversity Array Technology sequence-based SNP markers

The study revealed significant phenotypic and genetic diversity among the tropical inbred lines that may be used for maize improvement. Phenotypic diversity was expressed through significant genotypic variation within the study population. The presence of genetic diversity within the study population was confirmed by: 1) Observed gene diversity (GD) of 0.35, indicating the presence of moderate diversity. 2) The high level of heterozygosity observed (Ho) signifying the presence of genetic diversity within the subpopulation. 3) Higher genetic diversity within the subpopulation (65%). 4) Nine subpopulations generated from population structure analysis based on cross-entropy criteria and neighbor-joining hierarchical cluster analysis, emphasizing the high genetic variation within the 140 inbred lines. 5) Moderate divergence across the subpopulations revealed by the population pair-wise fixation index. The observed genetic diversity from the study could be used to enhance selection efficiency, broaden the genetic base of the breeding population, and enhance the formation of high-yielding stress-tolerant hybrids adapted for different agroecological zones.

6.2 Estimation of gene action for resistance to fall armyworm in tropical maize inbred lines

The evaluation of hybrids under FAW artificial and natural infestation for damage parameters and agronomic traits identified the gene action governing the traits. Fall armyworm leaf and cob damage, and ear aspect were controlled by additive gene effects, while plant height, ear height, and ear rot were regulated by both additive and non-additive gene effects. Additive gene was the predominant gene action in all the traits evaluated, implying that GCA effects mainly accounted for the variation among the parental inbred lines. Maize inbred lines with a significant negative GCA effect in fall armyworm damage parameters and ear rot were selected to be used for future improvement of the traits. Lines with significant GCA effect could contribute to favorable alleles for improving grain

yield in maize. Lines with significant positive GCA for plant and ear height are suitable for improving the height in maize.

Twenty (20) inbred lines (3,4,5,7,11,14,15,1819,21,22,23,24,25,26,27,28,29,31,36) were identified as new sources of fall armyworm leaf damage resistance alleles in maize and thirteen (13) inbred lines (6,7,8,15,17,18,20,21,23,25,26,27,31) identified as new sources of fall armyworm cob damage resistance genes in maize.

Eight (8) inbred lines 3,4,6,7,9,24,28,31, were identified as new sources of alleles for resistance to maize ear rot disease, and one (1) inbred line (Entry 27) was identified as a new source of alleles for maize grain yield improvement.

Twenty-three (23) hybrids with resistance to maize ear rot disease were identified (2x34, 7x11, 7x15, 8x25, 9x21, 10x19, 13x2, 14x32, 15x33, 16x30, 19x6, 20x29, 24x27, 25x3, 26x12, 26x13, 27x22, 28x22, 31x11, 31x16, 32x23, 32x36, 33x14)

Forty-two (42) new hybrids promising for increased plant height were identified. These were 1x20, 2x34, 3x11, 6x16, 6x17, 7x15, 7x17, 8x21, 8x25, 9x19, 9x21, 9x25, 10x35, 11x05, 11x30, 12x27, 15x33, 16x26, 16x33, 18x32, 19x6, 19x31, 20x8, 21x3, 21x31, 22x10, 23x29, 24x2, 25x3, 26x12, 26x14, 28x20, 28x34, 29x35, 30x14, 32x23, 32x36, 33x14, 33x24, 34x10, 34x29, 35x3.

Forty-eight (48) new hybrids (1x22, 1x34, 1x36, 3x17, 5x24, 6x15, 7x16, 7x17, 8x35, 9x25, 9:35, 10x21, 10x25, 11x33, 12x27, 13x1, 13x32, 14x27, 15x5, 16x26, 16x30, 17x05, 17x30, 18x02, 18x27, 19x3, 20x29, 21x7, 22x10, 23x10, 23x29, 24x2, 26x13, 26x18, 27x22, 28x23, 28x36, 29x19, 30x14, 32x22, 32x23, 33x12, 33x18, 34x8, 34x9, 35x6, 35x7, 35x31) were promising for reduced plant height in maize.

Forty-two (42) new hybrids from the study were promising for increased ear height in maize. The hybrids were 1x20, 2x34, 3x11, 6x16, 6x17, 7x15, 7x17, 8x21, 8x25, 9x19, 9x21, 9x25, 10x35,

11x05, 11x30, 12x27, 15x33, 16x26, 16x33, 18x32, 19x6, 19x31, 20x8, 21x3, 21x31, 22x10, 23x29, 24x2, 25x3, 26x12, 26x14, 28x20, 28x34, 29x35, 30x14, 32x23, 32x36, 33x14, 33x24, 34x10, 34x29, 35x3.

6.3 Genome-wide association studies and gene ontology functional enrichment analysis for resistance to fall armyworm in tropical maize inbred lines

The study identified genomic regions and candidate genes associated with resistance to FAW in tropical maize germplasm. Eight highly (8) significant SNPs associated with FAW leaf and cob damage traits confirmed the presence of native host plant resistance for FAW within the population that could be exploited when improving the trait. Thirty-two (32) candidate genes associated with FAW leaf damage in the study were identified based on their role in regulating various metabolic pathways involved in stress (biotic and abiotic) control. Seven (7) new genes and potential metabolic pathways associated with resistance to the pest; these included genes governing pathways regulating the biosynthesis of NAD/NADH, DIBOA-glucoside, benzoxazinoids, phenolic, and flavonoid pyrimidine deoxyribonucleotides, suberin monomers biosynthesis, glyoxylate cycle, and fatty acid degradation. New biological processes regulating plant defense response during FAW infestation in maize were discovered.

6.4 Recommendations

Inbred lines with resistance to fall armyworm can be used as parental lines for the accumulation of FAW-resistant alleles through recurrent selection.

Lines from genetically distinct sub-populations are recommended for marker-assisted hybrid maize breeding to maximize heterosis.

Lines with negative GCA effects for FAW and ear rot are recommended as new sources of resistant alleles during breeding

The promising new hybrids identified for various traits from the study need to be further exploited

for the improvement of those specific traits

The SNPs, candidate genes, pathways, and biological processes identified provide extensive genomic information that can be further investigated for FAW resistance in maize

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APPENDICES

Appendix 3.1 Artificial infestation of maize with Fall armyworm neonates on the 14th day after germination

Appendix 3.2. Fall armyworm leaf damage scoring in maize

Appendix 3.3 Combined mean performance for FAW damage and agronomic traits of 140 tropical maize inbred lines under artificial FAW infestation during 2023 seasons A and B in Kiboko

Genotype	LDS1	LDS2	LDS3	Plant height	Ear height	No. ears	Ear Aspect	Grain yield	Cob damage
CKL177012	3.29	5.85	5.63	141.32	61.05	10.47	2.94	0.52	1.98
CKL201115	3.26	5.77	5.77	169.76	87.59	10.94	2.59	0.58	1.88
CKL201171	3.26	5.79	5.76	146.54	74.27	11.63	2.62	0.51	1.69
CKL201243	3.26	5.67	5.58	152.41	90.68	11.94	2.49	0.55	1.70
CKL201274	3.25	5.72	5.65	127.74	74.97	12.44	2.68	0.58	1.73
CKL201279	3.27	5.71	5.60	162.92	90.03	11.96	2.62	0.54	1.97
CKL201281	3.25	5.68	5.55	147.38	86.20	15.75	2.52	0.65	1.61
CKL201283	3.28	5.68	5.53	161.03	73.11	10.38	3.15	0.38	1.78
CKL201285	3.27	5.70	5.57	138.73	66.62	11.49	2.69	0.61	1.71
CKL201286	3.25	5.75	5.58	148.50	81.76	12.12	2.86	0.49	1.85
CKL201292	3.26	5.69	5.80	122.52	65.12	11.01	2.75	0.58	1.87
CKL201294	3.25	5.75	5.54	133.38	71.23	12.76	2.63	0.58	1.72
CKL201297	3.24	5.82	5.78	132.78	72.45	11.73	2.68	0.62	2.08
CKL201303	3.26	5.85	5.65	166.06	100.67	12.06	2.56	0.55	1.81

CKL201307	3.27	5.79	5.72	136.75	76.58	11.21	2.83	0.54	2.00
CKL201314	3.25	5.74	5.55	144.37	82.54	13.21	2.66	0.67	1.84
CKL201319	3.25	5.70	5.55	110.30	62.57	11.05	2.61	0.52	1.95
CKL201320	3.31	5.79	5.60	116.25	49.73	11.45	2.91	0.58	2.09
CKL201328	3.25	5.67	5.54	142.57	73.54	12.22	2.94	0.50	1.91
CKL201330	3.29	5.78	5.59	141.78	62.09	10.76	2.78	0.44	1.90
CKL201331	3.27	5.78	5.75	150.11	65.04	13.16	2.65	0.52	1.72
CKL201341	3.30	5.67	5.74	141.90	73.93	12.10	2.87	0.54	1.88
CKL201343	3.27	5.82	5.83	154.21	65.81	10.80	2.66	0.53	1.72
CKL201346	3.25	5.73	5.71	144.98	72.19	13.02	3.03	0.61	1.97
CKL201353	3.25	5.72	5.65	134.82	67.72	13.79	2.93	0.54	2.20
CKL201363	3.29	5.84	5.76	148.01	73.05	13.44	2.87	0.58	2.17
CKL201366	3.27	5.87	5.89	190.21	79.56	12.42	2.81	0.60	1.83
CKL201368	3.31	5.92	5.63	121.70	71.81	13.35	2.79	0.49	1.90
CKL201381	3.30	5.76	5.50	140.62	65.84	10.28	2.74	0.44	1.86
CKL201384	3.26	5.68	5.78	153.45	69.36	12.00	2.78	0.49	1.93
CKL201385	3.28	5.78	5.71	145.10	78.42	11.11	2.75	0.68	1.91
CKL201388	3.27	5.78	5.63	136.97	68.54	9.89	2.88	0.42	1.80
CKL201389	3.27	5.70	5.75	152.17	77.67	11.84	2.71	0.62	1.69
CKL201391	3.29	5.80	5.54	126.91	64.14	11.46	3.07	0.57	1.95
CKL201407	3.26	5.58	5.44	126.03	64.17	13.64	2.88	0.52	1.91
CKL201409	3.32	5.87	5.78	154.83	66.09	11.07	2.86	0.55	2.17
CKL201410	3.28	5.77	5.63	149.17	81.30	10.51	2.69	0.48	1.89
Appendix 3.2 continued									
CKL201413	3.27	5.74	5.77	146.78	84.11	11.79	3.05	0.58	2.27
CKL201415	3.27	5.66	5.54	162.74	87.76	10.92	2.78	0.42	1.78
CKL201417	3.25	5.65	5.56	150.13	82.54	13.74	2.76	0.70	2.18
CKL201423	3.28	5.81	5.67	154.48	76.07	10.18	2.93	0.45	1.79
CKL201507	3.26	5.73	5.62	140.24	68.55	11.45	2.82	0.45	1.98
CKL201512	3.29	5.76	5.88	149.73	63.74	10.68	2.90	0.50	2.38
CKL201522	3.28	5.78	5.63	157.27	70.83	12.40	2.72	0.55	1.99
CKL201526	3.27	5.78	5.88	144.47	71.00	12.94	2.89	0.58	2.35
CKL201528	3.26	5.68	5.58	146.60	70.59	13.18	2.37	0.77	1.91
CKL201531	3.27	5.71	5.68	127.82	62.26	11.47	2.58	0.51	1.73
CKL201584	3.26	5.75	5.66	163.32	77.90	13.82	2.58	0.67	1.82
CKL201586	3.26	5.76	5.64	129.66	63.22	13.22	2.51	0.65	1.89
CKL201588	3.27	5.78	5.63	139.76	75.40	12.13	2.69	0.55	1.99
CKL201589	3.28	5.78	5.63	125.08	68.26	14.17	2.65	0.71	2.07
CKL201590	3.27	5.82	5.83	159.97	90.44	11.82	2.57	0.51	1.77
CKL201594	3.29	5.91	5.69	180.68	96.42	10.92	2.69	0.60	1.79
CKL201598	3.28	5.69	5.66	136.99	74.43	12.38	2.66	0.57	1.88
CKL201599	3.27	5.69	5.65	113.82	58.61	12.44	2.90	0.43	1.83
CKL21578	3.26	5.57	5.45	187.15	81.26	12.67	2.74	0.63	2.15
CKL21580	3.28	5.72	5.54	124.90	50.52	12.08	2.79	0.51	2.07

CKL21581	3.28	5.72	5.59	153.99	64.35	11.30	2.67	0.51	1.89
CKL21582	3.27	5.81	5.51	148.21	72.55	10.38	3.13	0.47	1.94
CKL21583	3.24	5.70	5.57	197.37	79.13	14.85	2.55	0.68	1.88
CKL21584	3.24	5.70	5.48	156.61	76.12	13.61	2.65	0.54	2.11
CKL21587	3.26	5.71	5.54	162.73	61.59	12.28	2.94	0.55	2.22
CKL21588	3.28	5.74	5.63	186.87	82.35	12.43	2.79	0.57	2.28
CKL21589	3.26	5.71	5.75	188.08	66.30	12.02	2.78	0.52	1.89
CKL21591	3.25	5.70	5.61	135.20	56.03	12.06	2.75	0.53	1.92
CKL21592	3.28	5.81	5.55	149.52	65.30	11.99	2.93	0.48	1.89
CKL21594	3.28	5.77	5.67	157.22	68.79	10.92	2.78	0.59	2.09
CKL21595	3.26	5.75	5.58	155.18	79.68	14.39	2.43	0.60	1.89
CKL21596	3.28	5.60	5.39	168.10	67.49	12.30	3.03	0.53	1.93
CKL21597	3.30	5.75	5.72	164.59	74.56	11.52	2.84	0.50	2.01
CKL21598	3.29	5.66	5.46	140.92	70.37	10.45	3.04	0.46	2.65
CKL21599	3.28	5.69	5.60	147.78	70.15	11.85	2.98	0.44	2.09
CKL21600	3.29	5.87	5.74	143.76	51.64	10.90	2.57	0.55	1.98
CKL21601	3.26	5.68	5.51	159.09	70.59	14.03	2.75	0.73	1.97
CKL21602	3.29	5.69	5.55	110.94	47.00	10.91	3.06	0.43	2.06
CKL21603	3.28	5.74	5.64	133.78	62.97	12.36	2.56	0.53	2.10
CKL21604	3.24	5.65	5.56	125.79	49.95	11.22	2.67	0.49	1.79
CKL21605	3.27	5.68	5.66	156.93	82.28	13.18	2.63	0.60	2.14
CKL21606	3.26	5.67	5.56	123.36	63.88	10.44	2.86	0.51	2.00

Appendix 3.2 continued

CKL21608	3.24	5.54	5.45	161.47	77.54	13.93	2.75	0.61	1.94
CKL21610	3.26	5.62	5.38	159.88	71.08	11.48	2.63	0.51	1.85
CKL21611	3.26	5.68	5.54	159.53	65.07	11.69	2.87	0.51	2.14
CKL21612	3.27	5.67	5.54	164.77	68.00	12.59	2.65	0.53	1.78
CKL21613	3.25	5.48	5.40	186.58	77.25	15.05	2.58	0.69	2.20
CKL21615	3.27	5.75	5.65	212.70	92.31	14.29	2.72	0.56	1.96
CKL21616	3.27	5.64	5.52	149.82	67.10	14.20	2.70	0.61	1.81
CKL21617	3.26	5.66	5.55	185.65	69.80	12.56	2.72	0.49	1.81
CKL21618	3.26	5.66	5.47	193.73	85.19	12.11	2.73	0.54	2.28
CKL21619	3.24	5.58	5.48	143.86	68.30	13.81	2.90	0.60	2.11
CKL21621	3.28	5.80	5.55	139.76	57.40	8.63	3.04	0.35	2.06
CKL21624	3.24	5.57	5.41	205.32	86.37	13.16	2.65	0.68	2.05
CKL21625	3.26	5.62	5.46	148.91	63.79	13.28	2.64	0.66	1.92
CKL21627	3.26	5.65	5.51	182.87	74.84	13.53	3.03	0.54	2.39
CKL21628	3.26	5.66	5.53	135.81	57.76	12.19	2.52	0.58	1.88
CKL21629	3.30	5.74	5.49	148.84	65.00	9.73	3.03	0.38	2.50
CKL21630	3.27	5.65	5.60	189.67	72.01	14.63	2.43	0.67	2.02
CKL21631	3.26	5.64	5.35	155.04	64.39	11.86	2.66	0.57	1.69
CKL21632	3.27	5.79	5.54	133.02	64.92	12.75	2.59	0.64	1.79
CKL21633	3.29	5.88	5.74	172.29	77.94	9.14	3.02	0.35	2.28
CKL21634	3.27	5.67	5.55	197.92	115.56	12.78	2.45	0.70	1.78

CKL21638	3.26	5.68	5.58	187.61	62.08	13.11	2.94	0.49	2.01
CKL21640	3.28	5.58	5.50	199.16	97.80	10.05	2.90	0.42	1.68
CKL21641	3.27	5.62	5.44	175.56	75.65	12.45	2.94	0.54	2.28
CKL21644	3.27	5.77	5.65	157.54	72.78	10.17	2.89	0.46	2.01
CKL21645	3.27	5.75	5.67	184.80	96.78	9.51	2.85	0.51	1.81
CKL21646	3.27	5.76	5.68	193.51	92.78	12.02	2.76	0.64	2.11
CKL21648	3.27	5.61	5.44	157.85	68.64	12.87	2.59	0.61	2.06
CKL21649	3.26	5.72	5.61	195.79	81.53	12.46	2.56	0.56	1.74
CKL21650	3.28	5.79	5.54	170.73	82.66	11.14	2.66	0.67	2.10
CKL21651	3.27	5.72	5.57	167.09	66.77	11.49	2.79	0.58	1.70
CKL21652	3.27	5.75	5.49	151.42	73.63	10.73	2.90	0.46	2.14
CKL21653	3.26	5.65	5.73	141.91	52.95	11.97	2.82	0.49	2.01
CKL21656	3.27	5.64	5.59	164.53	70.51	13.30	2.95	0.55	2.29
CKL21657	3.26	5.68	5.49	207.79	91.36	11.41	2.61	0.56	1.89
CKL21658	3.26	5.59	5.56	193.90	97.25	10.90	2.83	0.50	1.59
CKL21660	3.27	5.74	5.72	167.19	79.26	11.79	2.74	0.53	1.99
CKL21661	3.25	5.70	5.72	171.46	93.54	12.11	2.65	0.62	2.01
CKL21662	3.27	5.70	5.61	173.53	92.91	11.99	2.67	0.56	2.22
CKL21663	3.25	5.67	5.60	156.71	89.43	11.47	2.82	0.60	2.36
CKL21664	3.27	5.75	5.59	194.37	86.42	12.24	2.49	0.62	1.93
CKL21665	3.27	5.62	5.49	152.48	60.93	11.00	2.98	0.45	2.13
Appendix 3.2 continued									
CKL21667	3.27	5.65	5.56	201.99	67.08	11.31	2.66	0.45	1.97
CKL21668	3.25	5.58	5.42	176.72	75.03	12.69	3.04	0.57	2.07
CKL21670	3.27	5.69	5.52	175.66	97.49	14.21	2.32	0.64	1.83
CKL21671	3.28	5.74	5.65	156.47	78.05	11.42	2.92	0.47	2.68
CKL21672	3.26	5.82	5.80	175.16	85.76	12.55	2.80	0.59	2.22
CKL21681	3.27	5.80	5.62	165.47	85.80	9.15	3.03	0.37	1.94
CKL21682	3.28	5.77	5.67	172.87	87.03	9.94	2.96	0.40	1.87
CKL21683	3.27	5.68	5.75	150.36	86.05	9.22	3.17	0.44	2.23
CKL21686	3.26	5.56	5.50	177.18	67.67	11.46	2.89	0.39	1.81
CKL21688	3.28	5.64	5.52	139.80	61.30	11.65	2.81	0.55	2.19
CKL21694	3.28	5.83	5.71	160.99	62.97	11.93	2.59	0.67	2.03
CKL21696	3.26	5.60	5.46	182.62	75.58	12.90	2.83	0.68	2.38
CKL21698	3.26	5.73	5.73	144.09	69.01	9.25	2.73	0.45	1.79
CKSBL1008	3.24	5.59	5.50	183.14	85.29	13.56	2.74	0.66	1.88
CML125	3.25	5.53	5.52	204.26	84.31	11.96	2.42	0.69	1.81
CML24	3.23	5.37	5.39	125.44	61.49	12.42	2.47	0.51	1.60
Appendix 3.2 continued									
CML336	3.28	5.75	5.67	146.22	67.51	11.68	2.76	0.58	2.18
CML338	3.24	5.61	5.61	163.18	79.23	12.20	2.20	0.68	1.90
CML610A	3.26	5.82	5.77	149.81	75.26	12.21	2.39	0.61	1.77
Heritability	0.16	0.4	0.43	0.91	0.86	0.51	0.39	0.52	0.4

Genotype Variance	0.01	0.02	0.03	558.95	161.11	3.52	0.09	0.01	0.11
GenxLoc Variance	0.02	0.01	0	42.62	23.71	3.39	0.12	0.01	0.14
Residual Variance	0.07	0.1	0.19	141.77	56.83	6.81	0.32	0.04	0.38
Grand Mean	3.27	5.78	5.58	156.9	73.88	12	2.76	0.55	1.97
LSD 5%	0.19	0.32	0.41	21.47	13.89	3.77	0.66	0.24	0.72
CV	8.05	5.56	7.76	7.59	10.2	21.75	20.55	34.26	30.87

CV- Coefficient of variation; LSD- Least significant difference; LD1-FAW Leaf damage score at 7 days, LD2-14 days and LD3-21 days after artificial infestation; No Ears-Number of ears

Appendix 3.4. Fall armyworm-tolerant checks

Appendix 3.5. Fall armyworm susceptible checks

Appendix 4.1 Pollination of inbred lines during hybrid development

Appendix 4.2 List of 128 hybrids evaluated for combining ability under artificial FAW infestation during the 2023 seasons A and B in Kiboko, Kirinyaga, and Kakamega

CKL201346/CML610A	CML24/CKL21630	CKL21698/CKL201243	CKL21611/CML24
CKL201297/CKSBL10025	CKL21661/CKSBL10025	CKL21611/CKL201243	CKL201297/CML610A
CKL201346/CKSBL10025	CKL201243/CKL21653	CKL21584/CKSBL1008	CKL21661/CML610A
CKL187012/CKL21625	CKL201171/CKL21618	CKL201285/CML338	CKL21630/CKL177012
CKL21618/CML125	CKL201599/CKSBL10025	CKL201586/CML338	CML610A/CKL201285
CML336/CKL201598	CKL187012/CKL21611	DK777 (commercial hybrid)	CKL201115/CKL21618
CKL201586/CKL187009	CKL201598/CKL187012	CML444/CKL201297	CKL201171/DL21113
CML24/CKL21653	CKL187019/DL21115	CKSBL1008/CKL201346	CKL21584/CML444
CKL201331/CKL187011	CKL21625/CML24	WH507	CKL21618/CKL177012
CKL187009/CKL21698	CKL201415/CKL187012	CKL201285/CKL187010	CKL201331/CML336
CKL201243/CKL21630	CKL21653/CML125	CML338/CKL21611	DL21115/CKL201243
CML444/CKL201346	CKL187008/CKL201331	CKSBL10025/CKL201285	CKSBL1008/CKL201297
CKSBL1008/CKL201599	CML24/CKL21618	CKL21661/CKL187011	CKL21653/CML444
DL21113/CML125	CKL21630/CML444	DL21113/CML444	CKL21618/CML444
CKL201599/CML610A	CML444/CKL21661	CKL201115/DL21113	CML336/CKL201586
CKSBL1008/CKL21661	FAW2003 (FAW tolerant variety)	CKL201586/CKL187012	CKL187009/CKL21625
CKL201346/CML336	CKL187019/CKL21625	CML610A/CKL201415	CKL201285/CKL187009
FAW2001(FAW tolerant variety)	CKL187009/CKL21611	CML338/DL21115	DL21115/CML24
CKL201598/CML338	CKL21653/CKL177012	CKSBL10025/CKL201598	CKL201285/CKL187019
CKL187009/DL21115	CKSBL1008/CKL201331	CKL21630/CKL187008	CML336/CKL201285
CKL187019/CKL21611	CKL21698/CKL201115	CML338/CKL21625	CKL21625/CKL201115
CKL201586/CKL187010	CKL21653/CKL187008	CKL187008/CKL21661	CKL201243/DL21113

Appendix 4.2 continued

CKL21630/CML125	CKL201331/CKSBL10025	CKL187008/CKL201599	CKL201285/CKL187012
CKL187011/CKL201598	CKL21611/CKL201171	CML338/CKL21698	CML24/DL21113
CKL201346/CKL187011	CKL187011/CKL201285	DL21113/CKL177012	CKL177012/CKL201331
CKL21618/CKSBL1008	CKL201415/CKL187019	CKL21653/CKSBL1008	CML444/CKL201331
CKL201598/CKL187019	CKL187019/CKL21698	CKL21584/CML125	CKL201297/CML336
CML610A/CKL201586	CKL21584/CKL177012	DL21115/CKL201115	CKL177012/CKL21661
DL21113/CKL187008	CKL21625/CKL201243	CKL187008/CKL201297	CML444/CKL201599
CKL187011/CKL201415	WH508 (commercial hybrid)	CKL187012/DL21115	CKSBL10025/CKL201586
CKL201586/CKL187019	DL21115/CKL201171	CKL187008/CKL201346	CKL21698/CKL201171
CKL21625/CKL201171	CKL201243/CKL21618	CKL201598/CKL187009	CKL21630/CKSBL1008