CHARACTERIZATION OF THE GENETIC DIVERSITY OF *Cercospora zeina* IN KENYA AND MAPPING THE QTL FOR RESISTANCE TO GRAY LEAF SPOT AND *Turcicum* LEAF BLIGHT IN MAIZE

BY

OMONDI DENNIS ODUOR

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN GENETICS AND PLANT BREEDING

SCHOOL OF AGRICULTURE, FOOD SECURITY AND ENVIRONMENTAL SCIENCES

MASENO UNIVERSITY

© 2022

DECLARATION

I hereby declare that this dissertation is a representation of my original work which was done after registration for the degree of Master of Science in Genetics and Plant breeding, Maseno University and has not been submitted for any degree/ certificate in Maseno University or any other university. I have conducted this work myself and any information sourced out is acknowledged by way of reference.

Sign: ______________________ Date: ______________________

Omondi Dennis Oduor Registration No; Msc/AF/00031/016

Declaration by the supervisor

I hereby declare that I have read this thesis. This research has been conducted and the thesis developed under my supervision. This thesis is hereby submitted for examination with my approval as the student's supervisor.

Prof. Mathews Dida

Sign: ______________________ Date: ______________________

School of agriculture, food security and environmental sciences.

Maseno University.

Prof. Dave Berger

Sign: ______________________ Date: ______________________

Faculty of Natural and Agricultural Sciences

Department of Plant and Soil sciences

Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria.

Dr. Yoseph Beyene

Sign: ______________________ Date: ______________________

Senior maize breeder

International maize and wheat improvement center (CIMMYT) - Nairobi.

ACKNOWLEDGEMENTS

This study was supported by the Kenya- South Africa joint science and technology research collaboration. The grants received from the National Research Fund, Kenya and the National Research Foundation (NRF), South Africa (grant # 105806) towards this research is hereby acknowledged.

I wish to extend my special and most sincere gratitude to my supervisors;

Professor Mathews Dida, Thank you so much for your time and patience. Indeed you are a great professor who is able to explain complex topics and make them look very simple. I truly appreciate the help, guidance and financial support I received from you when conducting my research work.

Professor Dave Berger, Thanks for your humility and being willing to share with me so much knowledge and experience as well. Your strong attention to detail approach has inspired me to be more diligent on my work. Thank you so much for caring about our well-being as students.

Dr. Yoseph Beyene, Thank you for all you did for this project. You played a very important role in shaping my ideas towards the implementation of this project. Many thanks for providing the necessary materials such as seeds and pesticides.

Dr. Irene Barnes, You are gifted at explaining things in a clear fashion for students to comprehend, I am extremely grateful for your guidance and assistance throughout this research work, particularly for working together with us during collection of the samples in Kenya.

Dr. David Nsibo thank you very much for your mentorship. I appreciate the great zeal and enthusiasm that you have towards sharing wisdom with your students. This, you taught me that benefits not only the receiver but also the giver. When I started my research work I was very green and thanks to your teaching I have gathered so much knowledge within the last two years. I will always be grateful for everything you have taught me.

It is my wish to express my heartfelt thanks to Feroze Ferris for your technical support with regards to performing fungal isolation and culturing of the fungus. I was fortunate to work with you.

I also thank Prof. Mathews Dida and Prof. Dave Berger for including me into the Kenya-South Africa bilateral project and for their financial support in facilitating my travel to and stay in South Africa to be trained on practical aspects of molecular work.

I would also like to thank the Dean School of Agriculture, food security and environmental sciences Prof. George Odhiambo and Prof. Harun Ogindo, the departmental head for their seamless efforts towards supporting us. I also appreciate the school of graduate studies at Maseno University for giving me the chance to fulfill my potential here.

I wish to thank my parents for their generous support. They have been committed to my studies and have taken the initiative to advise me on the most appropriate career path.

My sincere appreciation to Molecular Plant-Pathogen's Interaction (MPPI) and Cereal Foliar Pathogen Research (CFPR) group members particularly Mr. Nicky Olivier, Richard Kotze, Ingrid Marais, Trystan, Kevin Scheepers, Tanya, Eugene, Carol Ann for their assistance with most of the lab work and their suggestions as well.

I am also grateful to the Plant Sciences Complex 4th floor lab, University of Pretoria for the use of their equipment. I also thank Ms. Gladys Shabangu for her assistance with gene scan at the sequencing facility within the Forestry and Agricultural Biotechnology Institute.

Since this study required the movement of plant materials from one country to another, I wish to acknowledge the technical staff at Kenya plant health inspectorate service for their invaluable assistance. It is through them that I was able to acquire the phytosanitary certificate to enable the movement of maize leaves from Kenya to South Africa.

I wish to also mention Mr. Andrew Chavangi of CIMMYT who guided me through statistical analysis.

DEDICATION

This work is dedicated to my mother Mrs. Lydia Rawago for her unrelentless support.

ABSTRACT

Gray leaf spot (GLS) caused by *Cercospora zeina* in Africa and *Turcicum* leaf blight (TLB) caused by *Exserohilum turcicum* are a major threat to maize production due to the associated grain yield losses. Resistance in the currently grown maize hybrids could be overcome by high levels of genetic diversity that characterize *C. zeina* and *E. turcicum* populations. However, little is known concerning the population structure and diversity of *C. zeina* in Kenya. The objectives of this study were first to characterize the genetic diversity of *C. zeina*. Secondly to map the quantitative trait loci (QTL) conditioning resistance to GLS and TLB in the double haploid (DH) population from CML511×CML546. The genetic diversity of *C. zeina* and the role of sexual recombination in this population was determined by collecting GLS infected maize leaves from four counties in Kenya. The genomic DNA for the 129 successful isolates were assayed using previously designed mating type (MAT) primers and genotyped using 11 microsatellite markers. The *CTB7* (cercosporin toxin biosynthesis 7) test confirmed that all the isolates sampled were *C. zeina* as they all produced PCR products of 618 bp. The population exhibited high levels of gene diversity (H_e=0.445), slightly high gene flow (Nm=3.85) and high level of polymorphism. In addition, the four counties were characterized by nearly equal distribution of the two mating types, providing evidence that it could be undergoing sexual recombination. Occurrence of sexual recombination could be responsible for the high genetic diversity. STRUCTURE analysis revealed that the population clustered into four sub-groups according to the four counties. The PhiPT value of 0.15 (p=0.001) corroborated with AMOVA tests was significant to provide evidence for partial population differentiation. QTL mapping was achieved by evaluating the DH population in Maseno and Kabianga in a 5×46 alpha lattice design during the long rains of 2018 and 2019. The disease incidence for the plots were scored on a scale of 1-9 and the best linear unbiased predictions determined using META-R statistical software. Marker genotyping of the population was performed using 1250 markers in diversity arrays technology (DArTseq). Linkage map construction and QTL analysis were conducted in QTL IciMapping v4.1. Nine GLS resistance QTLs were mapped on the chromosomal bins 1.06, 1.07, 1.11, 2.04, 2.06, 3.04, 3.05, 4.1 and 7.04. Fourteen TLB resistance QTLs were detected on the chromosomal bins 1.02, 1.08, 2.05, 2.06, 2.07, 3.01, 3.04, 4.02, 4.08, 5.03, 6.05, 7.03, 8.08 and 10.04. The QTLs were detected in at least two environments. The highest phenotypic variance was conditioned by *qGLS1_190* (16.60%) for GLS and *qTLB8_171* for TLB (13.65%). Disease resistance was negatively correlated with flowering time suggesting higher resistance in the late maturing genotypes. These findings will enhance proper identification of the pathogen causing GLS and GLS management programs. The identified QTLs and their flanking markers could be validated and fine mapped in future work for use in breeding for resistance to GLS and TLB.

TABLE OF CONTENTS

Title page i
Declaration ii
Acknowledgements iii
Dedication v
Abstract vi
Table of Contents vii
Abbreviations and Acronyms xii
List of Tables xiii
List of Figures xiv
List of Plates xv
List of Equations xvi
List of Appendices xvii
CHAPTER ONE: INTRODUCTION 1
1.1 Background of the Study 1
1.2 Statement of the Problem 6
1.3 Objectives of the Study 7
1.3.1 General objective 7
1.3.2 Specific objectives 7
1.4 Hypotheses 7
1.5 Justification of the study 7
1.6 Significance of the Study 8
CHAPTER TWO: LITERATURE REVIEW 10
2.1 Maize: Importance and Biology of the Crop 10
2.1.1 Importance of Maize in Kenya 10
2.2 Gray leaf spot 11
2.2.1 Effects of GLS on maize (*Zea mays* L.) 11
2.2.2 Symptoms of Gray Leaf Spot 12
2.2.3 Historical perspective of *Cercospora zeina* 13
2.2.4 Geographic origin of the pathogen 14
2.2.5 Epidemiology of the pathogen 15
2.2.6 Infection cycle of Gray Leaf Spot 15
2.2.7 Cultural Characteristics of the pathogen 16
2.2.8 Taxonomy of *Cercospora zeina* 16
2.2.9 Dissecting the causal pathogen of gray leaf spot in Kenya 17

2.2.10 Identification using a species-specific diagnostic test ... 17
2.2.11 Mode of reproduction of the pathogen ... 18
2.2.12 Fungal pathogenesis ... 19
2.2.13 Production of Cercosporin ... 19
2.2.14 Genetics of resistance to gray leaf spot ... 20
2.2.15 Management of gray leaf spot ... 21
2.2.16 An overview of Previous QTL Studies on gray leaf spot ... 22
2.3 *Turcicum* leaf blight ... 23
2.3.1 Background information on *Turcicum* leaf blight ... 23
2.3.2 Taxonomy of *Exserohilum turcicum* ... 24
2.3.3 Symptoms of *Turcicum* leaf blight ... 25
2.3.4 Effect of *Turcicum* leaf blight on yield ... 26
2.3.5 Origin of *Exserohilum turcicum* ... 27
2.3.6 Disease Cycle of *E. turcicum* ... 27
2.3.7 Epidemiology of *Exserohilum turcicum* ... 28
2.3.8 High-risk Factors and Favorable Conditions ... 29
2.3.9 Infection strategy of the pathogen ... 30
2.3.10 Management of *Turcicum* Leaf Blight ... 31
2.3.11 Mode of Reproduction of the pathogen ... 32
2.3.12 Teleomorph formation from *E. turcicum* in nature ... 33
2.3.13 Impact of the Reproductive structure ... 34
2.3.14 Genetics of TLB Resistance ... 34
2.3.14.1 Qualitative resistance (Ht genes) ... 34
2.3.14.2 Pathogen races ... 37
2.3.14.3 Quantitative resistance ... 38
2.3.14.4 An overview of previous TLB resistance QTL studies ... 39
2.3.15 Distribution of *Turcicum* leaf blight ... 40
2.4 DNA markers ... 40
2.4.1 Microsatellite Markers ... 41
2.4.2 Single nucleotide polymorphisms (SNPs) ... 42
2.4.3 SNP Genotyping using Diversity Array Technology (DArT) ... 44
2.5 Population Genetics ... 45
2.5.1 Population Structure ... 49
2.5.2 Linkage Disequilibrium ... 49
2.6 Genetic Mapping ... 50

CHAPTER THREE: MATERIALS AND METHODS 52
3.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya 52
3.1.1 Materials 52
3.1.1.1 Reagents 52
3.1.1.2 Buffer compounds 52
3.1.1.3 Equipment 52
3.1.1.4 Media 52
3.1.2 Collection of GLS samples 52
3.1.3 Fungal isolations and DNA extractions 53
3.1.3.1 Fungal isolations 53
3.1.3.2 DNA extractions 54
3.1.4 PCR Analyses 54
3.1.4.1 Multiplex MAT gene PCR assay 54
3.1.4.2 Analysis of neutral markers 55
3.1.5 Gene scan analysis 55
3.1.6 Analysis of genetic diversity 56
3.1.6.1 Population differentiation 56
3.1.7 Population Structure 57
3.2 To determine the occurrence and role of sexual recombination 57
3.2.1 Gametic disequilibrium/ Analysis of recombination 57
3.3 Mapping of the Quantitative trait loci 58
3.3.1 Maize Genotypes 58
3.3.1.1 CML 511 58
3.3.1.2 CML 546 58
3.3.2 Field trials 58
3.3.2.1 Study sites and genetic material 58
3.3.2.2 Experimental Design 59
3.3.2.3 Artificial Inoculation 60
3.3.2.4 Phenotypic evaluation and Data collection 60
3.3.3 Genetic diversity among the genotypes 62
3.3.4 Genotyping 62
3.3.5 Statistical Analysis of the phenotypic data 62
3.3.5.1 Data Analysis 62
3.3.5.2 The BLUEs and BLUPs 63
3.3.5.3 Broad-sense heritability 64

3.3.5.4 Genetic correlations among environments 64
3.3.5.5 Genetic correlations between variables 65
3.3.5.6 Least significance difference 65
3.3.5.7 Coefficient of variation 65
3.3.5.8 Descriptive statistics 66
3.3.6 Construction of the genetic linkage map 66
3.3.7 QTL analysis 67
3.3.7.1 QTL comparison 67
CHAPTER FOUR: RESULTS 69
4.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya 69
4.1.1 The outcome of single spore isolations 69
4.1.2 Identification of the species associated with gray leaf spot 70
4.1.3 Genotyping of the SSR markers 73
4.1.4 Genetic diversity of *Cercospora zeina* in the four counties of Kenya 73
4.1.5 Population genetic differentiation, gene and genotypic flow 76
4.1.6 Population structure 79
4.1.7 Principal co-ordinate analysis 80
4.2 Occurrence of Sexual Recombination 83
4.2.1 Distribution of mating type genes among *Cercospora zeina* isolates 83
4.2.2 Linkage disequilibrium 84
4.3 Mapping the Quantitative trait loci 85
4.3.1 Phenotypic data 85
4.3.1.1 Disease severity data 85
4.3.1.2 Genetic diversity among the Genotypes 88
4.3.1.3 Distribution of disease severity scores 91
4.3.1.4 Agronomic data 95
4.3.1.5 The Weather data 96
4.3.1.6 Correlation between environments 98
4.3.1.7 Correlation between variables 100
4.3.1.8 Variance analysis 104
4.3.2 Construction of the genetic linkage map 104
4.3.3 QTL analysis 107
4.3.3.1 Quantitative trait loci and their genetic effects for GLS resistance 109
4.3.3.2 Quantitative trait loci for resistance to TLB. 110
4.3.3.3 Flowering time QTLs 110

4.3.3.4 Individual Location QTL ... 111
4.3.3.5 Overlapping QTL ... 112
4.3.3.6 Gene action ... 112
CHAPTER FIVE: DISCUSSION ... 113
5.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya ... 113
5.1.1 Identifying the causal pathogen of GLS in Kenya ... 113
5.1.2 Genetic diversity of *Cercospora zeina* in the four counties of Kenya ... 114
5.1.3 Partial population differentiation of *Cercospora zeina* in Kenya ... 115
5.1.4 Existence of partial population structure among *Cercospora zeina* populations .. 116
5.1.5 Further dissecting the partial population structure ... 117
5.2 Occurrence of sexual recombination in the population ... 119
5.2.1 Importance of this study ... 120
5.2.2 Limitations of this study ... 121
5.3 Mapping the QTL conferring resistance to gray leaf spot and *Turcicum* leaf blight ... 122
5.3.1 Heritability estimates ... 122
5.3.2 Effect of planting season on agronomic traits ... 123
5.3.3 Relationship between agronomic traits and disease resistance ... 124
5.3.4 Relationship between GLS and TLB ... 125
5.3.5 Quantitative Trait Loci associated with resistance to gray leaf spot ... 126
5.3.6 Quantitative trait loci associated with resistance to *Turcicum* leaf blight ... 128
5.3.7 Quantitative trait loci associated with Flowering time ... 130
5.3.8 Quantitative trait loci affecting plant height and ear height ... 132
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS ... 133
CONCLUSIONS ... 133
RECOMMENDATIONS ... 135
REFERENCES ... 136
APPENDICES ... 154

ABBREVIATIONS AND ACRONYMS

AFLP: Amplified fragment length polymorphism

AMOVA: Analysis of molecular variance

BecA: Biosciences eastern and central Africa

CIMMYT: Centro international de Mejoramiento de Mazy Trigo/ International Maize and Wheat Improvement Centre

CML: CIMMYT maize lines

CTAB: Cetyltrimethyl ammonium bromide

DH: Double Haploid

GPS: Global Positioning System

GLS: Gray leaf spot

IGSS: Integrated genotyping service and support

KARI: Kenya Agricultural Research Institute

MAT: Mating type genes

MAT1: Mating type locus

MAT1-1, 1-2: Mating type idiomorphs

MAT1-1-1: Mating type gene encoding an alpha box (α1) protein

MAT1-2-1: Mating type gene encoding a protein with a high mobility group domain

META-R: Multi Environment Trial Analysis with R for windows

NARL: National agricultural research laboratories.

PCOA: Principal Coordinate Analysis

PCR: Polymerase chain reaction.

QTL: Quantitative trait locus.

RAPD: Random amplified polymorphic DNA.

RFLP: Restriction fragment length polymorphism.

SCA: Specific combining ability.

SNP: Single Nucleotide Polymorphism.

SSR: Simple sequence repeats

TLB: *Turcicum* leaf blight.

List of Tables

Table 3. 1: Geographical locations for the field trials 59

Table 3. 2: Disease severity rating scale 61

Table 4. 1: The number of successful isolates retrieved from each geographical region 69

Table 4. 2: Gene diversity reported for the SSR markers and the sequence information 71

Table 4. 3: Indicators of genetic diversity for *Cercospora zeina* isolates 72

Table 4. 4: Private alleles observed in the four geographical locations in Kenya 75

Table 4. 5: Analysis of molecular variance (AMOVA) for the isolates 77

Table 4. 6: Actual frequency distribution of mating types 84

Table 4. 7: Averages of the weather data for the three different environments 96

Table 4. 8: Correlation coefficients between environments for GLS and TLB 98

Table 4. 9: Correlation coefficients between environments for flowering time 99

Table 4. 10: Correlation coefficients between variables for GLS and TLB 100

Table 4. 11: Estimates of means, ranges, genetic variance components ($\sigma^2 G$) and broad sense heritability (h^2) 103

Table 4. 12. Quantitative trait loci detected in all the three environments 107

Table 4. 13: QTL for resistance to gray leaf spot, Turcicum leaf blight and flowering time 108

Table 4. 14: The level of phenotypic variance explained in the various traits examined 109

List of Figures

Figure 3. 1: A map illustrating the six collection sites for GLS infected maize in Kenya 53

Figure 4. 1: Appearance of single plex diagnostic PCR for CTB7 gene region 70

Figure 4. 2: The Delta K graph 79

Figure 4. 3: Bar graphs from the STRUCTURE analysis 79

Figure 4. 4: Principal coordinate analysis (PCoA) plot 81

Figure 4. 5: Gel image of *Cercospora zeina* mating type genes...83

Figure 4. 6: Occurrence of disease lesions on the maize leaves 85

Figure 4. 7: Distribution of disease severity scores for the two parental genotypes 86

Figure 4. 8: Box plot showing progress of TLB and GLS combined across environments 87

Figure 4. 9: Dendogram showing the genetic diversity among the 230 genotypes 91

Figure 4. 10: Frequency distribution for disease severity data and days to anthesis 93

Figure 4. 11: Frequency distribution for the different agronomic traits 94

Figure 4. 12: Weather data across locations during the planting season 97

Figure 4. 13: Heat map of the Pearson correlation matrix between the different variables ..101

Figure 4. 14: Genetic linkage map for the DH population 106

List of Plates

Plate 1: Typical symptoms of gray leaf spot..13

Plate 2: Typical symptoms of *Turcicum* leaf blight...25

List of Equations

Equation 1: To estimate the variance components with covariate 63

Equation 2: To estimate the variance components without covariate 63

Equation 3: Estimating the broad sense heritability. 64

Equation 4: To estimate the genetic correlations among environments.................................64

Equation 5: To estimate genetic correlation between traits. 65

Equation 6: To estimate the least significance difference. 65

Equation 7: To estimate the coefficient of variation...65

List of Appendices

Appendix A: Distribution of mating type genes in the Kenyan population. 154

Appendix B: Averages for the different response variables across environments. 157

Appendix C: An overview of the experimental field in Kabianga 2018. 159

Appendix D: Diseased maize crop at early stages of infection. 159

Appendix E: Frequency distribution graphs for area under the disease progress curve, GLS and TLB. 160

Appendix F: Physical locations and genetic map positions for the identified QTL. 161

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Maize (*Zea mays* L.) is the most produced grain crop throughout the world, providing food, animal feed, and biofuel (Sucher et al., 2017). Maize is also considered a model crop for cytogenetics, functional genomics and genetic studies owing to its high levels of genetic and phenotypic diversity (Ding et al., 2015; Strable & Scanlon, 2009). For communities in Eastern and Southern Africa, maize is considered the principle staple food crop (Almeida et al., 2013; Kinyua et al., 2010; Shiferaw et al., 2011). Despite its importance, maize production in Kenya is still low with an estimated potential yield of 6 t/ha^{-1}, small holder farms achieve an estimated average production of 1.8 t/ha^{-1} (Munialo et al., 2019). This is partly due to the threat of highly destructive and virulent fungal pathogens limiting crop production in Kenya (Ngugi et al., 2000), with typical yield losses attributable to diseases in maize averaging at 9% worldwide (Oerke, 2006).

Turcicum leaf blight (TLB) caused by *Exserohilum turcicum* (Leonard & Suggs, 1974) and gray leaf spot caused by either *Cercospora zeina* (Crous et al., 2006) or *Cercospora zeae-maydis* (Tehon & Daniels, 1925) are both lethal and economically significant foliar diseases of maize in Kenya (Kinyua et al., 2010; Sserumaga et al., 2020). *Cercospora sorghi* var *maydis* was previously isolated from GLS lesions that were collected in Western Kenya (Kinyua et al., 2010). Preceding authors speculated that this pathogen could probably be saprophytic on the lesions as its ability to cause infection remains unclear (Muller et al., 2016; Nsibo et al., 2019). The two diseases (GLS and TLB) destroy the green leaf tissue resulting in reduced photosynthetic potential and the eventual decrease in grain yield (Saito et al., 2018). Gray leaf spot epidemics are characterized by formation of tan to gray lesions that resemble a matchstick in shape and grow in a parallel orientation to the leaf veins (Korsman et al., 2012) causing output losses of approximately 60-65% particularly when using susceptible lines (Ward et al.,

1999). *Turcicum* leaf blight epidemics are characterized by long elliptical cigar-shaped lesions on leaves that are gray-green in colour which turn dark due to formation of fruiting bodies (Welz, 1998). Gray leaf spot and TLB therefore are potentially serious threat to food security, crop productivity and nutrition in Kenya (Kinyua et al., 2010). *Turcicum* leaf blight has been reported to induce yield reductions of 36 to 72% on susceptible maize genotypes compared to resistant hybrids which do not exhibit significant reductions in yield under African tropical conditions (Berger et al., 2020).

Cercospora zeina and *E. turcicum* are ascomycete fungi belonging to the class Dothideomycetes (Crous et al., 2006) and exhibit shared aspects of pathogenesis and a necrotrophic lifestyle (Kotze et al., 2019). Though *E. turcicum* happens to be classified as a hemi-biotroph (Chung et al., 2010b; Kotze et al., 2019; Martins et al., 2019), it is not clear whether the pathogen is an actual hemi-biotroph (Human et al., 2020). Infection is initiated when spores land and attach on the leaf surface, the conidium then germinates and forms appressorium which produces a penetration peg that penetrates through the leaf cuticle and epidermis or stroma. *Exserohilum turcicum* grows intracellularly through the xylem while *C. zeina* grows between the cells during initial infection (Kotze et al., 2019; Zwonitzer et al., 2010).

The two fungi produce phyotoxins that aids in pathogen infection and disease development. *E. turcicum* produces a non-host specific HT toxin (named after the old species *Helminthosporium turcicum*) that enhance the process of pathogenicity and induces disease symptoms in the host (Galiano-Carneiro & Miedaner, 2017). *Cercospora zeina* does not produce the phytotoxin cercosporin in vitro while its sibling species *C. zeae maydis* produces the phytotoxin in vitro (Dunkle & Levy, 2000; Swart et al., 2017).

The two sibling species of *C. zeina* are morphologically similar but phylogenetically and culturally distinct. Only the necrotrophic *C. zeina* has been identified among isolates

collected from Africa (Dunkle & Levy, 2000; Meisel et al., 2009; Muller et al., 2016; Okori et al., 2003), demonstrating that *C. zeina* is widely prevalent in Africa and causes numerous infections. Molecular techniques such as species specific PCR diagnostics have been developed and have been successfully used to distinguish between the two species associated with GLS (Crous et al., 2006; Swart et al., 2017).

High relative humidity of approximately 95%, daily temperatures of above 20°C, early rains and regions characterized by misty mornings and warm humid afternoon are the predisposing conditions for TLB and GLS infections (Stromberg & Donahue, 1986; Ward, 1996). The most commonly used strategies for GLS control include cultural control; deep tillage, application of chemical fungicides and host plant resistance (Munkvold et al., 2001). In addition to health problems posed by chemicals fungal pathogens may develop resistance to fungicides (Miles et al., 2012). Thus, the most effective and economical way to manage GLS is by planting resistant hybrids (Lopez-Zuniga et al., 2019; Mammadov et al., 2015). Biocontrol strategies such as foliar application of *Bacillus* spp. has been shown to reduce TLB infections by 40-56% at 39 days post inoculation (Sartori et al., 2017).

Groenewald et al. (2008) documented that understanding the mode of reproduction of the pathogen is critical to design more effective control strategies against fungal pathogens. There is evidence that *C. zeina* is undergoing sexual recombination based on the distribution and frequency of mating type genes at the MAT 1 locus. The pathogen is heterothallic in nature, having both MAT 1-1 and MAT 1-2 idiomorphs that harbor the mating locus coding sequences of mating type genes MAT 1-1-1 and MAT 1-2-1 (Groenewald et al., 2006). Such ratios of MAT idiomorphs are critical to determine the predominant mode of reproduction. Populations that undergo sexual recombination are characterized by mating type ratios that do not significantly deviate from 1:1 and high levels of genetic diversity (Milgroom, 1996; Bolton et al., 2012).

A previous study investigated the genetic diversity of *C. zeina* isolates collected from three East Africa countries (Kenya, Uganda and Rwanda), the population was characterized by lack of population structure and high gene flow was reported among the isolates from the different countries (Okori et al., 2003). However, the study did not investigate the distribution of the mating type genes in the population as an indicator of potential sexual recombination in the population. In addition, the sample size (15 isolates from Kenya) used in this population study was relatively small.

A high genetic diversity of *C. zeina* populations has previously been reported in South Africa (Muller et al., 2016; Nsibo et al., 2019), part of which is driven by sexual recombination, high levels of gene flow and lack of population differentiation between different regions (Muller et al., 2016). Recombination allows fungal pathogens to generate more fit genotypes and such recombining pathogen populations pose a greater risk of breaking down host resistance genes or developing fungicide resistance (McDonald & Linde, 2002). Such studies provide support for resistance breeding programs and have important implications for the design of integrated disease management programs (Ferguson & Carson, 2004; Munialo et al., 2019).

The degree of genetic diversity in *C. zeina*, has not been extensively examined in Kenya as compared to other fungal diseases such as *E. turcicum* of northern corn leaf blight (Borchardt et al., 1998). The first and second objectives of the present study were therefore to determine the genetic diversity of *C. zeina* across four counties in Kenya and the role sexual recombination plays in driving its population structure.

Resistance to GLS is quantitative meaning that it is controlled by several genes each with a small effect on disease resistance, while resistance to TLB is inherited both quantitatively and qualitatively (Chen et al., 2015; Kolkman et al., 2020). Qualitative *Ht* genes have been extensively used in commercial hybrids (Ferguson & Carson, 2007) especially in

temperate environments that are characterized by relatively lower disease pressure. While to manage TLB in African tropical conditions, quantitative resistance is the best option for durable resistance (Galiano-Carneiro & Miedaner, 2017). The *Ht* genes provide partial resistance against TLB by suppressing sporulation, lesion expansion and growth rate. However they may not be durable with their expression being modified by temperature and light intensity, in addition they are easily broken down by evolving fungal populations (Welz & Geiger, 2000). Qualitative resistance to GLS has not been reported and therefore resistance is mainly quantitative with additive gene action (Berger et al., 2014). Poland et al. (2009) reported that qualitative resistance in necrotrophic pathosystems tends to be rarely available, this limits their utility in maize breeding programs. The study also indicated that such quantitative resistance locus are not easily broken down due to their smaller effects and broader specificity.

Mapping of the quantitative trait locus (QTL) based on linkage analysis is a powerful tool for identifying the genomic regions associated with the trait of interest. This is supported by the fact that there are over 10,000 published articles on QTL mapping in different species out of which 360 articles with over 1000 QTLs are directly linked to different traits of maize (Yan et al., 2011). Previous QTL studies have mapped QTLs for resistance to GLS and TLB on all the ten maize chromosomes (Berger et al., 2014; Chen et al., 2015). Therefore the second objective of the present study was to determine the quantitative trait loci underlying resistance to TLB and GLS using a double haploid population of CML511 and CML546.

Cui et al. (2014) identified phenotyping, genotyping with molecular markers and genetic map construction as the crucial steps in QTL identification. In the current study DArTseq genotyping was applied to analyze the genomes of 230 genotypes of maize and a total of 1250 DArT markers were used to genotype the population. Sequencing with Diversity Arrays Technology involves a combination of the complexity reduction of DArTs with genotyping-by-sequencing platforms (Kilian et al., 2012; Raman et al., 2014). In addition,

DArTseq as a hybridisation based technique relies on complexity reduction method to simultaneously type several thousand loci in a single assay (Akbari et al., 2006). The platform originally developed for rice, offers a high throughput but low cost genome-wide genotyping technique (Jaccoud et al., 2001).

Through the process of complexity reduction, DArTseq markers are able to detect a large number of DNA polymorphisms that result from single base changes and insertion or deletion. This is conducted by scoring for the presence or absence of DNA fragments in genome-wide representations generated from samples of genomic DNA (Akbari et al., 2006; Sánchez-Sevilla et al., 2015). The genotypic data from DArT analysis was used as input data in QTL IciMapping, which is a free software that can be downloaded from http://www.isbreeding.net/. QTL IciMapping is a powerful tool for the construction of genetic linkage maps and mapping of quantitative trait loci in bi-parental populations (Meng et al., 2015).

1.2 Statement of the Problem

Gray leaf spot is associated with losses of 60-65% on susceptible hybrids while *Turcicum* leaf blight contributes to maize yield losses of 36-72%, in Sub-Saharan Africa. The two fungal diseases destroy the leaf green tissue and significantly contribute to decrease in grain yield, hence a threat to food security. Planting resistant hybrids is the most effective management strategy. However, quantitative resistance to GLS and TLB is a complex trait. Conventional breeding strategies are challenged by the possibility of negative genetic linkage drag, a situation that can be resolved through QTL identification. However, temperate germplasm were mostly used in the previous QTL mapping studies under temperate experimental conditions, hence may not produce effective results for African tropical environments. Initial QTL mapping studies in Kenya used molecular based markers such as RFLP. However, due to their

low resolution it has become increasingly challenging to use some of these QTLs in breeding programs.

1.3 Objectives of the Study

1.3.1 General objective

To determine the genetic diversity of *Cercospora zeina* and map the Quantitative trait loci (QTL) associated with resistance to gray leaf spot and *Turcicum* leaf blight disease of maize for improved disease resistance.

1.3.2 Specific objectives

1. To analyze the genetic diversity of *Cercospora zeina* as the causal pathogen of gray leaf spot in Kiambu, Meru, Nakuru and Tharaka Nithi counties of Kenya.
2. To determine if sexual recombination is taking place in the population of *Cercospora zeina* collected from Kiambu, Meru, Nakuru and Tharaka Nithi counties in Kenya.
3. To identify the QTLs associated with gray leaf spot and *Turcicum* leaf blight resistance in CIMMYT DH population (CML 511×CML 546).

1.4 Hypotheses

1. *Cercospora zeina* is not the only causal pathogen of gray leaf spot in Kenya and it is not genetically diverse.
2. The population of *Cercospora zeina* collected from the four counties in Kenya does not deviate from the 1:1 mating ratio expected in populations undergoing sexual recombination.
3. Progenies of maize double haploid CML 511×CML 546 do not possess multiple QTL associated with gray leaf spot and *Turcicum* leaf blight resistance.

1.5 Justification of the study

Development and deployment of disease resistance traits is an effective way for the control of gray leaf spot and *Turcicum* leaf blight. This requires a better understanding of the mechanisms

underlying quantitative disease resistance (Benson et al., 2015). In addition disease resistance breeding programs would also benefit from characterizing the composition and genetic variation of the *Cercospora* spp. population (Dunkle & Levy, 2000).

The extensive spread of GLS necessitates the development of resistant germplasm. Small scale farmers in developing countries would benefit from hybrids that have been bred for resistance to foliar diseases of maize. These farmers have limited access to chemical control and application of fungicides adds to the cost of producing maize (Berger et al., 2014). In addition, fungicide application for GLS control is not reliable due to the high genetic diversity of the pathogen that leads to dispersal of fungicide resistance (Muller et al., 2016). Thus identification of these QTL would hasten the breeding of resistant hybrids through marker-assisted breeding of resistant QTLs into the genome of elite susceptible inbred lines (Xu et al., 2014).

Knowledge on the level of *Cercospora zeina* genetic diversity coupled with the mating type distribution will provide indirect evidence on whether sexual reproduction occurs in the population or not. An advanced understanding of the mode of reproduction and genetic diversity of the fungus is crucial for improved management of GLS (McDonald, 1997). Furthermore Crous et al. (2006) emphasized the need to conduct population-level studies to examine the level of diversity present in populations of *C. zeina*, and to identify whether sexual recombination plays a role within such populations.

1.6 Significance of the Study

The present study demonstrates the usefulness of the *CTB7* diagnostic test as a tool for rapid and precise identification of the *Cercospora* species associated with gray leaf spot disease of maize. The identification of *C. zeina* as the only pathogen causing gray leaf spot disease of maize among the isolates collected in Kenya, is important to inform future studies on the disease as to the pre-dominant species within the country. The high genetic diversity of *C. zeina*

and lines of evidence supporting sexual recombination reported is important to understand the pathogen dynamics. This is equally important to inform crop protection agencies in Kenya.

In comparison to traditional approaches used in mapping studies, the use of double haploid lines as the biparental population and genotyping by sequencing technologies such as DArTseq, improved efficiency and resolution in QTL mapping. The major QTL identified for GLS resistance *qGLS1_190* and *qTLB8_171* for resistance to *Turcicum* leaf blight are significant QTL that could be deployed in maize breeding programs to improve resistance to the two infections. A major QTL for resistance to both *Turcicum* leaf blight and gray leaf spot has been reported as *qTLB3_27*. This QTL could be important in pyramiding for resistance genes. Some of the previously identified QTL have been validated in this study showing the importance of such QTL regions. A major gene which plays a crucial role in flowering time has also been reported.

CHAPTER TWO
LITERATURE REVIEW

2.1 Maize: Importance and Biology of the Crop

2.1.1 Importance of Maize in Kenya

For a significant proportion of the Kenyan population, maize is predominantly the bread and butter for both the urban and rural communities, with a per capita consumption estimated at 103 kg/person/year (Abate, 2015). This translates to approximately 30 to 34 million bags (2.7 to 3.1 million metric tons) annually (Onono et al., 2013). The crop is very important in Kenya's food crop production system with roughly 38% of crop producers in Kenya growing maize (Onono et al., 2013). The average production and yield of maize in Kenya stands at about 1.8 t/ha^{-1} from small scale farmers in comparison to the potential yield of 6 t/ha^{-1} (Munialo et al., 2019). Drought, low soil fertility, pests and diseases have been mentioned as significant contributors to the low productivity and host plant resistance being the most suitable and cost effective control option. Gray leaf spot and *Turcicum* leaf blight have been mentioned as the most damaging foliar diseases of maize in Kenya (Beyene et al., 2019).

2.1.2 Biology of the crop and agricultural improvement

Maize (*Zea mays* L.) is a member of the Poaceae (Gramineae) family and exhibits a monoecious flowering habit. Cultivated corn has 10 pairs of chromosomes (n = 10) hence it is a diploid (2n = 20) monocot and is predominantly cross-pollinating with high level of heterosis (Holland & Coles, 2011). It has a genomic size of 2300 mbp that contains more than 32000 genes (Schnable et al., 2009). During the process of pollination, pollen from any tassel can randomly pollinate the silks on the ears of neighboring plants or even its own silks. Cells of a random maize plant have a nuclei with 20 chromosomes (Bennetzen & Hake, 2009). Maize undergoes double fertilization whereby the nucleus of one sperm fuses with the egg to form the zygote, which has 20 chromosomes. This number persists in the somatic cells of the plant,

and the nucleus of all new cells that appear during growth have the 2n number of 20 chromosomes (Darrah et al., 2019).

Factors such as domestication and crop improvement efforts have significantly limited the breeding of maize varieties for resistance to diseases due to the narrow genetic base of cultivated maize (Wallace et al., 2014). *Zea mays* together with five additional species belong to the genus *Zea*, *Zea mays* is classified further into four subspecies among them is the modern maize. Matsuoka et al. (2002) reported that *Z. mays* ssp. *parviglumis* is considered to be the immediate ancestor of cultivated maize (*Zea mays* ssp. *Mays*).

Teosinte (*Z. mays* subsp. *parviglumis*) is a wild relative of maize and can easily be hybridized with modern cultivated maize inbreds. Teosinte therefore has genetic material of potential value that could act as source of alleles conditioning resistance for the improvement of modern maize. However, such breeding programs are impeded by structural differences between teosinte and current maize inbreds (Liu et al., 2016). Several biotic and abiotic factors affect maize leading to significant loss in yield. Diseases constitute a major component of the biotic factors with gray leaf spot and *Turcicum* leaf blight being the most destructive and yield limiting foliar diseases of maize globally (Ward et al., 1999).

2.2 Gray leaf spot

2.2.1 Effects of GLS on maize (*Zea mays* L.)

Owing to the common occurrence of gray leaf spot infections in Africa, it has caused severe reductions in yield volumes and quality of grains (Ward et al., 1999). Maize foliar fungal diseases contribute to significant production losses hence present a threat to food productivity, food security and economies that depend on agriculture (Korsman et al., 2012). Yield losses ranging from 11 to 69% have been reported to be associated with GLS in the US and South Africa (Danson et al., 2008; Ward et al., 1999). Yield loss potential of 30-50% were reported in Kenya (Kinyua et al., 2010), while in South Africa the estimates were between 30- 60% (Ward, 1996). However complete yield loss may also occur under severe epidemics (Liu et al.,

2016; Ward et al., 1999). In contrast, such magnitude of potential loss are rarely observed, due to underlying quantitative disease resistance genes in most maize hybrids (Zwonitzer et al., 2010). It has also been reported that symptoms appearing before anthesis caused greater yield loss (Rupe et al., 1982). Potential losses may also vary depending on cultivar selection and environmental conditions (Xu et al., 2014).

Yield reductions are attributed to the loss of photosynthetic leaf area particularly during the grain filling stage, which results in a reduced number of kernels per ear (Menkir & Ayodele, 2005). Reduction in the photosynthetic potential of the crop results in the transfer of carbohydrates from stalks to the developing ear predisposing the crop to stem deterioration and root lodging. Severely blighted leaves are characterized by reduced sugar levels resulting in premature plant death and can cause up to 100% yield loss in conducive environments (Danson et al., 2008; Sibiya et al., 2012). Following a successful initial infection, lack of control measures coupled with conducive environmental conditions may also result in total crop loss (Saghai et al., 1996).

2.2.2 Symptoms of Gray Leaf Spot

Gray leaf spot lesions are expressed as gray to tan in color and are typically rectangular in shape (Plate 1). A distinguishing trait for GLS is that these lesions run parallel to leaf veins (Korsman et al., 2012). The disease infects the lower leaves first and is spread by air and rain splash to the upper leaves (Ward et al., 1999). Genetic background of the genotype influences the diagnostic symptoms expressed on the leaves The expression of fleck type lesions is mainly observed among resistant genotypes (Latterell & Rossi, 1983). Chlorotic lesions are observed among genotypes with moderate levels of resistance and necrotic spots characterize a susceptible genotype (Latterell & Rossi, 1983; Lipps, 1998). Benson et al. (2015) demonstrated that there is a positive correlation between the inter vein distance and GLS disease

development, suggesting that restriction of lesions between the major veins may serve as a host resistance mechanism.

Gray leaf spot is initially characterized by pinpoint lesions, encompassed in a ring of yellow haloes. When the leaf is viewed through a source of light, the early lesions appear transparent, mature lesions on the other hand exhibit complete opacity (Latterell & Rossi, 1983). Under favorable conditions, lesions coalesce to form large areas of dead leaf tissue (Stromberg & Donahue, 1986), resulting in blighting and eventually leaf death (Latterell & Rossi, 1983).

Plate 1: Typical symptoms of gray leaf spot.
Courtesy of Nsibo, 2019

2.2.3 Historical perspective of *Cercospora zeina*

The first documented evidence of gray leaf spot globally was in Illinois, USA in 1925 (Tehon & Daniels, 1925). In Africa, the disease was not reported until 1988 when the symptoms were observed in Kwa Zulu Natal South Africa (Ward, 1996), where it got to epidemic levels in

1991-1992 (Ward et al., 1999). The pathogen has spread gradually to a number of maize growing countries in Africa (Ward et al., 1999). The National Agricultural Research laboratories in Kenya gave the first report of the disease in 1995 (Kinyua et al., 2010). GLS infections have since spread to many maize growing regions in Kenya, probably due to increased adoption of no till farming practices. Such practices allow for the buildup and over-wintering of fungal inoculum on maize residues to cause infection in the subsequent season (Muller et al., 2016).

2.2.4 Geographic origin of the pathogen

The direction of migration of *Cercospora zeina* is not yet clear up to date. Previous studies indicated that the pathogen came from Africa, South America and to a less extent the United States (Crous et al., 2006; Dunkle & Levy, 2000). Probably the pathogen could have migrated into Africa accompanying maize imports from the U.S. (Ward et al., 1999). The hypothesis was however opposed by Dunkle and Levy (2000) who indicated that suppose the pathogen originated from the U.S. then it could have more likely been *Cercospora zeae-maydis* which is more predominant in the U.S.A. This was supported by Brunelli et al. (2008) who submitted that *C. zeina* did not migrate into Africa from the US since the genotypes of *C. zeae maydis* have also not been recovered from the African continent. Dunkle and Levy (2000) then concluded that *C. zeina* could have migrated to the U.S.A. from Africa due to the relatively high level of genetic diversity of African isolates. However, the limited number of samples used in this study, limits the ability of this study to fully describe the genetic diversity of the two *Cercospora* populations (Hsieh, 2011). A compatible inference was made by Crous et al. (2006), who further postulated that *C. zeina* could have migrated into Africa and the US on different host since maize has its center of diversity in South America and not Africa.

2.2.5 Epidemiology of the pathogen

Environmental factors have a major role to play in determining the level of severity caused by gray leaf spot. Damaging levels may not be attained when the prevailing conditions are not favorable for GLS development. Pathogen development therefore requires frequent and prolonged periods of high relative humidity (Latterell & Rossi, 1983). Relative humidity of 95% has been demonstrated to promote germ tube elongation. Fluctuations in relative humidity affect the latent period of infections (Ringer & Grybauskas, 1995). The intensity distribution pattern of rainfall also provide conditions that influence GLS incidence and severity with early rains promoting the development of primary lesions (Ward & Nowell, 1998). It is widely accepted that the pathogen thrives well in maize producing regions characterized by misty mornings, warm humid afternoon and cool nights (Mammadov et al., 2015; Rupe et al., 1982).

2.2.6 Infection cycle of Gray Leaf Spot

Cercospora zeina the causal pathogen of gray leaf spot displays a polycyclic life cycle (Kuki et al., 2018). This means that the inoculum from the previous season are able to survive on maize residue left in the field, the maize crop grown in the subsequent season is then vulnerable to infection (Paul & Munkvold, 2004). When the environmental conditions are favorable for disease development, the fungus colonizes on residues and produces conidia (Stromberg, 2009). The fungal conidia are then dispersed to newly established maize fields primarily by wind or rain splash (Lipps, 1998). The spores are then deposited onto the bottom leaves (Korsman et al., 2012), whereby the conidia germinate and penetrate the leaves through the stomata.

Typical GLS symptoms develop in about nine days after infection. The pathogen has the ability to produce large numbers of conidia, which progress to become secondary conidia within 14 to 28 days under favorable humidity conditions (Korsman et al., 2012). Sporulation may be delayed in moderately resistant genotypes (Beckman & Payne, 1983). The new spores are then

dispersed by wind or splashing rainfall to the upper leaves under favorable environmental conditions (Jenco & FW Jr, 1992). This initiates secondary cycles of infection. Diseased leaves that stay on the ground after harvest, where the stromata survive the intercrop period initiate the infection cycle in the subsequent season.

2.2.7 Cultural Characteristics of the pathogen

The characteristics and dimensions of the conidia and conidiophores on lesions/in planta was as described in Wang et al. (1998). The distinguishing characteristic is that *Cercospora zeina* have shorter conidiophores while *Cercospora zeae-maydis* have longer conidiophores (Crous et al., 2006). *Cercospora zeina* have colonies that exhibit slower growth rate on potato dextrose agar in comparison to those of *Cercospora zeae-maydis*. The two sibling species are morphologically similar but phylogenetically and culturally distinct. *Cercospora zeae-maydis* is known to produce cercosporin in vitro while *Cercospora zeina* does not produce the phytotoxin *in vitro* (Swart et al., 2017).

2.2.8 Taxonomy of *Cercospora zeina*

The genus *Cercospora* belongs to the class Dothideomycetes, a subclade that contains one of the largest groups of fungal pathogens a vast majority of which are asexual (Goodwin et al., 2001). *Cercospora* spp. are largely successful and are associated with spot like diseases on leaves, with a host range of about one hundred plant species such as maize, sugar beet, soybean and rice (Daub & Ehrenshaft, 2000). This success is attributed to the ability of these pathogens to secrete cercosporin during infection (de Jonge et al., 2018). The genus has a total of 659 species, three species in this genus have been associated with infections of gray leaf spot namely, the two sister species *Cercospora zeae-maydis* and *Cercospora zeina* and the third pathogen *Cercospora sorghai* var. *maydis* (Crous et al., 2006). The former two are morphologically similar but culturally and genetically distinct (Wang et al., 1998). The latter arises as a saprophyte on the GLS lesions caused by either *Cercospora zeae-maydis* or

Cercospora zeina. However, it lacks pathogenicity to sorghum and its pathogenicity on maize has not been confirmed (Crous et al., 2006). In addition to the genus and class, *C. zeina* belongs to the Ascomycota division, Pezizomycotina subdivision, Capnodiales order and Mycosphaerellaceae family (Hsieh, 2011).

2.2.9 Dissecting the causal pathogen of gray leaf spot in Kenya

C. zeina and *C. zeae-maydis* have previously been distinguished using DNA sequence analysis of the Internal Transcribed Spacer (ITS) regions and the 5.8S ribosomal DNA (Crous et al., 2006; Dunkle & Levy, 2000). The study observed that the ITS region of an isolate of *C. zeina* was more similar to the ITS region of *C. sorghi* var. *maydis* isolate in comparison to that of *C. zeae-maydis*. They also distinguished the two based on physiological characteristics, the conidia of *C. zeina* grew faster on artificial media compared to that of *C. zeae maydis*. In addition, isolates of *C. zeina* were unable to produce cercosporin in culture while isolates of *C. zeae-maydis* produced abundant cercosporin. Similar results were obtained by Crous et al. (2006) when they used histone H3 gene PCR reactions, elongation factor 1-α, actin and calmodulin gene regions to distinguish between the two species. Dunkle and Levy (2000) were able to ascertain that *C. zeae-maydis* is predominant in the USA while *C. zeina* is more widespread in Africa. Kinyua et al. (2010) conducted a molecular based study on the causal pathogen of GLS in Kenya and was able to distinguish between *C. zeae-maydis* group I and group II from *C. sorghi* var. *maydis*. The study identified *C. zeae-maydis* group II as the causal pathogen of GLS in Kenya. Following reclassification of *C. zeae-maydis* group II as *Cercospora zeina*, the study provided evidence that *Cercospora zeina* is the causal pathogen of GLS in Kenya but did not look at the diversity of the pathogen.

2.2.10 Identification using a species-specific diagnostic test

A diagnostic test was designed based on the species specific PCR primer sequence from the histone H3 gene to differentiate between the three species of *Cercospora* associated with GLS

(Crous et al., 2006). A number of years later an easier PCR assay has been developed; Primers, CTB7del R (GATGCGGGTGAAGTAGAAA) and CTB7del F (AAGAGTGCTTGTGAATGG) are therefore specific for *C. zeae-maydis,* and *C. zeina* respectively, and could be used to differentiate between the two species (Swart et al., 2017). These primers can be used to easily and rapidly identify *C. zeina* and *C. zeae-maydis* in single-plex PCR amplification. Other techniques that can be used to distinguish the two species include ITS, calmodulin, actin, histone H3 (his) and translation elongation factor 1 alpha (tef1-a) (Crous et al., 2006).

2.2.11 Mode of reproduction of the pathogen

Fungi in the genus *Cercospora* that includes up to 3,000 named species have been speculated to undergo asexual reproduction due to the fact that sexual structures had not been documented in this genus (Goodwin et al., 2001; Groenewald et al., 2006). To date no sexual stage has been successfully induced by crosses in the laboratory. In previous studies performed on *C. zeae-maydis* and *C zeina*, isolates were shown to be heterothallic, hence they require the fusion of two mating types MAT1-1 and MAT1-2 for them to successfully undergo sexual reproduction (Groenewald et al., 2006; Groenewald et al., 2008; Kim et al., 2013). In addition, the nearly equal distribution of the mating type genes (MAT idiomorphs) led researchers to conclude that there is a potential of the two species undergoing sexual reproduction (Groenewald et al., 2006).

Highly significant levels of genotypic diversity blended with an equal distribution of mating type genes could potentially imply that a population undergoes sexual reproduction, which results in genetic reshuffling within the population to enhance genetic variation, adaptation of the pathogen to the environment, its improved fitness during which resistant asexual spores could be produced (Groenewald et al., 2008; Milgroom, 1996). Previous studies reported greater genotype diversity for the isolates of *Cercospora zeina* populations studied

(Groenewald et al., 2006; Muller et al., 2016). Groenewald et al. (2006) concluded that suppose the pathogen was undergoing asexual reproduction, then it could be characterized by uneven distribution of the mating type genes and the presence of *MAT1-1* or *MAT1-2* idiomorph in the population.

2.2.12 Fungal pathogenesis

The potential ability of a microorganism to cause disease is termed as pathogenicity (Pirofski & Casadevall, 2012). Phytopathogens produce a series of virulent molecules that facilitate host infection (Kimura et al., 2001). These molecules are present in different chemical forms and tend to be either host specific or non-specific (Benson et al., 2015).

2.2.13 Production of Cercosporin

Cercosporin is a host non-selective, photo activated phytotoxin produced by a number of phytopathogens such as *Cladosporium* spp and *Cercospora* spp that is required for high levels of virulence by these fungi on plants (Daub & Ehrenshaft, 2000). Upon illumination, the photosensitizing perylenequinone reacts with oxygen molecules to produce reactive oxygen species (ROS) that causes oxidative injury to cellular components resulting in electrolytic leakage, decrease in membrane permeability, and the eventual death of cells (Daub & Ehrenshaft, 2000). Growth of pathogens and formation of spores are then supported by the nutrients released form the host cells (Daub & Ehrenshaft, 2000). Similar symptoms could be induced *in planta* upon the application of cercosporin alone. This makes the toxin a very crucial virulence factor for interaction between the pathogen and the host (Upchurch et al., 1991).

Different plant-associated *Cercospora* species have demonstrated their ability to produce cercosporin in vitro, these include *C. zeae-maydis*, *Cercospora kikuchii*, *Cercospora nicotianae*, *Cercospora beticola* and *Cercospora asparagi* (Jenns et al., 1989). However *C. zeina* isolates did not produce the toxin *in vitro* (Dunkle & Levy, 2000; Goodwin et al., 2001; Koshikumo et al., 2014). Many researchers speculated that the inability to produce cercosporin

in planta could be attributed to the lack of appropriate conditions to induce cercosporin production *in vitro*. Daub and Ehrenshaft (2000) suggested that accumulation of cercosporin in culture is affected by environmental and developmental factors.

Cercospora zeina isolates from Africa and partly from the US exhibit multiple deletions in the *CTB7* gene region inhibiting cercosporin production in comparison with the corresponding orthologues from *C. zeae maydis* isolates (Swart et al., 2017). From this distinction between *C. zeina* and *C. zeae maydis* isolates, Swart et al. (2017) designed the *CTB7* diagnostic PCR assay that was efficiently able to distinguish between the two pathogens based on amplicon sizes in agarose gel electrophoresis. The *CTB7*del primer pair, that represents the region of deletions in *C. zeina*, can be used in a singleplex PCR reaction to screen for the presence of *C. zeina.*

2.2.14 Genetics of resistance to gray leaf spot

Resistance to gray leaf spot in maize is a trait that is inherited quantitatively and conditioned by many minor QTLs acting in an additive manner (Mammadov et al., 2015). Both additive and non-additive gene effects have been reported to have a vital role in the resistance mechanism for different genetic materials of maize (Gevers & Lake, 1994). In addition to additive gene effects, different gene actions have been reported to condition resistance to GLS by previous studies, including dominant gene action (Derera et al., 2008). The prevalence of additive gene effects for resistance to gray leaf spot in maize was also observed in the studies of Bubeck et al. (1993); (Clements et al., 2000; Gordon, 2004; Juliatti et al., 2009).

Given the prevalence of additive gene effects in resistance to gray leaf spot and high heritability of the trait which is congruent with the fact that resistance in the inbred lines exhibits a significantly positive correlation with resistance in the hybrid (Menkir & Ayodele, 2005), there are higher chances of selecting for resistant lines through crosses and selfing. The advantage of the additive and dominance effects of the parental loci could be exploited to develop hybrids with resistant lines (Sibiya et al., 2012). Such insight on the nature of resistance and gene

effects are crucial to increase the chances for introducing favorable genes into the genomes of susceptible varieties and to optimally utilize genetic variability (Pozar et al., 2009).

2.2.15 Management of gray leaf spot

Methods to control GLS include application of foliar fungicides and cultivation of hybrids with resistance that is predominantly used by commercial farming systems (Nsibo et al., 2021), discontinuation of minimal till practices that has been practiced by smallholder farmers and host plant resistance (Benson et al., 2015). Considering the economic and environmental benefits of soil conservation, discontinuation of minimal tillage systems is not a sustainable management option. The use of crop rotation will be instrumental to reduce the level of inoculum left in the field for plant pathogen interactions. However its use may not be a comparatively effective control strategy (Gordon et al., 2006). Therefore, the management of GLS disease has focused mostly on the use of fungicides and deployment of maize hybrids with resistance to the disease (Munkvold et al., 2001).

Commercial maize production systems majorly use fungicide treatments for GLS control (Mallowa et al., 2015; Nsibo et al., 2019). The high genetic diversity reported within pathogen populations of *Cercospora* spp may result in the development of resistance towards fungicides either through mutation or recombination. Such cases were reported for *Cercospora sojina* isolates collected from soybean in Kentucky, Illinois and Tennessee (Zhang et al., 2012), and *Cercospora beticola* isolates collected from sugar beet leaves in Michigan (Bolton et al., 2014), that have exhibited increased tolerance to Quinone outside inhibitor (QoI) fungicides. To manage fungal diseases in this pathosystem, breeding for resistance to foliar diseases will be critical for small scale farmers in many parts of Africa who may not use chemicals due to their additional cost (Muller et al., 2016).

2.2.16 An overview of Previous QTL Studies on gray leaf spot

QTL analysis is a statistical method that involves crossing segregating parental populations that differ genetically in two or more complex traits (resistant variety crossed with a susceptible variety) and analyzing the segregating progeny so as to link trait measurements to known molecular markers (Asíns, 2002). Due to the complex nature of most candidate genes, performing marker analysis to locate these QTLs helps in understanding the genetic mechanisms underlying causal polymorphisms, facilitating their subsequent utilization in maize breeding programs by researchers. Quantitative disease resistance (QDR) can be defined as a type of resistance that results from the cumulative action of several genes with minor effect and mostly characterized by continuous distribution of scores (French et al., 2016; Niks et al., 2015).

A number of previous studies have reported several QTLs responsible for resistance to gray leaf spot using different parental lines, mapping populations and across a range of environments. And to date more than 57 QTLs have been identified on every maize chromosome distributed on all the 10 chromosomes (Benson et al., 2015; Berger et al., 2014; Bubeck et al., 1993; Clements et al., 2000; Juliatti et al., 2009; Mammadov et al., 2015; Shi et al., 2007; Xu et al., 2014; Zhang et al., 2012). Intriguingly, seven consensus QTLs have been detected on chromosomal bins 1.06, 2.06, 3.04, 4.06, 4.08, 5.03, and 8.06 (Benson et al., 2015; Berger et al., 2014; Wisser et al., 2006). Identifying QTL hotspots by comparing the markers flanking the QTL region across published studies is important to dissect the genomic regions underpinning the complex trait (Berger et al., 2014). Some studies however have reported inconsistency of QTL positions across environments (Bubeck et al., 1993), while others were consistent (Gordon et al., 2006). Juliatti et al. (2009) reported that QTLs for resistance to GLS tend to be stable across different environments.

Several studies have employed $F_{2:3}$ and the double haploid populations for QTL identification (Mammadov et al., 2015; Zhang et al., 2017). The double haploid is an immortal population, implying that it allows replication in different environmental conditions Furthermore DH populations can be speedily generated through cell culture and are highly homozygous (Mammadov et al., 2015; Seymour et al., 2012). Gordon (2004) and Bubeck et al. (1993) used the $F_{2:3}$ population for QTL identification, which limits the number of environments in which each population could be tested due to the fact that this breeding population is not immortal.

Kibe et al. (2020a) combined the use of linkage mapping and genome wide association study to detect the significant SNPs and QTLs conditioning resistance to gray leaf spot in an IMAS diversity panel comprising of biparental populations and a set of 410 double haploid lines in Kenya. GWAS method detected 10 significantly associated SNPs on chromosomes 1, 2, 6, 7 and 8 explaining 6 to 9% of the phenotypic variance, 14 QTLs on chromosome 1, 4, 5, 6, 8 and 9 explaining 6-8% of the phenotypic variation were detected through joint linkage association mapping (JLAM) explaining a phenotypic variance of 0.1 to 15.7% while 22 QTLs were identified through linkage mapping. The authors reported moderate heritability values and prediction accuracies and negative but significant correlation between GLS resistance and flowering time. The use of different mapping populations such as linkage mapping and JLAM restricted the region flanking the QTLs and validated the QTLs across populations (Kibe et al., 2020a).

2.3 *Turcicum* leaf blight

2.3.1 Background information on *Turcicum* leaf blight

The hemi-biotrophic fungal disease *Turcicum* leaf blight (TLB) is caused by *Exserohilum turcicum* which is an ascomycete fungus (Passerini), Leonard and Suggs (1974). This is a very destructive foliar wilt disease of maize that contributes to significant yield losses in many tropical and temperate environments worldwide (Poland et al., 2011). Balint-Kurti et al. (2010)

reported that *E. turcicum* is a hemi-biotrophic fungus rather than a pure nectrotroph. This is due to the fact that *E. turcicum* feeds on dead leaf tissue following an initial biotrophic phase and then transition to a necrotrophic phase (Sucher et al., 2017). *Turcicum* leaf blight can also affect crops in other genera such as *Sorghum bicolor* (causing sorghum leaf blight) (Zhang et al., 2020) however, maize is the most important host (Romero, 2016). In addition to maize, alternate hosts of *E. turcicum* include *Sorghum halepense*, *Sorghum bicolour,* barley, oat, rice and *Zea mays* spp. *mexicana* (Romero, 2016).

2.3.2 Taxonomy of *Exserohilum turcicum*

The pathogen was formerly called *Helminthosporium turcicum* after which, Cooke and Ellis redefined the disease two years later in the United States (Romero, 2016). *Setosphaeria turcica* represents the sexual stage. *Exserohilum turcicum*, can be classified phylogenetically in the kingdom Fungi, subkingdom; Dikarya, phylum Ascomycota, cup fungi subphyulum Pezizomycotina, class Dothideomycetes, subclass Pleosporomycetidae, order Pleosporales, family Pleosporaceae, and genus *Exserohilum* (http://www.mycobank.org).

2.3.3 Symptoms of *Turcicum* leaf blight

Plate 2: Typical symptoms of *Turcicum* leaf blight.

Turcicum leaf blight is most frequently characterized by gray-green to tan colored elliptical or cigar shaped lesions that develop on all the leafy structures of the maize plant (Plate 2). The lesions can range from one to six inches long and run parallel to the midrib but are not restricted by the leaf veins (Welz, 1998).

The initial symptoms of TLB first appear as small chlorotic flecks on the leaves after infection (Kloppers & Tweer, 2009) and are characterized by a few lesions scattered in the lower leaves, except when plants are infected by a large number of conidia from the atmosphere. As the disease progresses, lesions enlarge and change to greenish gray spots as they spread along the leaves. As lesions mature, they develop a brown elliptical-shape with distinct dark zones that are associated with fungal spore production (Wathaneeyawech et al., 2015). Provided that optimal infection conditions persist, the lesions progress onto the upper canopy as well. In the advanced stages of the disease or where there is no host resistance, multiple lesions coalesce

and almost all of the leaves may be entirely blighted resulting in complete destruction of the foliage (Wathaneeyawech et al., 2015).

2.3.4 Effect of *Turcicum* leaf blight on yield

Turcicum leaf blight is an economically critical foliar disease of maize and poses a significant threat to maize cultivation worldwide. TLB can cause moderate to- severe yield losses, with typical losses in the range of 15 to 30% and it is widespread across maize growing regions globally (Kloppers & Tweer, 2009; Poland et al., 2011). Factors such as cultivation of susceptible hybrids, appearance of the disease early in the season and high humidity may worsen the associated losses to be as high as 30-50% (Perkins & Pedersen, 1987). Provided there are optimal conditions during disease development and severe TLB infection occurs before flowering such losses may exceed 50% in maize final yields (Ding et al., 2015). Yield losses of 18 to 62% have been demonstrated in controlled experimental trials on yield under high levels of TLB infection (Shankara & Gowda, 2011). Berger et al. (2020) documented yield reductions of 36 to 72% on susceptible hybrids under controlled conditions in Kwa Zulu Natal South Africa.

Since *E. turcicum* is a necrotrophic pathogen (Galiano-Carneiro & Miedaner, 2017), TLB can suddenly reduce the yield of maize by damaging the photosynthetically active leaf tissues during the critical grain-filling period (Raymundo et al., 1981). When the disease incidence of *Turcicum* leaf blight exceeds 50%, there could be a 91% reduction in the ability of leaves to photosynthesize. As the disease progresses, lesions coalesce to blight the entire leaf surface resulting in loss of the photosynthetic leaf material (Kloppers & Tweer, 2009). *Turcicum* leaf blight therefore reduces the amount of chlorophyll which produces the carbohydrate, fat and protein required by the plant (Setyawan, 2016). If considerable green leaf area is destroyed the vigour and yields are reduced, starch formation is also inhibited and the kernels become chaffy. In addition, the extensive leaf damage during seed set and fill predisposes infected plants to

stalk rots and lodging which may be accompanied by a reduction in yield, feed value and grain quality (Raymundo et al., 1981).

2.3.5 Origin of *Exserohilum turcicum*

Vavilov (1928) hypothesized that centers of origin were characterized by high levels of genetic diversity. In a study conducted by Borchardt et al. (1998) to examine the population structure of *Exserohilum turcicum* isolates from tropical and temperate environments. A multidimensional scaling of *E. turcicum* populations revealed that the Mexican isolates could be at the center, possessing the highest number of polymorphic bands amongst all the populations examined.

Random amplified polymorphic DNA markers analysis also revealed a high gene diversity for the Mexican population, which was the highest numerically but not significantly as high as the Kenyan (Embu) population. The Shannon-Wiener index of genetic diversity, was significantly high in Mexico (0.96) and Embu (0.98). These results were compatible with results reported by Muiru et al. (2010) who also reported a high genotypic diversity of *E. turcicum* isolates collected from Kenya. An isolate that was retrieved from southwestern France and marked "African alleles" indicated that it could have migrated from Africa (Borchardt et al., 1998). Galiano-Carneiro and Miedaner (2017) reported that Mexico was the most likely centre of origin for *E. turcicum.*

In view of this development, it is more likely that *Exserohilum turcicum* could have migrated from Central America if the pathogen coevolved with its host *Zea mays* (Zhang et al., 2020) or East Africa if it coevolved with its host *Sorghum bicolor* (Borchardt et al., 1998).

2.3.6 Disease Cycle of *E. turcicum*

The life cycle of the fungus causing TLB is closely linked to that of maize (Human et al., 2016). *Exserohilum turcicum* overwinters as mycelia and conidia on infected corn residues left on the soil surface at the end of the cropping season, when maize is harvested (Human et al., 2016).

Conidia may be transformed into chlamydospores which are thick-walled resting spores that allow survival of the fungus between seasons (Romero, 2016). At the start of the following growing season, new conidia germinate on the old corn residue to initiate primary infections (Weems & Bradley, 2018). The conidia are transported via wind or rain splash to lower leaves of young corn plants (Kloppers & Tweer, 2009).

Infections and disease development are favored by temperatures between 20 and 25°C, warm weather, heavy dew of at least 4 hours and 90 to 100% relative humidity (Galiano-Carneiro and Miedaner, 2017). Such favorable conditions, enable development of TLB lesions (about 2×10-15 cm) on lower leaves, which later spread and cause severe damage on the upper leaves. TLB lesions develop to produce new spores on susceptible genotypes, enabling the disease to spread aggressively (Wathaneeyawech et al., 2015). This secondary spread of the inoculum from lower to upper leaves, results from dissemination of the conidia by wind, thus continuing the infection cycle (Galiano-Carneiro & Miedaner, 2017).

2.3.7 Epidemiology of *Exserohilum turcicum*

Exserohilum turcicum initially exhibits and maintains a hemibiotrophic phase, proliferating in living tissue for a duration that can last from a few hours to several days (Sucher et al., 2017). The pathogen subsequently switches to a necrotrophic lifestyle to feed on dead leaf tissues (Hurni et al., 2015).

Under optimum conditions, conidia undergo either wind or water dispersal to initiate new infections on healthy maize plants (Romero, 2016). Secondary infections are promoted by the subsequent dispersal events of airborne conidia. Thereafter, the conidia are spread from plant to plant and across fields by wind driven rain, the fungi will reproduce to form spores on the lesions, which will subsequently serve as source of inoculum to initiate new infections (Romero, 2016).

2.3.8 High-risk Factors and Favorable Conditions

Infection of corn by *Exserohilum turcicum* is influenced by three environmental and two biotic factors; light, dew temperatures, dew period, plant age and inoculum concentration (Levy and Cohen, 1982). TLB becomes epidemic especially in regions with cool climates at night, frequent rainfall, moderate temperatures ranging between 20°C and 25°C during the day, low radiance and 90 to 100 percent relative humidity (Wathaneeyawech et al., 2015). Such conditions prevail on major maize producing regions in Sub-Saharan Africa, China, India and Latin America. Such regions also fall within the midaltitude range of 900-1600m above the sea level and provide favorable environmental conditions for the development of *Exserohilum turcicum* due to the prolonged dew periods and moderate temperatures (Welz & Geiger, 2000). Because of the ability of the pathogen to survive from year to year in infected corn debris, cultural practices that maintain more infected residue on the surface such as adoption of conservation tillage practices and increased acreage under continuous maize cropping has led to greater levels of crop residue that harbor disease inoculum. Maize crop residue infected with *E. turcicum* residues will serve as the primary inoculum source for TLB development (Weems & Bradley, 2018). A field with a history of TLB or the existence of TLB in nearby fields combined with the ability of the fungus to survive from one year to another contributes significantly to the development of infections in subsequent years making TLB a perpetual problem.

To increase productivity, maize farmers/producers select hybrids with a potential for high yield per hectare over those with high disease ratings. Some of these hybrids could be susceptible and have poor disease ratings for TLB hence would contribute towards disease development and high levels of disease incidence (Wise & Mueller, 2011). This is an indicator that some of the lines associated with high yields may lack the requisite resistance genes (R-genes) to reduce the impact of TLB on maize production (Weems & Bradley, 2018).

Development of the disease early in the season prior to corn tasseling is also a high-risk factor for severe disease later in the growing season. The infection can also progress vigorously during favorable weather conditions after corn silking (Perkins & Pedersen, 1987). Some isolates of *E. turcicum* may also infect other grasses such as Johnson grass (*Sorghum halepense*), barley, oat, rice, millet, Sudan grass, and *Zea mays* spp. *mexicana*, but these fungal isolates may not infect corn (Romero, 2016; Wathaneeyawech et al., 2015).

2.3.9 Infection strategy of the pathogen

Pathogenesis is the manner in which the disease progresses within the host-pathogen system. It is characterized by inoculation, fungal colonization of the respective host, production of spores and their eventual dispersal (Chung et al., 2010b). From the lesions on infected leaves, the conidia arises from the conidiophore to act as primary source of inoculum that will initiate new infections. The spores are dispersed to spread the disease from the host to another plant (Kotze et al., 2019).

Following successful conidial attachment and germination on the host surface, germ tube growth and appressorium formation, the infection hypha is formed from the apical cells of the appressorium to penetrate the leaf cuticle and epidermal cell wall (Muiru et al., 2010). *E. turcicum* initially exhibits a biotrophic lifestyle during the first part of its lifecycle. During this phase, the hyphae penetrates the leaf epidermal cells and doesn't kill the host cells and tissues as it advances towards the vascular bundles. The infective hyphae then enters the xylem whereby the hyphae grows extensively all through the xylem cells for 6–8 days (this could be 7 days on susceptible varieties) without causing any destructive effects or conspicuous symptoms (Hilu & Hooker, 1964). Within 7 to 13 days after infection, the pathogen exhibits a transition in its lifestyle to being a necrotroph and this marks the onset of the appearance of TLB lesions (Human et al., 2020).

As the disease progresses, the hyphae grows to completely block the minor veins and cause 80-90% blockage on major veins at 14 days post inoculation (Kotze et al., 2019). It has been reported that hyphal growth is reduced in resistant genotypes as compared to susceptible genotypes (Muiru et al., 2010).

2.3.10 Management of *Turcicum* Leaf Blight

Management of TLB in maize production systems is achieved through a combination of methods. TLB has been successfully managed through the application of fungicides and host plant resistance (Galiano-Carneiro & Miedaner, 2017). Fungicides inhibit production of the disease and actively stops production of new spores and reduce overall severity and numerous fungicides have been manufactured for their application on maize and to control TLB. Different fungicides have diverse modes of action, active ingredients and target specific sites within the infection cycle of *E. turcicum*.

Previous studies indicated that the use of sulfur compounds, phenyl pyrroles and carboxamides was highly efficient towards controlling mycelial growth of *E. turcicum* (Galiano-Carneiro & Miedaner, 2017). Different chemical families were tested for their ability to stop the production of HT-toxin in *E. turcicum*, among the 18 chemical compounds on trial, mancozeb was found to be highly effective in reducing the production of HT toxin (Wathaneeyawech et al., 2015). However cases of failure in the control of other *Cercospora* species using fungicides has been reported such as the resistance of *Cercospora beticola* isolates to strobilurin fungicides (Vaghefi et al., 2016).

Traditional management practices such as ploughing deep to break down corn residues on the soil surface and significantly reduce the amount of inoculum, crop rotation with a non-host species and away from maize fields are widely used in the control of TLB (Human et al., 2016). This reduces the amount of overwintering inoculums and the risk of TLB in the future.

Biological control agents, such as bacterial *Enterococcus* and *Bacillus* species have also been used to reduce the negative effects of TLB (Sartori et al., 2017).

Planting resistant hybrids that suppress pathogen attack is the most effective and economical way to manage TLB (Technow et al., 2013). Different types of resistance are available in corn through the deployment of horizontal (multi-gene) and vertical (single gene) resistance (Weems & Bradley, 2018). Hybrid resistance can help reduce disease severity by reducing the developmental rate and spread of the pathogen and development of new haplotypes, retarding other activities of the pathogen such as spore production. This can also be accomplished by lengthening the latency and incubation period and reducing the expression of symptoms (Welz & Geiger, 2000).

2.3.11 Mode of Reproduction of the pathogen

Mating type loci (sex locus) are functional drivers conditioning the ability of fungi to undergo sexual reproduction. *Exserohilum turcicum* undergoes sexual heterothallism and is in possession of a single MAT locus that has two alleles MAT 1-1 and MAT 1-2 controlling the mating type gene (Fan et al., 2007). For sexual reproduction to occur isolates of both mating types should be in close proximity (Human et al., 2016). In contrast, isolates collected from northern China were classified into three major mating types: MAT1, MAT2 and MAT12. Isolates in MAT12 group could mate MAT1 or MAT2 isolates, but they could not mate each other or themselves. In this case isolates possessing MAT12 could not mate with other isolates with MAT12 (Fan et al., 2007). The MAT12 was first discovered in China, and its discovery made it more difficult to understand the genetic control of mating types in *S. turcica*, because the MAT12 might increase the mating frequency of *S. turcica* in field.

Previous studies documented the existence of the two opposite mating types and their equal distribution among isolates collected from tropical regions such as Kenya, Mexico, Southern United States and Southern China (Borchardt et al., 1998; Bunkoed et al., 2014; Ferguson &

Carson, 2004). In addition, high genetic variability and genetic linkage disequilibrium in such areas has also been reported (Fan et al., 2007). This suggests the existence of mixed modes of reproduction in the lifecycle of *E. tucicum*, (Milgroom, 1996; Nieuwoudt et al., 2018) and as such sexual hybridization is likely taking place in the population on old leaves late in the season (Human et al., 2016).

In temperate regions such as Europe, United States Corn Belt and Northern China strong gametic phase disequilibrium, appearance of only one mating type gene or unbalanced distribution of mating type genes has been reported (Borchardt et al., 1998; Ferguson & Carson, 2004). This indicates the probable lack of sexual reproduction in such populations (Vaghefi et al., 2016). Most studies have reported the occurrence of both mating types in the population (Bunkoed et al., 2014).

The pathogen may reproduce sexually in the laboratory by producing ascocarp (pseudothecia) on synthetic media containing sterilized plant materials. In the laboratory, ascocarp forms when two different mating types are grown on barley grain or straw (Luttrell, 1958).

2.3.12 Teleomorph formation from *E. turcicum* in nature

Although mature ascocarps and ascospores had been successfully produced in Sach's medium for *E. turcicum* by Fan et al. (2007) it was not until 2013 that the sexual stage was reported in two fields under maize cultivation in Thailand (Bunkoed et al., 2014).

Bunkoed et al. (2014) induced the formation of sexual structures of *E. turcicum* (Pseudothecium, bitunicate ascus and ascospores) by making 18 crosses between six *E. turcicum* isolates of two opposite mating types that had been selected from the population to obtain 36 cross combinations. The crosses were evaluated in vitro on potato dextrose agar on maize (*Zeae mays*) leaves. The isolates underwent sexual reproduction to produce pseudothecia after 3 weeks at 23°C in oscillating light dark cycles of 16 hours and 8 hours respectively. The

appearance of the pseudothecia (ascocarp) and ascospores were as described in Bunkoed et al. (2014) and such descriptions were consistent with those made by Luttrell (1958).

2.3.13 Impact of the Reproductive structure

Sexual reproduction enhances the pathogen's ability to overcome host resistance by rapidly evolving to generate new strains with diverse virulence profiles (Bunkoed et al., 2014), thus promoting more fit genotypes and virulence on differential maize cultivars (Milgroom, 1996). In contrast, pathogens that reproduce asexually are characterized by deleterious mutations, small amounts of gene flow and small sizes of the effective population, hence they are associated with reduced risks (McDonald & Linde, 2002).

2.3.14 Genetics of TLB Resistance

TLB has been sustainably managed through the deployment of resistant varieties and resistance is either broad spectrum or race specific (Welz & Geiger, 2000; Wisser et al., 2006). Quantitative resistance is pathogen specific, durable and is under the control of multiple genes acting in an additive manner with minor phenotypic effects, whereas qualitative resistance is considered to be race specific and is under the control of single Ht genes. Both mechanisms of resistance are critical for the control of TLB and have been extensively utilized to breed for resistance (Pratt & Gordon, 2010).

2.3.14.1 Qualitative resistance (Ht genes)

This form of resistance is considered to be effective against biotrophs (Balint-Kurti & Johal, 2009). Qualitative TLB resistance genes are available in a number of maize germplasm and are mainly conditioned by *Helminthosporium turcicum* (*Ht*) genes. Race-specific genes (*Ht1*, *Ht2*, *Ht3*, and *HtN*) are reported to protect maize against specific races of *E. turcicum* and confer high levels of resistance.

Introgression of qualitative Ht genes into the genomes of susceptible cultivars has been observed to result in delay in the formation of symptoms, reduced disease response and disease

severity. However, their effectiveness may be affected by the emergence of very virulent races probably due to the high genetic variability arising from sexual recombination in the population (Weems & Bradley, 2018; Welz & Geiger, 2000).

The gene *Ht1*, identified from the field corn inbred GE440 and popcorn cultivar Ladyfinger, confers a chlorotic lesion type, is partly dominant and it is characterized by greatly suppressed sporulation in chlorotic lesions (Galiano-Carneiro & Miedaner, 2017). This gene was previously mapped on chromosomal bin 2.08 positioned on the long arm of chromosome 2 (Welz, 1998). Severity of TLB was reduced by the *Ht1* gene, with yield losses for *Ht1* hybrids ranging from 6 to 28% in comparison to losses reported from susceptible hybrids not carrying the *Ht1* gene that ranged from 21 to 56% (Pataky & Ledencan, 2006).

Ht2 gene originating from a breeding material from Australia exhibits a similar resistance reaction as *Ht1* but with enhanced necrosis on leaves (Weems & Bradley, 2018). *Ht2* also shows partial dominance and was identified to be located on the long arm of bin 8.06 on chromosome 8 (Ding et al., 2015; Galiano-Carneiro & Miedaner, 2017). The gene *Ht3* is the sole resistance gene that was successfully transferred from *Tripsacum floridanum* into maize (Van Inghelandt et al., 2012) and was identified to be located on chromosomal bin 7.04 (Zhang et al., 2007). *Ht3* gene is associated with chlorotic-lesion resistance and is closely similar to that of plants with *Ht2* (Hooker, 1981). The fore mentioned studies have reported the independence of *Helminthosporium turcicum 2* (*Ht2*) and *Helminthosporium turcicum 3* (*Ht3*). However, Yang et al. (2021) reported that *Ht2* and *Ht3* are identical and could be found on the same physical interval, and they designated the domain as *Ht2*/*Ht3*. The study revealed that the domain encodes ZmWAK.RLK1, a resistance gene associated with delay in the appearance of lesions. The authors further postulated that the two qualitative genes are probably different versions of the same gene. The *Sht1* locus has been shown to inhibit the expression of *Ht2/Ht3* and *Htn1* gene (Chung et al., 2010b; Yang et al., 2021).

The recessive gene *ht4*, originates from a breeding material from the United States (maize synthetic BS19) (Pataky & Ledencan, 2006). This gene resides on the short arm of chromosome 1 to condition chlorotic halos-like symptoms on the phenotype (Carson, 1995).

Htn1 gene (formerly known as *HtN*) was transferred from the Mexican Pepitilla corn into the modern maize genotypes in the 1970s and its mechanism of action is different from the other qualitative *Ht* genes such as *Ht1*, *Ht2* or *Ht3*. The *Htn1* gene confers quantitative resistance and it's partially resistant to TLB (Galiano-Carneiro & Miedaner, 2017). It is critical to ensure reduced number of lesions, delays lesion formation by approximately 4 weeks and delays the sporulation of lesions (Welz & Geiger, 2000). *Htn1* gene was identified to be located on chromosomal bin 8.05 on the long arm of chromosome 8 and it is functional against most TLB races (Hurni et al., 2015).

Other *Ht* genes include *HtP* that was identified to be located on the long arm of chromosome 2, bin 2.08, *HtM* and *HtNB* mapped on bin 8.07 (Ogliari et al., 2005). Nearly all *Ht* genes are dominant or partially dominant making *Turcicum* leaf blight unique among the necrotrophic diseases (Welz & Geiger, 2000).

The use of *Ht* genes as sources of resistance to control TLB has several limitations; numerous pathogenic races of *E. turcicum* have been documented and are virulent to the major *Ht* genes. In addition, such qualitative resistance genes tend to be broken down by more virulent *E. turcicum* races (Van Inghelandt et al., 2012). The strength of Ht genes is however dependent on the genetic background and environmental conditions such as light and temperature (Thakur et al., 1989; Yang et al., 2021).

This limits the practical value of Ht genes and their use in maize breeding programs due to the risk of major gene resistance breakdown (McDonald & Linde, 2002; Welz & Geiger, 2000). In addition, the expression of *Ht2*, *Ht3* and *Htn1* is modified by *Sht1* gene, which is a dominant suppressor gene, this complicates their use through backcrossing (Simcox & Bennetzen, 1993).

The Ht genes can be transferred into the genomes of maize cultivars to condition resistance in temperate regions where the disease pressure is low (Galiano-Carneiro & Miedaner, 2017). Tropical environments on the other hand are characterized by high disease prevalence, high genetic diversity of *E. turcicum* and highly abundant pathogen population, in such conditions *Ht* genes can only provide partial resistance to *E. turcicum* (Weems & Bradley, 2018).

2.3.14.2 Pathogen races

There are several physiological races of *E. turcicum* that have the ability to break down qualitative resistance genes such as Ht1, Ht2, Ht3, or Htn1. To date, more than seven pathogenic races of this fungus with distinct functions have been identified and include races 0, 1, 2, 12, 23, 23N and 123N. Based on six different host resistance genes, there are 64 potential races of *E. turcicum* (Pataky & Ledencan, 2006). The inheritance of virulence to *Ht1* gene is controlled by a single gene in *E. turcicum*. This results in the Flor's gene for gene interaction between the *Ht1* gene of the host and the avirulence gene of the pathogen (Galiano-Carneiro & Miedaner, 2017).

Symptom response of maize differential lines introgressed with the individual Ht genes when inoculated with different *E. turcicum* races provides theoretical basis to define the E. *turcicum* races (Weems & Bradley, 2018). Pathogenic races are therefore designated based on their virulence or avirulence to corresponding *Ht* genes in maize, for instance race 1 is pathogenic to maize lines possessing the *Ht1* resistant gene whereas *Ht* genes can prevent pathogenic attacks from race 0 strain of *E. turcicum* (Weems & Bradley, 2018). Resistance conferred by *Ht2*, *Ht3* and *Htn1* genes can be overcome by race 23N, while maize genotypes that do not have any resistance genes are easily infected with race 0 and race 1 (Ogliari et al., 2005). Race 123N can trigger infections on all maize genotypes with the conserved *Ht* genes and this is attributed to the fact that race 123N exhibits high virulence complexity (Welz, 1998). Ferguson and Carson (2004) also reported existence of several races of the pathogen and variability even

within the races. The expression of these *Avr* genes in *E. turcicum* undergo modifications as a result of fluctuations in environmental conditions such as temperature and light intensity (Welz & Geiger, 2000).

2.3.14.3 Quantitative resistance

In environments where populations of *E. turcicum* exhibit high genetic diversity and the disease pressure is also high, broad-based race non-specific resistance to TLB is preferred (Galiano-Carneiro & Miedaner, 2017). Complex traits such as resistance to TLB are controlled by polygenic genes with minor effects (Ding et al., 2015) that are distributed throughout the genome (Chen et al., 2015; Poland et al., 2011; Van Inghelandt et al., 2012; Welz & Geiger, 2000; Wisser et al., 2006). Twenty nine QTLs conditioning resistance to TLB have been identified so far in several populations (Chen et al., 2015). However, major QTLs associated with resistance to TLB are hardly available, and the large variation in TLB resistance presents a major challenge and has been attributed to the accumulation of small effect polygenic genes/QTLs (Ding et al., 2015; Poland et al., 2011).

In general, quantitative TLB resistance is highly heritable, durable under field conditions and is under the control of numerous small effect QTLs (Galiano-Carneiro & Miedaner, 2017). In addition it is not affected by changes in environmental conditions such as temperature and light intensity (Welz & Geiger, 2000). The mode of gene effect differentiates with vegetative or reproductive stage of the maize plant, with additive gene action being more important in juvenile plants (Carson, 1995) and dominance effect increases as the disease progress after anthesis (Schechert et al., 1999). In conclusion resistant genotypes typically exhibit delay in the appearance of lesions, the lesions are fewer in number and smaller in size. However, sporulation is not reduced when compared to susceptible hosts (Welz & Geiger, 2000).

Up to the present moment, previous studies have documented more than 197 QTLs from 27 published articles for resistance to *Turcicum* leaf blight. These are representing QTLs that were

detected in a minimum of 2 environments and a population size of no less than 100 genotypes. Thorough investigation revealed that some QTLs were detected on the same chromosomal bin such as 1.05/1.06, 3.04, 4.06, 7.02, 8.03, 8.05 and 9.02 and probably these are QTL hotspots for resistance to TLB (Miedaner et al., 2020).

2.3.14.4 An overview of previous TLB resistance QTL studies

Previous QTL mapping studies conducted more than a decade ago were able to characterize the QTLs conditioning quantitative resistance to TLB in Kenya. Dingerdissen et al. (1996) used the interval mapping method to evaluate an $F_{2:3}$ mapping population from Mo17 crossed with B73. The segregating population of Mo17 (resistant) and B73 (susceptible) were evaluated over three counties in Kenya, namely Embu, Kitale and Kiambu (Muguga). The authors reported a QTL for area under disease progress curve on chromosomal bin 7.03. The qualitative Ht2 gene was also mapped on chromosome 8L. Similar results were reported by Freymark et al. (1993) who had previously detected consistent QTLs at similar positions with an additional 1S.

The $F_{2:3}$ mapping population of Lo951 (susceptible) and CML202 (resistant) cross comprising of 194-256 genotypes were phenotypically tested in Embu and Kakamega. One hundred and ten RFLP markers were used to genotype the population. Six QTLs significantly associated with incubation period and area under disease progress were detected on chromosomes 2, 3, 5, 8 and 9. Two QTLs originated from Lo951 that explained up to 69% of the phenotypic variance (Schechert et al., 1999). Similar population and the same number of RFLP markers were used by Welz et al. (1999) to find out if the QTLs were effective at different stages of plant development. The authors detected 9 significant QTLs on chromosomes 2, 4, 5, 8 and 9 that were associated with different resistance components in the combined analysis. The expression of these QTLs were affected by the different development stages. Most of the QTLs were effective after flowering time. The results of these mapping studies were summarized by Welz

and Geiger (2000) who identified QTL regions that were common across populations on chromosomal bins 3.05, 5.04 and bin 8.05.

2.3.15 Distribution of *Turcicum* leaf blight

Turcicum leaf blight is highly prevalent in major maize (*Zea mays* L.) growing regions globally that are characterized by favorable environmental conditions such as moderate temperatures and high humidity (Poland et al., 2011). The major corn producing countries affected by TLB include Canada, the United States, Mexico, Ecuador, southwest Brazil, Argentina, India, north-eastern and northern China, Uganda, Kenya and Tanzania where the pathogen has caused significant losses in yield (Borchardt et al., 1998; Romero, 2016). Such suitable environmental conditions are widely experienced in tropical highlands, that are further characterized by small scale farmers and food insecurity situations are widespread (Poland et al., 2011). Adipala et al. (1993) reported that *Turcicum* leaf blight is widespread in East Africa, but is particularly destructive in the wet, warm and humid areas around the Lake Victoria.

2.4 DNA markers

Molecular markers are highly abundant within the genome making them suitable for an array of applications such as linkage map construction. DNA markers represent differences in the genetic architecture of individuals within the same or different species and they occupy specific positions within chromosomes called loci that reveal sites of diversity in DNA (Collard et al., 2005). Gel electrophoresis stained with either ethidium bromide or silver may be used to visualize such genetic differences.

Apart from the use of molecular markers to analyze the level of genetic variation within a population and identification of a specific cultivar (Collard et al., 2005), DNA markers have been extensively used to verify the genetic purity of lines (Sserumaga et al., 2020), to resolve questions regarding phylogenetic issues and to describe the genetic structure of a population

(Muller et al., 2016). In addition, molecular markers are used to construct linkage maps that contribute towards the identification of QTLs for important traits (Sansaloni et al., 2020).

An ideal genetic marker should be diagnostic, (it should be able to discriminate between varieties that either express or do not express the trait of interest in a wide range of germplasm). An ideal marker should also be highly informative, selectively neutral, reproducible and exhibit random distribution in the genome (Collard & Mackill, 2008). Most molecular markers are selectively neutral due to their position in non-coding regions of DNA.

Amplified fragment length polymorphisms (AFLP) and random amplified polymorphic DNA (RAPD) markers have been extensively used to conduct population genetics analyses of fungal populations (Okori et al., 2003). However, both AFLP and RAPD have their technical or operational limitations that are overcome by simple sequence repeat markers (Selkoe & Toonen, 2006).

2.4.1 Microsatellite Markers

Microsatellites are traditional marker types and are also called short tandem repeats (STR), simple-sequence repeats (SSR), simple sequence length polymorphisms or variable number tandem repeats (VNTR). Microsatellites consist of repetitive DNA sequences typically 1–10 nucleotides found within the genome. The flanking region of a simple sequence repeat locus includes the DNA surrounding a microsatellite locus and is composed of a highly conserved sequence across individuals of the same species. Primers specific for these regions can be designed to bind to the flanking region for use in the PCR amplification of a microsatellite and to screen for polymorphism (Selkoe & Toonen, 2006).

Simple Sequence Repeat markers are ideal for analyses in population genetics because they are widely distributed across the genome, codominant, (Xu et al., 2014), highly polymorphic and are amplified by PCR using specific primers designed to flank SSR regions. In addition, microsatellites are multi-allelic and possess a high mutation rate. Simple sequence repeats also

occur frequently and are randomly distributed throughout the genomes of all eukaryotic organisms in coding and non-coding sequences. This makes them robust, technically simple to score statistically and readily shared among research groups (Collard et al., 2005). The development of multiplexing has also promoted moderate level throughput in studies involving SSR markers as it allows several marker loci to be tested simultaneously. Historically, SSR markers have been used to characterize the genetic diversity and population structure of plant pathogens. This is attributed to the fact that they do not require prior sequence information and they exhibit widespread accessibility. In addition, SSR markers do not need specialized equipment to perform the analysis (Naegele et al., 2021).

SSR markers also have several challenges that complicate their use in population genetics; Microsatellite markers are species-specific making transferability of microsatellite markers across species difficult, therefore new primers have to be developed for each new species (Sansaloni et al., 2010; Selkoe & Toonen, 2006). In addition, microsatellite markers are less suitable for association studies due to the occurrence of microsatellite alleles of identical size but different ancestral origin. The SSR markers are also under selection themselves and typically suffer from reproducibility across laboratories (Seeb et al., 2011). Developing SSR markers is also time consuming, relatively costly and technical. Microsatellite markers are thus important and will be employed in this study to determine the genetic diversity of *C. zeina* isolates collected from Kenya.

2.4.2 Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs pronounced “snips”) involve variations in a single base pair site in the genome and this translates to the difference in the DNA sequence from one individual to another. Each SNP marker represents the difference in a single nucleotide which is the building block of DNA, for instance SNP may replace cytosine (C) with Thymine (T) in a certain DNA sequence. Single nucleotide polymorphism markers exist in abundance and are

extensively distributed within the genome. In fact SNP markers are much more numerous than any other DNA marker in the genome. However, their rate of mutation (10^{-9}) is much lower than SSR markers (10^{-4}) (Xia et al., 2020).

The distribution and occurrence of SNPs throughout the genome vary among species, with estimates of one SNP per 60-120 bp being reported in corn genes (Rafalski, 2002). Nasu et al. (2002) reported an average of one SNP every 89 bp in rice. Therefore SNPs can contribute directly to a detectable phenotypic effect as a result of linkage disequilibrium (Nasu et al., 2002).

Single nucleotide polymorphism markers are the method of choice in the identification of plant varieties and cultivars, high density linkage map construction, QTL analysis, molecular breeding and genome-wide linkage analysis (Xia et al., 2020). This is attributed to their abundance and codominance. In addition, SNPs are to a greater extent abundant in the non-coding sections of the genome and are often associated with genes making them highly important in locating for instance disease genes (Nasu et al., 2002).

Apart from the above, the use of SNP markers for analysis of linkage has several advantages namely: molecular analysis can be conducted at the early stage of crop development, hence this requires only a small quantity of DNA, it allows systematic processing of a large number of samples from sowing to SNP genotyping in 96-well plates and it is more efficient on time and labor as there is no electrophoresis needed (Nasu et al., 2002). However, SNP markers are predominantly biallelic, a characteristic that makes them less descriptive than multiallelic markers, as a result their capacity to discriminate among individual loci is limited. SNP markers are majorly utilized for genotyping and linkage mapping (Syvänen, 2001).

The high level of polymorphism reported for SNP markers coupled with their widespread abundance in the genome together with the development of SNP genotyping platforms have great implications for adoption of marker assisted selection in the future (Rafalski, 2002). Such

high level of polymorphism has previously been documented in some crop species such as maize, where 80% of the amplicons sequenced from diverse individuals were found to be polymorphic and this facilitates SNP identification. Availability of inbred lines makes read-off of haplotypes relatively straightforward (Rafalski, 2002).

An array of SNP genotyping platforms have been developed, however, currently no superior platform has been universally adopted. Array-based methods such as Single feature polymorphism (SFP) and Diversity Array Technology (Jaccoud et al., 2001) detection offer prospects for lower-cost marker technology that can be utilized for whole-genome sequencing (Collard & Mackill, 2008).

2.4.3 SNP Genotyping using Diversity Array Technology (DArT)

Diversity arrays technology (DArT) is a complexity reduction, DNA hybridization-based technique that simultaneously scans several hundreds to thousands of polymorphic markers in a single assay. DArT was developed in 2000 as a technology based on hybridization, for resolving DNA polymorphisms and does not require prior sequence information for the trait of interest before genotyping (Jaccoud et al., 2001). Diversity arrays technology performs allele calling for all probes of all the different sequences of genomic DNA by scoring as either present or absent with regards to the target sequence (Sansaloni et al., 2010).

Diversity arrays technology was originally developed for rice as a model crop with a genomic size of 430 Mbp (Jaccoud et al., 2001). Due to the fact that DArT is not species specific, its high throughput capabilities and wide genome coverage, DArT has been subsequently applied across numerous plant species and plant pathogens (Jaccoud et al., 2001).

DArTseq involves the use of Diversity arrays technology in combination with next generation sequencing. In combination with endonucleases, this targets low copy regions of the genome instead of the repetitive DNA fragments to achieve genome reduction (Wenzl et al., 2004). A high number of informative SNP markers can then be detected across the genome.

There is growing interest towards the use of DArT, and this can be attributed to the fact that DArT allows the process of data acquisition to be automated, relatively targets low-copy regions of the genome and it is cost competitive. In addition, diversity arrays technology have the capacity to deliver hundreds to thousands of polymorphic markers for genetic diversity studies and they are also efficient to generate high density linkage maps for marker genotyping. The use of DArT technique also suffers a number of limitations, the most important one being the dominant mode of inheritance. Dominant markers are predominantly less informative for linkage map construction. This can be remedied by the use of a large number of markers and a bigger population size (Sansaloni et al., 2010).

2.5 Population Genetics

A population can be defined as a group of sexually interbreeding individuals that occupy the same geographic location and undergo reproductive continuity from one generation to the next. Population genetics is the study of the variation in allele and genotype frequencies within a population and it involves an observation of how this variation changes over time and space (McDonald & Linde, 2002). According to Evanno et al. (2005), population structure is frequently measured using the Wright's F statistics as previously demonstrated by Sewall Wright in 1931. The pathogen population dynamics may be determined by the environment from where the samples originated, but then their genetic structure is dependent on the genetic make-up of the individual isolates and not the geographic origin of the phenotypes. In addition isolates from different geographical regions, may not be genetically distant, suggesting that gene flow occurs between different regions (Evanno et al., 2005).

The Hardy–Weinberg law forms the fundamental principle of population genetics. Population genetics focuses on genetic processes such as mutation, genetic drift, gene flow, mating systems, and natural selection that lead to evolution and genetic change over generations and seasons (McDonald & Linde, 2002). The Hardy-Weinberg law therefore predicts a balanced

equilibrium in allele and genotype frequencies provided there is no mutation, no natural selection, no genetic drift, no gene flow and the population is large enough and randomly mating. An interaction amongst the evolutionary forces have an influence on the level of genetic structure of the pathogen population (McDonald & Linde, 2002). It is important to take into account that mutation acts as the primary source of new alleles in a population while other forces stabilize the distribution of alleles in the population.

Gene diversity and genotypic diversity are two types of genetic diversity that form an important component of genetic structure. Gene diversity refers to the probability of observing two distinct alleles at an individual locus following the sampling of two haploid individuals from a population (Linde, 2003). The level of gene diversity is directly related to the number of alleles at a locus and therefore increases as the number of alleles increases in a population and the gene frequencies move towards a state of equilibrium (McDonald & Linde, 2002). Factors such as large population size, older population and high gene flow tend to increase the number of alleles in a population and therefore affect gene diversity (McDonald & Linde, 2002). Gene flow is particularly important as it involves the movement of alleles between geographically differentiated populations in the process introducing old virulent genes into a new population. Therefore high gene flow, contribute to a greater gene diversity.

Genotypic variability which is a measure of multilocus genotypes in a population is affected by selection, genotypic flow, genetic drift and mode of reproduction of the pathogen. The occurrence of sexual recombination within a population and equal distribution of mating type genes results into high levels of genotypic diversity (Milgroom, 1996), while pathogens that recombine asexually exhibit low genotypic diversity. Therefore the level of genotypic variability existing within a pathogen population is suggestive of the mode of reproduction (McDonald & Linde, 2002). Directional selection leads to an increase among a certain genotype in the population. Genotype flow which is the movement of genotypes between

populations in distant geographical locations leads to the introduction of new genotypes into a new geographical location (McDonald & Linde, 2002).

Population genetics focuses on the five forces that drive pathogen evolution and this has considerable implications for disease management. Plant pathogens pose a great risk in agroecosystems as they continually evolve to break down host resistance genes or to overcome other control methods such as fungicide application. Such an evolution is driven by the processes of mutation and recombination leading to high degree of genotype diversity (Muller et al., 2016). Hence the knowledge on the pathogen population structure and its evolutionary forces is a valuable arsenal to design better control strategies in order to maintain efficacy of the fungicides and to prolong the durability of resistance genes (McDonald, 1997).

Quite a number of studies have examined the population dynamics of pathogen populations affecting diverse crops. Gurung et al. (2011) investigated the genetic diversity of *Mycosphaerella graminicola* that causes Septoria leaf blotch in *Triticum aestivum*. In this study, 330 isolates were analyzed using 17 Simple sequence repeat markers. The authors reported high levels of gene diversity (H_e = 0.56) and significant levels of genetic differentiation ranging from 0.000 to 0.621. Among the 330 isolates analyzed in this study there were 306 unique genotypes.

Muller et al. (2016) investigated the population dynamics of *C. zeina* isolates in South Africa based on a sample size of 369, gathered from different commercial farms and reported high values for gene diversity (H_e=0.35) and gene flow (Nm= 5.51). This population was further characterized by low levels of genetic differentiation from AMOVA analysis and the absence of a clear population structure. Structure analysis revealed lack of population structure which was supported by the results of PCoA analysis. To identify whether the population genetics of *Cercospora zeina* is influenced by the cropping system, Nsibo et al. (2019) examined isolates collected from smallholder farms in relation to those from commercial farms using SSR marker

analysis. Isolates from smallholder farms were characterized by higher genetic diversity compared to commercial farms. The Shannon-Wiener and Stoddart-Taylor measures of genotypic diversity were significantly greater for smallholder farms (0.45) relative to the commercial farm sites (0.37). STRUCTURE analysis revealed the existence of partial population structure within the population from both commercial and small holder farming systems (Nsibo et al., 2019).

Kim et al. (2013) analyzed the genetic diversity and structure of *Cercospora sojina* populations collected from soybean fields in Arkansas using previously characterized SSR markers and reported high values for genetic diversity coupled with low index of differentiation. Nei's unbiased gene diversity was comparatively high and was within the range of 0.42 to 0.58 with low genetic differentiation that averaged at 0.084. This is a clear indication that significant genetic exchange existed within the population. In a different study on the population dynamics of *C. sojina*, Shrestha et al. (2017) identified the existence of population structure within *C. sojina* isolates collected from 2 locations in Tennessee and analyzed using 49 SNP markers. The population from Tennessee clustered into 3 subgroups based on Bayesian clustering algorithm, with high levels of gene diversity (H_e = 0.377) and genotypic diversity (Shannon Wiener I = 0.5571). However gene flow was not recorded for both studies (Kim et al., 2013; Shrestha et al., 2017).

Okori et al. (2003) investigated the genetic diversity of *C. zeina* within East Africa using a combination of AFLP and RFLP data sets. The authors reported presence of population structure between the African and US isolates of *C. zeina* and the lack of it among the isolates of *C. zeina* collected from Uganda, Rwanda and Kenya. The absence of genetic differentiation was also reported for *Cercospora sorghi* in Uganda (Okori et al., 2003). This lack of population structure could be attributed to the high level of gene flow within the East African population of *C. zeina* (Nm = 49.5).

2.5.1 Population Structure

Structure is a Bayesian clustering method that assigns individuals into specific clusters on the basis of their genetic makeup at multiple loci. The software structure is based on an algorithm that enables it to identify the number of clusters that include more than two populations. The number of clusters is estimated using the log probability for each value of K (Pritchard et al., 2009). This algorithm has been widely used to detect the genetic structure of a population, analysis of migration, the degree of admixture within a population and hybridization and also to detect cryptic genetic structure in a population (Evanno et al., 2005).

A number of studies have proven that the software Structure is efficient in assigning individuals to their populations of origin. In addition, structure works well with both dominant and codominant markers and accounts for the presence of Hardy-Weinberg equilibrium in the population (Evanno et al., 2005).

2.5.2 Linkage Disequilibrium

Agapow and Burt (2001) demonstrated that the index of association displays a steady increase with a buildup in the number of loci, the authors then came up with the standardized index of association, an approximation that is widely used. The ability of standardized index of association to take into account the number of loci tested makes it a more powerful measure of association (Agapow & Burt, 2001). The standardized index of association has an advantage over classical index of association, since it does not depend on the number of loci examined (Agapow & Burt, 2001; Mahalingam et al., 2020).

The index of association was used to assess if loci are linked as previously described by Agapow and Burt (2001). Linkage among markers provides evidence for sexual recombination while lack of it demonstrates that the population is clonal.

2.6 Genetic Mapping

The ultimate goal of a plant breeder is to assemble all the desirable genes into a single genotype (Collard & Mackill, 2008). Numerous traits of importance to agriculture and the plant breeder such as tolerance to environmental stress, yield, quality and expression of disease resistance are conditioned by polygenes and are frequently referred to as complex traits. The genomic regions containing genes associated with such complex traits are known as quantitative trait loci (QTL) (Collard et al., 2005). The aim of many genetic mapping studies is to identify quantitative trait loci (QTL) that are responsible for phenotypic variation.

Plant pathogens continuously undergo evolution and this enables them to overcome host resistance, therefore breeding programmes should continuously develop and incorporate new forms of resistance genes for plant protection. With the technological advancements in the use of molecular markers, it is possible to identify the QTLs underlying such complex traits for incorporation into the genomes of major crop species (Asíns, 2002).

There is preference towards the use of double haploid lines in breeding programs and in quantitative trait locus mapping. Double haploid lines are completely homozygous and can be attained in two generations compared to conventional breeding that require seven or eight generations (Prasanna et al., 2021). Double haploid lines are formed following initial crossing of the two parental lines in the first year (Choi et al., 2019). The subsequent year, the F1 hybrids are then crossed with a haploid inducer as the male parent (source of pollen) (Choi et al., 2019; Jacquier & Widiez, 2021). The haploid inducer line should be homozygous for the dominant anthocyanin (purple) color marker (R1-Najavo). The marker expression within the endosperm is used to distinguish between a diploid and a haploid (Dwivedi et al., 2015). This result in the formation of haploid embryos in seeds (Jacquier & Widiez, 2021). Maternal embryos are then doubled through the use of chemicals such as colchicine or other doubling agents to form

homozygous DH lines (Dean, 2018; Kurimella et al., 2021; Ochatt & Seguí-Simarro, 2021; Weyen, 2021).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya

3.1.1 Materials

3.1.1.1 Reagents

Liquid nitrogen (Sigma- Aldrich, cat. no. 00474), Taq polymerase and dNTPs Mix (10 mM)

3.1.1.2 Buffer compounds

CTAB, also known as hexadecyltrimethyl ammonium bromide (Sigma- Aldrich cat. no. BCBR9369), β-mercaptoethanol (bME; Sigma-Aldrich, cat. no. M6250), Chloroform, Ethylenediaminetetraacetic acid disodium (Na-EDTA), TRIS-HCl Ph, NaCl and Polyvinylpyrroidon.

3.1.1.3 Equipment

Mortar and pestle precooled to 70°C, Centrifuge capable of spinning 2-ml polypropylene tubes at 14,000 rpm at room temperature (HERMLE Labortechnik Z206 A), Water bath capable of maintaining 65°C, Fume hood, Microcentrifuge tubes and micropipettes, Thermal cycler.

3.1.1.4 Media

75 ml V8 vegetable juice, 1.5 g $CaCO_3$ (Calcium Carnonate) and 11 g Bacteriological agar.

3.1.2 Collection of GLS samples

Maize leaves exhibiting GLS symptoms were collected from four counties in Kenya namely, Kiambu, Nakuru, Meru and Tharaka Nithi (Figure 3.1). Collection of samples was conducted during the long rains of August 2018, during which 50-100 leaves were collected per site. Sampling was done in a diagonal design as described in Muller et al. (2016), to ensure consistency. The samples were collected from two distinct agro ecological zones based on altitude (Origa, 2011). These included Zone I (Subukia, Vicar and Meru 2) and Zone II (Meru 1, Kiambu and Tharaka Nithi). There were two fields for Meru (Meru 1 and Meru 2), two fields for Nakuru (Subukia and Vicar 1) and only one field for Tharaka Nithi and Kiambu. KALRO-

Ruiru is majorly for coffee but not exclusively coffee. The station had some maize fields at the time of sampling.

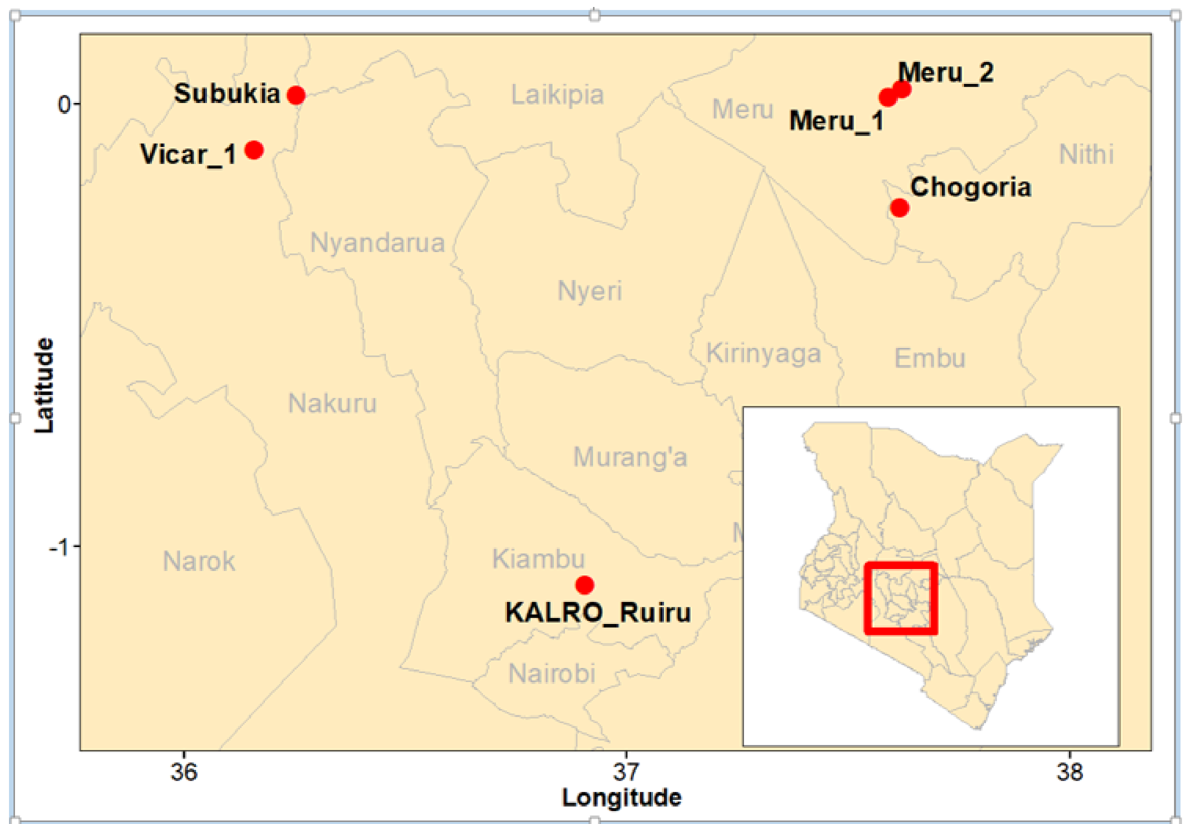


Figure 3. 1: A map illustrating the six collection sites for GLS infected maize in Kenya

3.1.3 Fungal isolations and DNA extractions

3.1.3.1 Fungal isolations

The V8 juice agar media was prepared using a mixture of 100 ml of V8 juice (Campbell soup company), 1.75g of Calcium Carbonate $CaCO_3$ (Sigma-Aldrich C4830), 10g of bacteriological agar (Sigma-Aldrich A6686), L-asparagine (Sigma-Aldrich A4159) and 500 ml Distilled water (Supelco EM3234). A flame sterilized hypodermic needle was used to pick conidia typical of *Cercospora zeina* from the lesions on the maize leaf with the aid of a dissecting microscope, which was then transferred to new V8 agar plates. Subsequent sub-culturing was conducted to produce enough mycelia for DNA extraction (Muller et al., 2016; Nsibo et al., 2019).

3.1.3.2 DNA extractions

DNA extraction was performed using the CTAB procedure of DNA extraction developed by Stewart and Via (1993). Following DNA extraction, the integrity of the gDNA was confirmed using agarose gel electrophoresis, a Nano Drop^{TM} 1000 spectrophotometer was then used to establish concentration of nucleic acid in the supernatant (Thermo Fisher Scientific Inc.). Only the samples that contained sufficient amount of nucleic acid were qualified for subsequent processes. This was then diluted to a working concentration of 30ng/µl (Korsman et al., 2012; Meisel et al., 2009).

3.1.4 PCR Analyses

From the genomic DNA, a number of PCR-based procedures were then conducted; to confirm that gray leaf spot disease is caused by *Cercospora zeina* in the four counties sampled; the CTB7 gene region, which was characterized by Swart et al. (2017) was amplified as a diagnostic test.

The CTB7 diagnostic test involved an initial denaturation step for three minutes at 95°C. This was followed by 95°C denaturation for 35 cycles with each cycle going for 30 seconds, primer annealing at 60°C for 30 seconds, 72°C extension step for 1 minute, a 72°C final extension step for 10 seconds and a rest at 25°C. The PCR products were then separated on an agarose gel and visualized under UV (Groenewald et al., 2006).

3.1.4.1 Multiplex MAT gene PCR assay

Using the genomic DNA, *C. zeina* MAT multiplex PCR was performed to establish distribution of the mating types for *C. zeina* isolates using primers previously developed by Muller et al. (2016). PCR conditions to amplify the MAT gene region included an initial three minutes denaturation step at 94°C, a 35 cycle denaturation process at 94°C for 20 seconds each, 30 seconds annealing of primers at 62°C, 40 seconds primer extension at 72°C, five minutes final extension at 72°C followed by a hold at 25°C.

Separation of the resulting PCR products was conducted in electrophoresis unit (Bio-Rad) containing 2% agarose gel in 1X Tris acetate EDTA (TAE) stained with 0.3 µl Ethidium bromide per 100ml TAE. A size standard 100 bp DNA ladder (New England Biolabs Inc; NEB #B7025) was loaded onto the first well to estimate allele size. The loaded samples were then electrophoresed at 80 Volts, 400Amp for 1 hour 45 minutes. The resulting gels were then placed under UV light in the Bio-Rad Gel Doc™ XR+ (Bio-Rad Hercules, 170-8299) and Image lab software was used to visualize the size of the fragments and the level of polymorphism (Groenewald et al., 2006).

3.1.4.2 Analysis of neutral markers

Analysis of neutral markers was then conducted i.e. SSR markers of each isolate to find out the diversity of the pathogen in the field whereby, PCR reactions with primers that flank the SSR regions in the genome of *C. zeina* were conducted.

Primers were fluorescently labelled at the 5' end with one of the dyes and pooled into either panel 1 or panel 2 based on the recommendations of Muller et al. (2016). Fluorescently labelled primer pairs were used in PCR amplification using the same conditions as described above. Product sizes were then separated using ABI capillary electrophoresis and then identified using GeneScan® software (Muller et al., 2016).

3.1.5 Gene scan analysis

Following separate amplification of the markers and their confirmation using gel electrophoresis, the PCR products were brought into either panel 1 or panel 2 (Muller et al., 2016; Nsibo et al., 2021). A 1:10 dilution series was then prepared to a total volume of 10 µl with respect to its optimal dilution concentration. A mixture of one microliter fluorescently labelled PCR product, 9.86 µl of HiDi Formamide (Thermo Fisher scientific) and 0.14 µl of Gene Scan 500LIZ dye size standard (Thermo Fisher Scientific) was prepared and added onto the 96 well plates as described by Muller et al. (2016). The aliquots were then denatured using

the thermal cycler and placed on an ABI 3500xl Genetic Analyzer (Thermo Fischer Scientific) for fragment analysis (Nsibo et al., 2021). GENEMAPPER software v4.1 (Applied Biosystems, Foster City, CA) was used to analyze the peaks and electropherograms. The scores were then captured in Microsoft excel and used for data analysis (Muller, 2014 Thesis dissertation).

3.1.6 Analysis of genetic diversity

The level of genetic diversity existing in the four counties was analyzed using a set of eleven SSR markers. Two datasets, the clone corrected and the non clone corrected were used in this analysis, whereby clone corrected data set was obtained by multilocus sequence concatenation and had only the unique haplotypes for each population while the non-clone corrected data set had all the isolates sampled in the population. Haplotypes with the same multilocus genotypes were considered clonal (Muller et al., 2016). The data set not subjected to clone correction was utilized to estimate the Stoddart and Taylor's measure of genotypic diversity (G) whereby $G=1/\Sigma p_i^2$, in which p_i is the frequency of the $_i$th genotype (Stoddart & Taylor, 1988).

The data set resulting from clone correction was used to estimate other measures of genetic diversity such as Nei's gene diversity index (H) and allelic frequency. Nei's gene diversity index for estimating the diversity of markers was assessed by calculating the gene diversity (Nei, 1978) using GenAlEx v 6.502 (Peakall & Smouse, 2012). Nei's unbiased gene diversity (Nei, 1978) was computed for each population using the formula $He=1-\Sigma x_\alpha^2$, where x_α is the frequency of the αth allele.

3.1.6.1 Population differentiation

AMOVA in GENALEX v6.502 (Peakall & Smouse, 2012) was used to statistically estimate the level of molecular variance among the isolates collected from four different geographical locations with probability values based on 999 permutations (Excoffier et al., 1992). This was supplemented with PhiPT analysis to estimate if there is population differentiation based on the geographic location from which the samples were collected (Muller et al., 2016).

Population differentiation was based on the Weir's theta (θ) statistic (Agapow & Burt, 2001). The genetic distance between isolates was computed in GENALEX v 6.502 and visualized using principal coordinate analysis (PCoA) in GENALEX v 6.502 (Peakall & Smouse, 2012).

3.1.7 Population Structure

Structure analysis was conducted to infer whether or not there was population structure and was examined using Bayesian clustering algorithm in Structure v 2.3.4 (Pritchard et al., 2000). The data were analyzed with the following parameters: 20 iterations with independent runs of K= 1 to 20 at 100,000 burn in period followed by 1,000,000 Markov Chain Monte Carlo simulations for the eleven microsatellite markers (Shrestha et al., 2017). The results obtained from STRUCTURE, were subjected to Structure Harvester to determine the most appropriate value of Delta K, using Evanno's method (Earl & vonHoldt, 2012; Evanno et al., 2005).

3.2 To determine the occurrence and role of sexual recombination

3.2.1 Gametic disequilibrium/ Analysis of recombination

The index of association (I_A) and the standardized index of association rBarD were estimated using the Multilocus v1.3b software (Agapow & Burt, 2001) with the randomization parameter set at 999. Both the index of association and the standardized index of association were used to estimate the gametic disequilibrium. Measures of association such as I_A and the rBarD were used to analyze the degree of recombination and clonality in the population.

3.3 Mapping of the Quantitative trait loci

3.3.1 Maize Genotypes

3.3.1.1 CML 511

This inbred line resulted from the crossing and selfing of CML389/CML176 and is well adapted to mid-altitude tropical conditions. It belongs to the heterotic group B. It exhibits intermediate levels of maturity (75 days to anthesis), intermediate plant height (128 cm tall). The inbred line is moderately resistant to gray leaf spot (CIMMYT, 2015).

3.3.1.2 CML 546

This maize line originated from SYNUSAB2/SYNELIB2 and is resistant to quite a number of common foliar diseases experienced in tropical conditions. It is highly resistant to maize streak virus and gray leaf spot (score of 1.3 reported) and tolerant to low nitrogen. It is moderately susceptible to *Turcicum* leaf blight (score of 3 reported) and of intermediate level of maturity (CIMMYT, 2015). The present study used a tropical× tropical germplasm that consisted of the parental and the inbred lines.

3.3.2 Field trials

3.3.2.1 Study sites and genetic material

The experiment was conducted in two geographically distinct locations; Maseno University field site and Kabianga.

3.3.2.1.1 Kabianga

A farmer's field in Kabianga was used for the field trials. Kabianga is located in Rift Valley, Kericho County, and Belgut Sub County in Kenya. The area receives mean annual precipitation of 1637.04 mm. Maximum annual temperatures recorded in the area average at 26.76^{o}C annually, minimum temperatures are about 10.67^{o}C annually with mean temperatures of 18.10^{o}C (FAO, 2018).

3.3.2.1.2 Maseno

Maseno University is situated at Latitude 0°0'0" S, Longitude 34°33'36" E. Maseno University field demonstration site is located in Kisumu county, Kisumu Town West sub county. The area receives a mean annual precipitation of 1618.44 mm with a bimodal distribution. Maseno is elevated at an altitude of 1500m above the sea level. The maximum temperatures recorded in the area average at 29.05ºC annually, minimum temperatures are about 12.57ºC annually with mean temperatures of 21.50ºC (FAO, 2018). Table 3.1 shows the sites where the field experiments were conducted, latitudes, longitudes and the season during which the experiments were conducted.

Table 3. 1: Geographical locations for the field trials

Population	Study Site	Altitude (masl)	Latitude	Longitude	Season
CML511×CML546 First Set	Kabianga	1780	0°25'24.1"S	35°07'31.7"E	March – August 2018
CML511×CML546 Second Set	Maseno university	1500	0°00'18.2"S	34°35'43.5"E	March – August 2018
CML511×CML546 Third Set	Maseno University	1503	0°00'07.0"S	34°35'41.9"E	March-August 2019

m asl; meters above the sea level.

3.3.2.2 Experimental Design

Two hundred and thirty (230) entries (228 double haploid lines and two parents) were planted in a 5×46 alpha lattice design, randomized and replicated three times at each site, according to the CIMMYT's field book (Ayala Hernández et al., 2020). Experimental plots consisted of 3 m long single rows with the rows spaced at 0.6 m apart. Adjacent plots were planted 0.75 m apart with an alley of 1.2 m at the end of each plot. Each plot was planted with 12 seeds, with 2 seeds getting planted per hill. Thinning was later conducted to one plant per hill. Border rows

of susceptible genotypes to act as spreaders of the pathogen were also planted (Galiano-Carneiro & Miedaner, 2017).

3.3.2.3 Artificial Inoculation

The population were exposed to natural infection by GLS since this was the period when higher disease pressure was experienced. To intensify disease severity on the maize plants, the plants were artificially inoculated. Maize leaves with symptoms of gray leaf spot from the previous season were collected. The leaves were ground using a grinder and stored at 4°C. 3g of the powdered material was picked and placed at the whorl of each plant at the V8 stage (Berger et al., 2014; Lehmensiek et al., 2001).

3.3.2.4 Phenotypic evaluation and Data collection

Gray leaf spot and TLB disease severity were scored on a per row basis using a 1–9 scale, as shown in table 3.2. GLS disease ratings were taken once per week for at least four weeks from when disease symptoms first appear, then the GLS mean disease score for each plot were then calculated by averaging the scores from the time points (Berger et al., 2014). All the data were collected using the CIMMYT's field book (Ayala Hernández et al., 2020). The weather data was retrieved from the Maseno University field demonstration site.

Table 3. 2: Disease severity rating scale

A		Disease severity diagnostics
1	=	No GLS or TLB lesions visible on the entire plant
2	=	Close inspection of each leaf is necessary to find lesions
3	=	Lesions are more easily seen but are majorly restricted to leaves lying below the ears
4	=	Individual lesions are just becoming visible on the ear leaf and the leaves above the ears
5	=	Lesions are more visible on the leaves above the ears, with the infections capturing <10% of the top leaves.
6	=	Lesions are more easily seen on the leaves above the ear leaf with infections covering >10% of the leaf area.
7	=	GLS and TLB lesions dominating the leaf area on all the leaves with 50% of the maize leaf surface diseased
8	=	GLS and TLB lesions prevalent on all the leaves of the maize plant with 80% of the maize leaf surface diseased
9	=	GLS and TLB lesions prevalent on all the leaves of the maize plant with the maize plant exhibiting a gray appearance with >80% of the maize leaf area diseased

A= scores on a 1-9 scale (Source: Berger et al., 2014)

Agronomic data collected included;

1. **Days to anthesis:** The number of days from planting to when 50% of the plants in a plot were shedding pollen.
2. **Days to silking:** The number of days from planting to when 50% of maize crops in a plot were showing silk.

3. **Plant height:** Ten representative plants were selected and measured from the ground level to the first branch of the tassel. Measurements were taken in centimeters.
4. **Ear height:** Ten representative plants were measured per plot from the ground level to the point of ear placement.
5. **Number of plants:** Plants on either side of the row were removed after which the remaining plants were counted per plot.
6. **Developmental stage of maize plants**: Every week the development stage of the maize plants was scored using the Purdue University "Leaf Collar" scale based on the vegetative and reproductive stage of the crops.

3.3.3 Genetic diversity among the genotypes

Based on the data recorded for the different disease severity traits and agronomic traits, hierarchical clustering was conducted using NCSS software 2021 v21.0.3. The analysis was run with unweighted pair group as the clustering method, Euclidean as the distance type and standard deviation as the scale type to construct the diversity dendogram (NCSS, 2021).

3.3.4 Genotyping

The double haploid lines were planted in the green house. Two to three weeks after seedling emergence leaf samples were collected based on the CIMMYT laboratory procedure (Warburton, 2005). The leaf samples were then sent to BeCA-ILRI for genotyping and marker identification using 1250 DArT markers.

3.3.5 Statistical Analysis of the phenotypic data

3.3.5.1 Data Analysis

META-R (Multi-Environment Trial Analysis with R for windows) version 6.0 was used to provide the calculus of best linear unbiased estimators (BLUEs) and Best linear unbiased predictions (BLUPs). In addition to BLUEs and BLUPs, META-R_v6.0 was also used to

compute the genetic correlations among all the variables and among environments, Least Significance Difference (LSD), grand mean, variance components, coefficients of variation (CV) and broad-sense heritability for all the variables. BLUPs of the GLS disease scores were used to calculate Area under disease progress curve (AUDPC).

3.3.5.2 The BLUEs and BLUPs

The BLUEs and the BLUPs were used to calculate the adjusted means for disease severity scores for TLB and GLS, plant height, ear height, days to silking, days to anthesis and the area under the disease progress curve which were the response variables. In the parameter setting window, alpha lattice was set as the experimental design. In the variable selection panel, the columns in the input files were selected to be the factor names with environment, replicate, block and genotype as the independent variables. Management was set as the grouping factor. BLUEs and BLUPs of each line was used for all traits to minimize the effect of variation attributable to environmental factors (Ding et al., 2015).

For analysis of individual environments using a lattice design, the model below was used to calculate the BLUEs and BLUPs and to estimate the variance components.

Equation 1: To estimate the variance components with covariate

$$Y_{ijkl} = \mu + Env_i + Rep_j(Env_i) + Block_k(Env_i\ Rep_j) + Gen_l + Env_i \times Gen_l + Cov + \varepsilon_{ijkl}$$

Equation 2: To estimate the variance components without covariate

$$Y_{ijkl} = \mu + Env_i + Rep_j(Env_i) + Block_k(Env_i\ Rep_j) + Gen_l + Env_i \times Gen_l + \varepsilon_{ijkl}$$

From the above equation Y_{ijkl} represents performance of the trait of interest, μ corresponds to the all-inclusive mean, Env_i the effect of the ith environment, $Rep_j(Env_i)$ the effect of the ith environment within the jth replication, $Block_k(Env_i\ Rep_j)$ represents the effect of the jth incomplete block within the ith environment and the jth replication, Gen_l the effect of the lth genotype, $Env_i \times Gen_l$ the environment by genotype interaction, Cov the covariate effect and ε_{ijk} is the error variance (http://hdl.handie.net/11529/10201).

When calculating the best linear unbiased estimates (BLUEs), genotypes and covariates were considered as fixed effects of the model while other terms were included as random effects of the model. The covariate was considered as the fixed effect of the model while all other terms were included in the random effects of the model to estimate the best linear unbiased predictions (BLUPs).

3.3.5.3 Broad-sense heritability

Heritability for the different traits was calculated as the ratio of the estimated genotypic variance to the estimated phenotypic variance (Knapp et al., 1985). Broad sense heritability of a specific trait at an individual location was estimated using the formula below:

Equation 3: Estimating the broad sense heritability.

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{ge}^2/\text{nEnvs} + \sigma_e^2/(\text{nEnvs} \times \text{nreps})},$$

Where σ^2_g corresponds to the genotypic variance, σ^2_e the error variance, σ^2_{ge} variance attributed to the genotype by environment interaction, nreps the number of replications, nEnvs the number of environments where the study was conducted (Alvarado et al., 2020). For estimating broad-sense heritability, all terms were considered random, except the covariate.

3.3.5.4 Genetic correlations among environments

META-R statistical software was used to estimate the genetic correlations between variables and environments (Alvarado et al., 2020). The following equation from Cooper et al. (1996) was used to estimate the genetic correlations among environments.

Equation 4: To estimate the genetic correlations among environments

$$\rho_{g(jj')} = \frac{\rho_{p(jj')}}{h_j h_{j'}},$$

From the above equation $\rho_{g(jj')}$ corresponds to the phenotypic correlation between environments j and j′; $h_j h_{j'}$ the heritabilities at environments j and j′ respectively.

3.3.5.5 Genetic correlations between variables

The genetic correlation between traits/variables was determined using the following formula;

Equation 5: To estimate genetic correlation between traits.

$$\rho_g = \frac{\overline{\sigma_{g(jj')}}}{\overline{\sigma_{g(j)}\sigma_{g(j')}}},$$

From the above genetic correlation equation, $\sigma_{g(jj')}$ represents the arithmetic mean for all pairs of genotypic covariance accumulated from between trait j and j'. $\sigma_{g(j)}\sigma_{g(j')}$ corresponds to the arithmetic average for all the pairs of geometric means originating from among the genotypic variance components of the variables.

3.3.5.6 Least significance difference

The least significance difference at 5% level of significance was estimated using the following formula;

Equation 6: To estimate the least significance difference.

$$\mathrm{LSD} = t_{(1-0.05, df_{error})} \times \mathrm{ASED},$$

Where

t = is the aggregate Student's t distribution

0.05 = alpha selected level of significance to 5%

df_{error} = is the degrees of freedom for variance of error

SD = is the standard deviation

3.3.5.7 Coefficient of variation

The coefficient of variation was determined using the formula below;

Equation 7: To estimate the coefficient of variation

$$\mathrm{CV} = 100 \times \frac{\mathrm{SD}}{\text{Grand mean}}.$$

3.3.5.8 Descriptive statistics

From the disease scores for GLS and TLB, the area under the disease progress curve was estimated. The frequency histogram of disease severity scores for GLS and TLB was also drawn to show the distribution of the scores within the population.

The genetic variance and heritability for days to anthesis, days to silking, ear height, plant height, GLS scores and TLB scores in the mapping population were estimated using the ANOVA/AOV functionality in QTL IciMapping software (http://www.isbreeding.net).

3.3.6 Construction of the genetic linkage map

The genetic linkage maps were constructed using the MAP functionality of QTL IciMapping v.4.1 (http:// www.isbreeding.net) where mapping QTL was conducted across the three locations within each population (Almeida et al., 2013).

Preparing input files for linkage map construction; In the parameter setting window for general information, three was set as the population type because the study used a double haploid population. Kosambi was set as the mapping function, marker position as the marker space type, 500 as the number of markers and 230 as the size of the mapping population. The general information category also comprised of genotypic data from DArTseq genotyping. The scores for all the SNP markers and polymorphic DArTseq markers were transformed into genotype codes ("A," "B") in accordance to the scores of the parents (Akbari et al., 2006; Barilli et al., 2018). Anchor information for all markers was also included in the input file.

Three steps were followed in linkage map construction; grouping, ordering and rippling. Logarithm of odds score was set at 3.00 for grouping. Ordering was performed using the ordering instruction with the nnTwo Opt algorithm. Sum of adjacent recombination frequencies (SARF) as the criterion and window size of 5 as the amplitude was used to ripple the marker sequence and to fine tune the chromosome orders. All the outputting functionalities were checked and the map was drawn using the MAP functionality (Meng et al., 2015).

3.3.7 QTL analysis

Quantitative trait loci for the different traits were detected using the BIP functionality of QTL IciMapping v.4.1 (Meng et al., 2015) based on the ICIM. In the parameter setting window for general information, one was set as the indicator, seven as the mapping population type as this was a double haploid population, kosambi as the mapping function, CentiMorgan as the unit for the marker space, 10 as the number of chromosomes and 230 as the size of the mapping population.

The input file consisted of the general information, the chromosome information (how many markers are available in each chromosome), the definition of each chromosome or linkage group, the marker type or genotype data and the phenotypic data (Meng et al., 2015). The linkage map used in this functionality was from the MAP linkage map construction. The best linear unbiased predictions (BLUPs) for the different traits were used as the input files for QTL identification across the different environments (Littell et al., 2007).

In the parameter setting window two methods were selected for QTL analysis namely, SMA: single marker analysis and ICIM-ADD: Inclusive composite interval mapping of additive and dominant QTL. Deletion was selected for the missing phenotype. An LOD threshold of 2.5 and 1000 permutations at $\alpha = 0.01$ were set to declare the significant QTL. Following selection of the mapping methods and setting of the parameters, the start button was then clicked to run the analysis. The results were displayed on the project window (Meng et al., 2015). The percentage of total phenotypic variance explained by individual QTLs was determined using stepwise regression.

3.3.7.1 QTL comparison

To ascertain the actual locations of the QTLs for all the traits on the chromosomes, the physical position of the identified QTLs were assigned to chromosomal bins available at the maize

genetics and genomics database (http://www.maizegdb.org/data_center/map) as described by Berger et al. (2014).

CHAPTER FOUR
RESULTS

4.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya

4.1.1 The outcome of single spore isolations

Samples collected from different locations in Kenya and successfully isolated were as indicated in Table 4.1. The isolates were obtained from Meru (n = 50), KALRO-Ruiru (n = 43), Tharaka-Nithi (n = 12) and Nakuru (n = 11) counties in Kenya and were collected during the long rainy season in August 2018.

Table 4. 1: The number of successful isolates retrieved from each geographical region

Location	County	Altitude (m asl)	Latitude	Longitude	Number of isolates
Subukia	Nakuru	2070	0°0'36"N	36°15'19"E	9
Vicar 1	Nakuru	2170	0°6'53"S	36°9'35"E	2
Chogoria	Tharaka-Nithi	1630	0°14'43"S	37°37'5"E	12
Meru_1	Meru	1770	0°1'25"N	37°37'17"E	50
Meru_2	Meru	1960	0°0'17"N	37°35'25"E	26
KALRO Ruiru	Kiambu	1600	1°5'55"S	36°54'22"E	43

Source: Google Maps 2019

4.1.2 Identification of the species associated with gray leaf spot

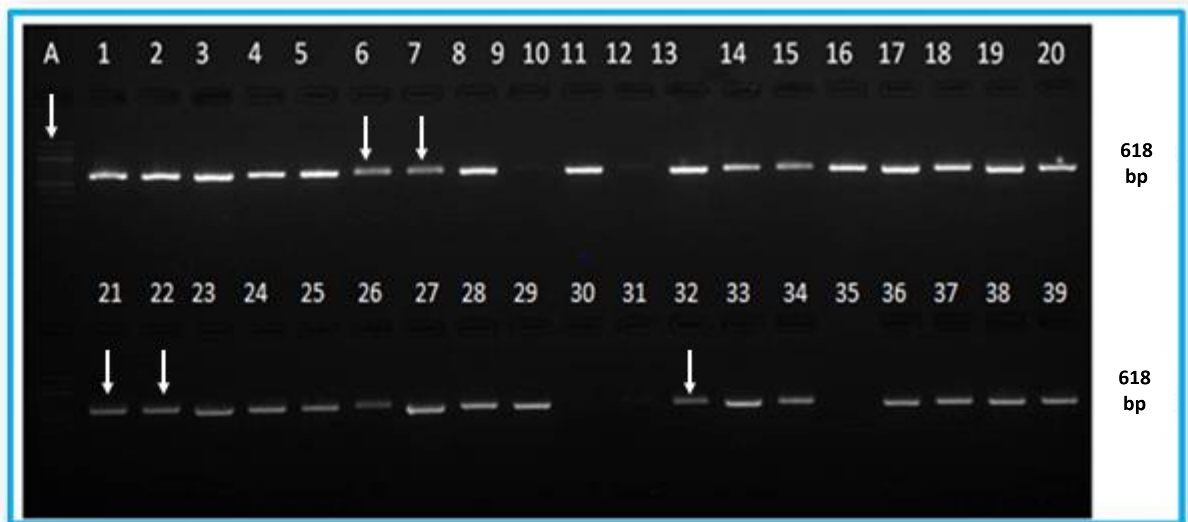


Figure 4. 1: Appearance of single plex diagnostic PCR for CTB7 gene region

39 representative isolates were visualized on the gel. Lane A represents 100 bp DNA ladder (New England Biolabs) used for sizing, Lane 1 represents CMW 25467 (positive control) and Lane 2-39 represents other *Cercospora zeina* isolates. Bands with the arrow above could be seen in the original image but faint in this image.

In the course of the present investigation, 129 isolates were used to determine the genetic diversity of *Cercospora zeina* in the four counties of Kenya as shown in table 4.1. Following amplification of the CTB7 diagnostic gene region with CMW 25467 as the positive control, all the isolates analyzed produced DNA products of 618 base pairs (bp) in length identical to the CMW 25467 strain of *C. zeina* (Figure 4.1). This confirmed the identity of all the isolates collected and analyzed in this study as *Cercospora zeina*. Figure 4.1 illustrates the appearance of the amplification profiles of *Cercospora zeina* subjected to a 2% agarose gel electrophoresis and visualized in the Bio-Rad Gel DocTM XR+ using the Image lab software. One hundred and seven isolates (82%) were successfully amplified on the 2% agarose gel and produced clear bands. All the *C. zeina* isolates investigated yielded amplicons of the expected size. There was no band for the negative control PCR of *C. zeina* (Figure 4.1).

Table 4. 2: Gene diversity reported for the SSR markers and the sequence information

Name of the locus	Primer sequence	Amplicon size (bp)	Panel	Recurrent alleles among locations (bp)	No. of alleles per locus	Nei's unbiased gene diversity
CzSSR01	F: AATTAATCGTAAGCACGACGA R: CTCCCTCCACAACCACAACT		2	155, 158	6	0.486
CzSSR04	F: GGTTAGCGTGTAGCCGAGTT R: CGACCAAGTGCTTGTCAAC		2	462	8	0.413
CzSSR05	F: CTTCGACTACGTTGCGTTGA R: AGCCCTTGACAGCACTGACT		1	242, 245	6	0.668
CzSSR06	F: CAGAAAGAAGGCACCAAAGC R: GAGCAGGTTTAGTCGGAGGA		2	223	4	0.329
CzSSR07	F: CAAGAATGCCAATGATGCTG R: GTCTCCTTTCTGGCGAAGTG		2	200	10	0.677
CzSSR08	F: GTAACTCCGCGAGATTCCTG R: AGCAGCAGCAGCAGTAACAA		2	196, 199	5	0.551
CzSSR10	F: GCGTTACTTCGAAGGTGCTT R: GTTGGTCGTTTGTTTTGTCCT		1	175	7	0.336
CzSSR12	F: GAAGGCTTTTCTCTCGCAAA R: TTGTCCCTCGGTCGCTTAT		1	240, 244	9	0.576
CzSSR13	F: GAGAGATAGTTGCGGCGT R: GATGATGATTTGAGGAGTGTTG		1	329	2	0.023
CzSSR15	F: CATTCTTTGTCCGCGTTC R: CACTCACTTCCCACATAC		2	245, 251	7	0.457
CzSSR18	F: ATGCGTCAAAATCACACTTTC R: AAAGCGTCTCCTCATCGATAC		1	134, 137	4	0.504
CzMAT1-1	F: TCACCCTTTCACCGTACCCA R: CACCTGCCATCCCATCATCTC	631				
CzMAT1-2	F: CGATGTCACGGAGGACCTGA R: GTGGAGGTCGAGACGGTAGA	409				
CzCTB7del	F: AAGAGTGCTTGTGAATGG R: GATGCGGGTGAAGTAGAAA	618				

The specific primers for the SSR markers were previously designed by Muller et al. (2016), mating type primers designed by Groenewald et al. (2006) and *CTB7* primers designed by Swart et al. (2017). There were no reported amplicon size for the SSR markers

Table 4. 3: Indicators of genetic diversity for *Cercospora zeina* isolates

Location	N[a]	MLG[b]	P_a[c]	% P[d]	CF (%)[e]	I[f]		H_e[g]	
						NCC[h]	CC[i]	NCC[h]	CC[i]
Tharaka Nithi	8	8	2	82	0.00	0.670	0.670	0.461	0.461
Kiambu	34	25	12	91	0.27	0.638	0.688	0.341	0.370
Meru	76	63	13	100	0.17	0.795	0.824	0.456	0.474
Nakuru	11	11	4	91	0.00	0.870	0.870	0.521	0.521
Total	**129**	**107**	**31**		**0.17**	**0.763**			

[a] The sum total of isolates collected per county.
[b] The sum total of multilocus genotypes observed per county.
[c] Sum total of private alleles per locus.
[d] Per-cent polymorphism.
[e] Clonal fraction estimated by the formula CF=1 – (total of unique genotypes/sum total of isolates).
[f] Shannon-Wiener index of multilocus genotypic diversity.
[g] Nei's unbiased measure of gene diversity (Nei, 1978).
[h] The data set before clone correction.
[i] The data set following clone correction.

4.1.3 Genotyping of the SSR markers

All the 11 microsatellite markers used in the present study to evaluate the diversity of *C. zeina* were polymorphic with a mean of 6.18 alleles identified per locus (Table 4.2). This study identified CzSSR07 as the most polymorphic locus harboring 10 alleles, while the least frequent locus was CzSSR13 with two alleles. A total of 68 alleles were obtained from the analysis of 11 SSR loci (Table 4.2). Nei's unbiased gene diversity spanned between 0.023 (CzSSR13) and 0.677 (CzSSR07). The evenness of alleles at each locus fluctuated between 0.63 and 1.0, with an average evenness of 0.83.

4.1.4 Genetic diversity of *Cercospora zeina* in the four counties of Kenya

Manual scoring of peaks using the GENEMAPPER software v4.1 revealed a total number of 68 alleles from the 129 isolates of *C. zeina* collected from four counties in Kenya namely, Kiambu, Meru, Nakuru and Tharaka Nithi. The number of alleles per locus ranged from two to ten with an average of six alleles per SSR marker. CzSSR07 exhibited the highest number of alleles at ten while CzSSR13 exhibited the lowest number of effective alleles at two (Figure 4.2).

Among the 129 *Cercospora zeina* isolates examined, 107 distinct haplotypes were detected as shown in Table 4.3. Clone correction of the 129 *Cercospora zeina* isolates led to the discovery of 107 multilocus genotypes with a clonal fraction of 0.17 (Table 4.3). Twenty five unique haplotypes were identified in Kiambu county and 63 haplotypes were identified in Meru County. The other two counties Tharaka Nithi (Chogoria) and Nakuru did not show any unique haplotypes. There were no shared haplotypes between the four different counties in Kenya. This could be due to lack of movement of haplotypes between different geographical locations. Nei's unbiased gene diversity for all SSR markers was comparatively high averaging at 0.45 while Shannon-Wiener index was also high averaging at 0.74. The level of polymorphism was

significantly high for all the isolates with Meru exhibiting percentage polymorphism of 100% (Table 4.3).

Nei's unbiased gene diversity (Nei, 1978) estimates was within the range of 0.341 to 0.521 for the non clone corrected data set with a mean value of 0.445, while for the clone corrected data Nei's gene diversity fluctuated between 0.370 and 0.521 with a mean value of 0.456. This reveals a high level of gene diversity within all the four geographical locations in Kenya. Nakuru population exhibited the highest gene diversity (h= 0.521) while Kiambu population was characterized by the lowest gene diversity (h=0.370) both for clone corrected and non-clone corrected data sets.

The Shannon-Wiener index of genotypic diversity was significantly high for both clone corrected and non-clone corrected data sets. The non clone corrected data set had a Shannon Wiener index of (I= 0.743) while the clone corrected data had a Shannon Wiener index of (I=0.763). Population wise, Nakuru had the highest Shannon-Wiener index (I = 0.870) and Kiambu had the lowest index (I = 0.638).

Table 4. 4: Private alleles observed in the four geographical locations in Kenya

Marker	Alleles restricted			
	Tharaka Nithi	Kiambu	Meru	Nakuru
CzSSR01		161	137	119
CzSSR04		366	450	
CzSSR05				251, 260
CzSSR06		218, 220		
CzSSR07	227	242	194, 248,	
CzSSR08	202	205		
CzSSR10		172, 244	169, 194	178
CzSSR12			214, 217, 236,256	
CzSSR13			349	
CzSSR15		158, 176, 194, 200		
CzSSR18			119, 131	

Private alleles are unique alleles only found in the specific geographic regions.

The *C. zeina* isolates collected from Kenya exhibited a number of private alleles for the different microsatellite markers CzSSR01, CzSSR04, CzSSR05, CzSSR06, CzSSR07, CzSSR08, CzSSR10, CzSSR12, CzSSR13, CzSSR15, CzSSR18 (Table 4.4). The distribution of the microsatellite markers was also restricted to the different geographical locations as indicated in Table 4.4. The Meru population had the highest number of private alleles and Tharaka Nithi population had the lowest number of private alleles. The presence of private alleles indicates relatively low rates of migration between populations.

4.1.5 Population genetic differentiation, gene and genotypic flow

Analysis of molecular variance (AMOVA) results for the *Cercospora zeina* SSR markers revealed that sources of variation from within and among the counties contributed to the genetic variance (Table 4.5).

Table 4. 5: Analysis of molecular variance (AMOVA) for the isolates

Source of variation	df[a]	SS[b]	MS[c]	Eσ[d]	Per-cent of the total variance	PhiPT (p-value)[e] NCC	PhiPT (p-value)[f] CC	N_m[g] NCC[i]	N_m[h] CC[j]
Among Populations	3	28	9.23	0.32	12	0.15 (0.001)	0.12 (0.001)	2.83	3.85
Within Populations	103	257	2.49	2.49	88				
Total	106	285		2.82	100				

[a] df: degrees of freedom

[b] SS: Sum of squares for the observations made

[c] MS: Mean of squares for the observations made

[d] Eσ: Estimated variance

[e] PhiPT: A measure of the population differentiation for the non-clone corrected data set.

[f] PhiPT: A measure of the population differentiation within the data set following clone correction

[g] N_m: An estimate of gene flow between different geographical locations for the non-clone corrected data set

[h] N_m: An estimate of gene flow between different geographical locations for the clone corrected data set.

[i] NCC: Non clone corrected data set.

[j] CC: Clone corrected data set.

Results of the analysis of molecular variance show that significant genetic diversity was distributed within populations as indicated in Table 4.5. Variations within counties contributed 85% while among counties contributed 15% of the total variation for the non-clone corrected dataset. For clone corrected data set within population source of variation contributed to 88% of the variation while among populations contributed 12% of the total variation. The results from AMOVA point towards a partial population differentiation among counties. This is consistent with the significant values for population differentiation obtained for both clone corrected (PhiPT=0.12, p = 0.001) and non-clone corrected data sets (PhiPT = 0.15, p= 0.001) (Peakall & Smouse, 2012).

The gene flow (Nm) values estimated for the clone corrected data set and non-clone corrected data sets were Nm = 3.85 and Nm= 2.83 respectively (Table 4.5). These estimates evaluated the level of gene flow between Tharaka Nithi (Chogoria), Meru, Kiambu and Nakuru counties. These values were relatively high. These high estimates of gene flow among the four counties in Kenya, indicate that there could be slight exchange of genetic material between different populations. From these observations it is evident that the population of *Cercospora zeina* across the four counties exhibits partial genetic differentiation.

4.1.6 Population structure

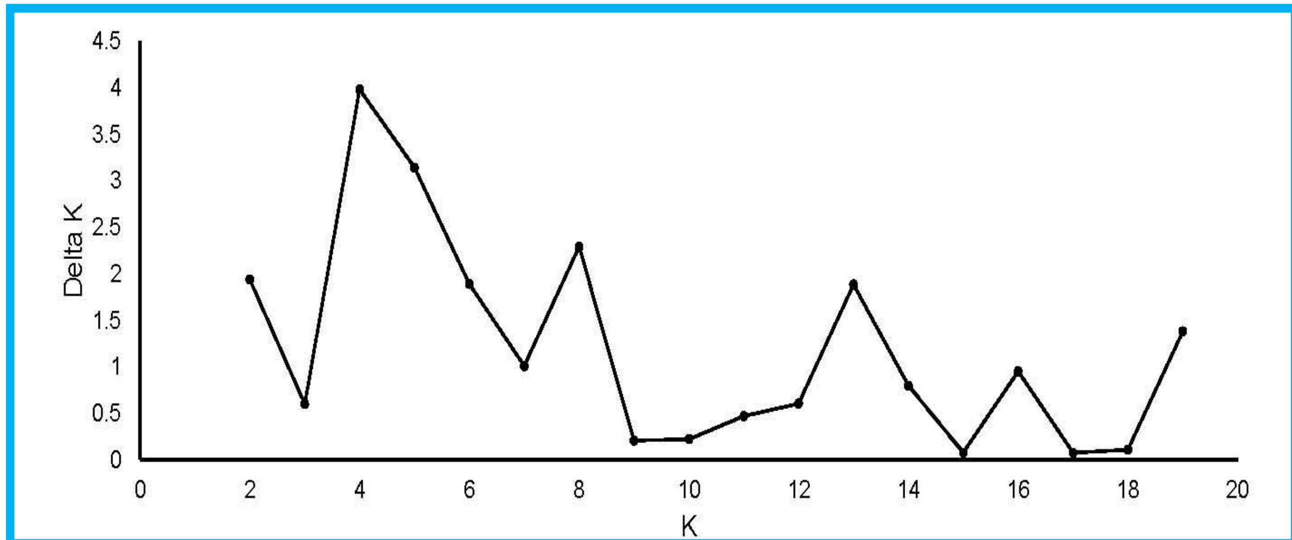


Figure 4. 2: The Delta K graph

The maximum peak of Delta K (K = 4) indicates the most likely number of populations for *Cercospora zeina*.

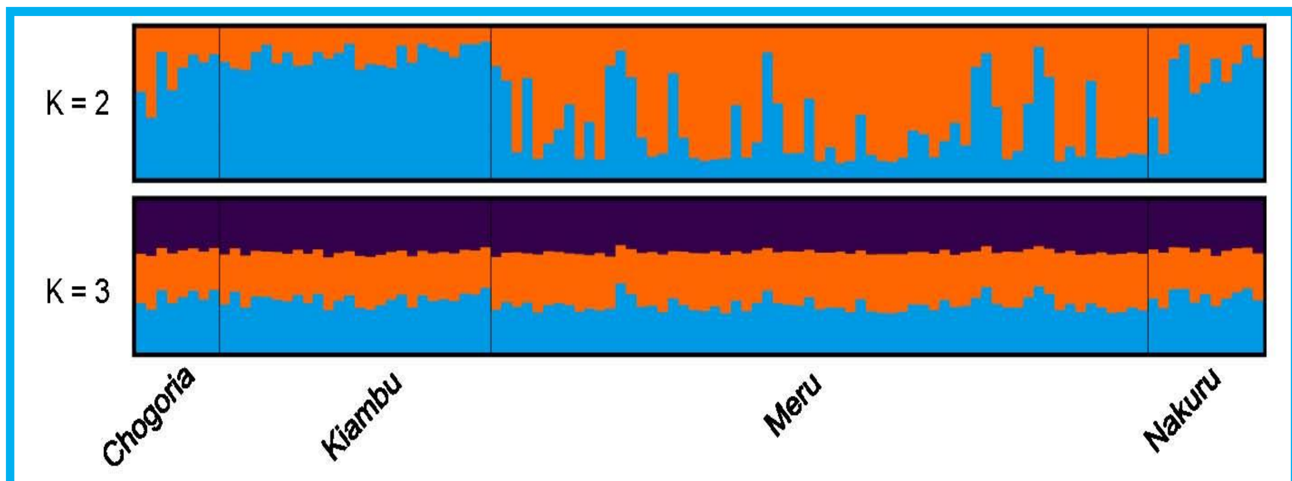


Figure 4. 3: Bar graphs from the STRUCTURE analysis

Each vertical line represents one multilocus genotype (MLG) and the black vertical line separate subgroups of individuals. There is partial population structure as indicated by the bar graphs.

A sampling population was defined as a set of isolates collected from a single geographical location in one season (Human et al., 2016). Population structure of the clone corrected data set was investigated using the software STRUCTURE v2.3.4 based on the Bayesian clustering

algorithm using the 11 microsatellite markers scored. STRUCTURE analysis successfully identified the existence of partial population structure within the *C. zeina* isolates collected from Kenya. As revealed by the STRUCTURE analysis, there was a clear peak at K= 4 on the Delta K graph as shown in Figure 4.2. This predicted four as the most probable value of delta K. In addition peaks were also reported at K = 2, K = 8, K = 13, K = 16 and K = 19. The individual populations were assigned to four different clusters, based on the four different geographical locations. Some level of admixture is also reported in the Kenyan population as the isolates had membership in all the clusters.

Significant population structure was evident among the different counties (Meru, Nakuru, Kiambu and Tharaka Nithi) and the isolates could be differentiated into the four distinct clusters as shown in figure 4.3. Based on the fact that there were four distinct genetic clusters within the data set, it can be inferred that there is evidence for partial population structure within the Kenyan population of *Cercospora zeina*. This is probably due to slight migration between different geographical locations. The results presented here are based on 20 iterations with K ranging from 1 to 20, runs of 1,000,000 Markov chain Monte Carlo following a burn in period of 10,000 iterations.

4.1.7 Principal co-ordinate analysis

The level of genetic similarity among the samples from different counties was visualized using principal co-ordinates analysis (PCoA). Principal coordinates analysis (PCoA) plot for the isolates of *Cercospora zeina* from Kenya exhibited partial population differentiation. Such partial population structure was particularly important for the counties of Meru and Kiambu that exhibited significant population differentiation into distinct clusters on the PCoA plot (Figure 4.4).

As evident in the PCoA plot, isolates from the same geographical location were mostly clustered together especially for Meru and Kiambu isolates. Tharaka Nithi (Chogoria) and

Nakuru however, did not show separate clustering according to the different counties as exhibited in the PCoA plot (Figure 4.4).

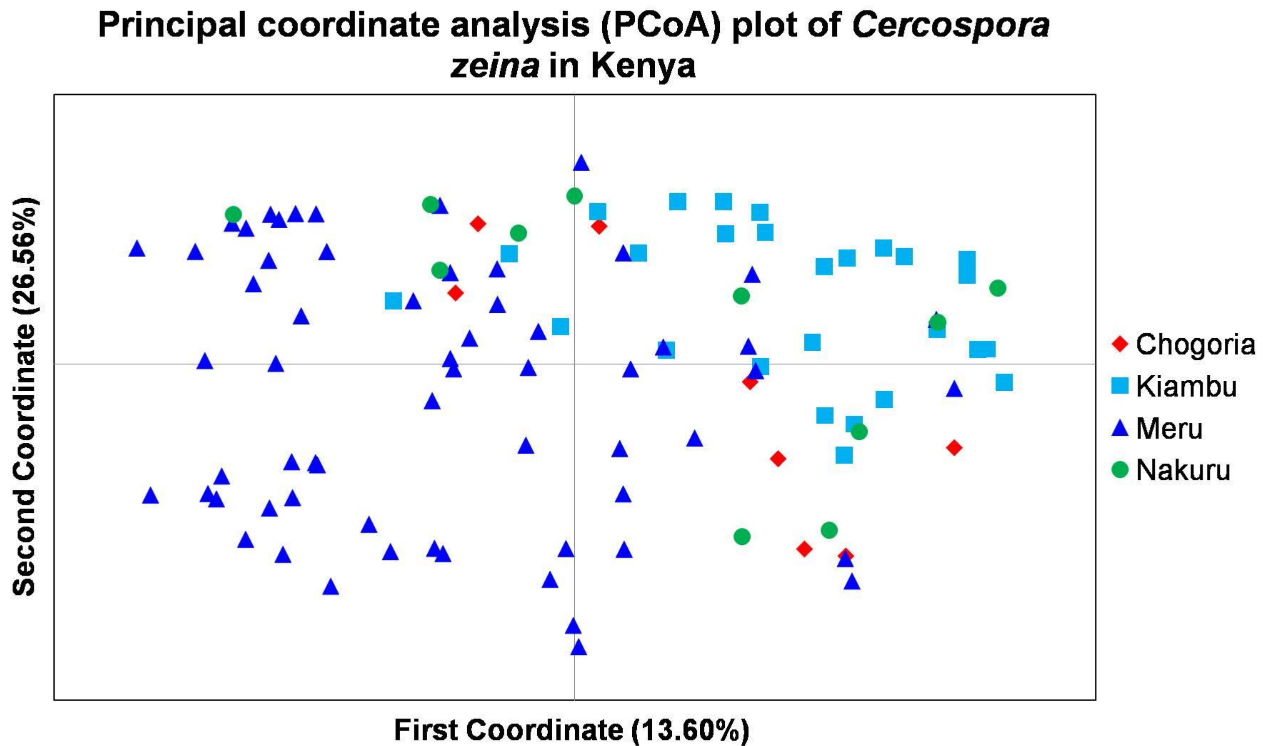


Figure 4. 4: Principal coordinate analysis (PCoA) plot

[1]PCoA plot illustrates the occurrence of partial population differentiation among haplotypes of *Cercospora zeina* collected from different geographical regions in Kenya. The first coordinate expresses 13.6% of the total variation, while the second coordinate expresses 26.56% of the total variation.

From the principal co-ordinates analysis, it can be inferred that the number of genetic clusters is equal to four (Figure 4.4). PCoA analysis provides a confirmation of the results obtained by STRUCTURE and AMOVA indicating that *C. zeina* from maize in Kenya highlands exhibits partial population differentiation. To a greater extent isolates from Meru clustered separately, occupying their own space matrix and slightly mixing with isolates from Chogoria (Tharaka Nithi), Nakuru and Kiambu. Isolates from Tharaka Nithi (Chogoria) county were ordinated closer to the isolates from Nakuru and Kiambu counties indicating more similarity. The occurrence of *Cercospora zeina* isolates from Meru in clusters where the Chogoria (Tharaka Nithi) and Kiambu isolates exist suggests some level of gene flow of the pathogen across different counties. The results from this particular study suggest that there was slight exchange

of genetic material between geographical locations. It is important to note that the structuring of the population is not that clear thus the four clusters inferred are not discrete.

4.2 Occurrence of Sexual Recombination

4.2.1 Distribution of mating type genes among *Cercospora zeina* isolates

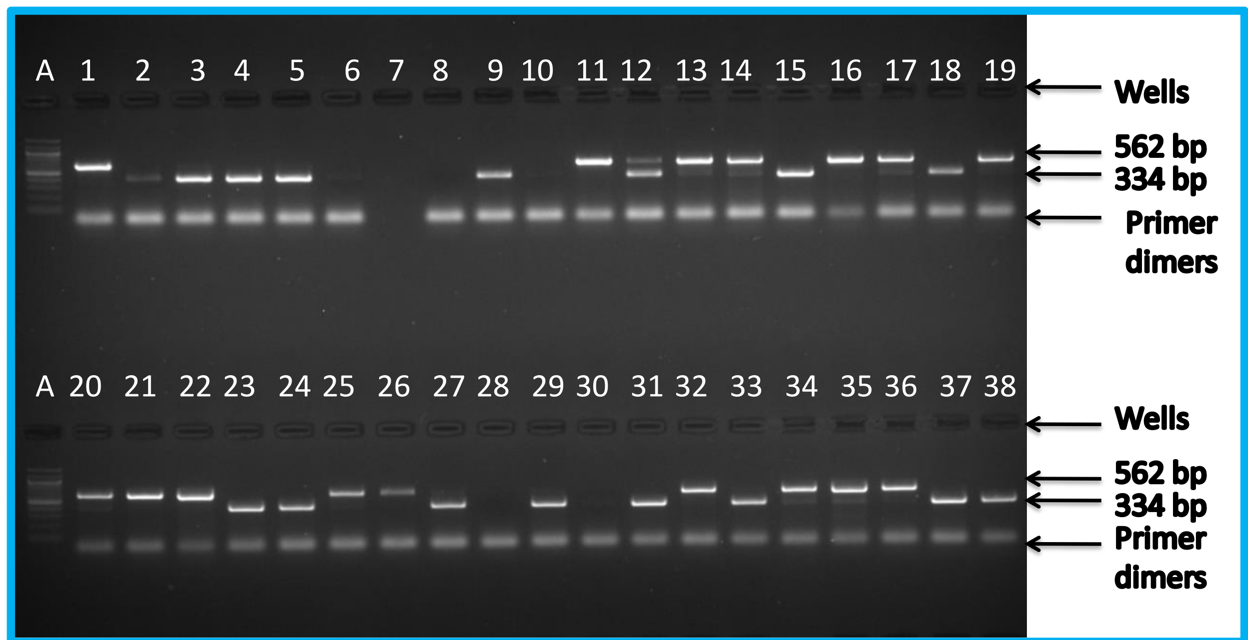


Figure 4. 5: Gel image of *Cercospora zeina* mating type genes

[1]Lane A represents 100 bp DNA ladder (New England Biolabs), Lane 1 is the amplicon for the positive control (CMW 25467). Lane 11, 13, 14, 16, 17, 19, 20, 21, 22, 25, 26, 32, 34, 35, 36 indicate isolates that harbor MAT 1-1-1 (562bp) and Lanes 2, 3, 4, 5, 6, 9, 10, 12, 15, 18, 23, 24, 27, 29, 30, 31, 33, 37, 38 indicate isolates that possess MAT 1-2-1 (334bp).

The mating type genes were successfully amplified using the *C. zeina* diagnostic mating type primers indicated in table 4.2 and showed either the MAT 1-1-1 or MAT 1-2-1 amplicons on the gel. A total of 129 isolates were assessed using this multiplex MAT PCR assay. Each produced a single amplicon matching a PCR product of either 562 bp (MAT1-1-1) or 334 bp (MAT1-2-1) as displayed in figure 4.5. Both MAT idiomorphs were present in all the four geographic locations. Among the 129 isolates of *C. zeina* from Kenya, there were 62 out of 129 isolates with the MAT1-1 idiomorph and 52 out of 129 isolates with the MAT 1-2 idiomorph (Table 4.6). About 15 isolates were not successfully amplified and could not be visualized on the gel (Appendix A). In general the Kenyan population exhibited a frequency that does not significantly deviate from the expected 1:1 ratio of MAT1:1 to MAT1:2 (χ^2=0.877). Chi- square test was performed for the individual locations to determine if the gene ratios significantly deviated from the expected 1:1 ratio of MAT idiomorphs within the

locations that are randomly mating. A look at the individual locations, Tharaka Nithi (χ^2=1.00), Meru (χ^2=0.24), Kiambu (χ^2=0.57) and Nakuru (χ^2=0.4) revealed that these populations did not significantly deviate from the expected 1:1 ratio of the two MAT idiomorphs (Table 4.6). Therefore based on the chi-square results, there is failure to reject the null hypothesis stating that the populations do not significantly deviate from the expected 1:1 ratio. The hypothesis is directly linked to the occurrence of sexual recombination.

4.2.2 Linkage disequilibrium

Table 4. 6: Actual frequency distribution of mating types

Location	**N**[a]	**MAT1**	**MAT2**	**Ratio**[b]	χ^{2c}	I_A[d]	**rBarD**[e]
						CC[g]	**CC**[g]
Tharaka Nithi	8	4	4	1.0	1.00 (0.32)	0.43 (0.079)	0.05 (0.079)
Meru	76	36	32	1.1	0.24 (0.35)	0.22 (0.001)	0.03 (0.001)
Kiambu	34	16	12	1.3	0.57 (0.50)	0.06 (0.338)	0.08 (0.338)
Nakuru	11	6	4	1.5	0.4 (0.47)	0.29 (0.102)	0.04 (0.102)
Subtotal	129	62	52	1.19	0.877		

[a] Actual number of isolates per geographical location (non clone corrected).

[b] Ratio of mating type 1-1: mating type 1-2 isolates

[c] χ^2 value calculated for the deviation from the expected 1:1 ratio at 95% level of significance.

[d] I_A: Index of association for $p > 0.05$.

[e] rBarD: Standardized index of association for $p > 0.05$ as calculated in Multilocus.

[g] clone corrected data set.

The microsatellite data revealed that only the Meru population exhibited significant linkage disequilibrium for the clone corrected data sets (Table 4.6). Therefore the null hypothesis of no linkage disequilibrium (at p >0,001) was rejected in Meru (rBarD =0.03, p<0.001001). The rest of the population from individual geographical locations such as Tharaka Nithi (rBarD =0.05, p=0.079), Kiambu (rBarD = 0.08, p=0.338) and Nakuru (rBarD = 0.04 p= 0.102) exhibited no linkage disequilibrium, hence failure to reject the null hypothesis of no linkage disequilibrium.

4.3 Mapping the Quantitative trait loci

4.3.1 Phenotypic data

Phenotyping for GLS and TLB resistance was conducted in three environments (Maseno 2018, Kabianga 2018 and Maseno 2019). Typical cigar-shaped tan to gray lesions were widely observed in the three environments for *Turcicum* leaf blight while gray to tan and sharply rectangular lesions characteristic of GLS was observed for lines susceptible to GLS infections. Though GLS pressure was not as high as TLB and most symptoms became visible after anthesis. The lower leaves were subjected to more infections than the upper leaves (Figure 4.6).

Figure 4. 6: Occurrence of disease lesions on the maize leaves

4.3.1.1 Disease severity data

The mean disease severity scores for GLS were 1.5, 3.38 and 2.16 in Kabianga long rains 2018, Maseno long rains 2018 and Maseno long rains 2019 respectively, while the mean disease severity scores for TLB were 4.45, 5 and 4.28 in Kabianga 2018, Maseno 2018 and Maseno 2019 respectively. The average area under the disease progress curve (AUDPC) for GLS were 39.9, 70 and 47.12 in Kabianga 2018, Maseno 2018 and Maseno 2019 respectively. The average AUDPC for TLB were 118.74, 121.39 and 85.34 in Kabianga 2018, Maseno 2018 and Maseno 2019 in that order. For both GLS and TLB, the resistant inbred lines had relatively lower AUDPC.

The R5 stage (kernels are denting) marked the peak for TLB using the rating scale of Purdue (http://extension.entm.purdue.edu/fieldcropsipm/corn-stages.php). This corresponded to approximately 115 days after planting in Maseno and approximately 129 days after planting in Kabianga.

In Kabianga CML511 had an average score of 2.5 at the final score while CML546 had an average score of 2.8 at the final average disease severity score for gray leaf spot. In Maseno 2018 CML511 had an average score of 3.08 at the final score then CML546 had an average score of 5.99 at the final score for GLS disease severity (Appendix B). In Maseno 2019 CML511 had an average score of 2.30 at the final score then CML546 had an average score of 3.47 at the final score (Appendix B). This evaluations show that CML511 is slightly more resistant than CML546 to GLS and CML546 is moderately susceptible to GLS.

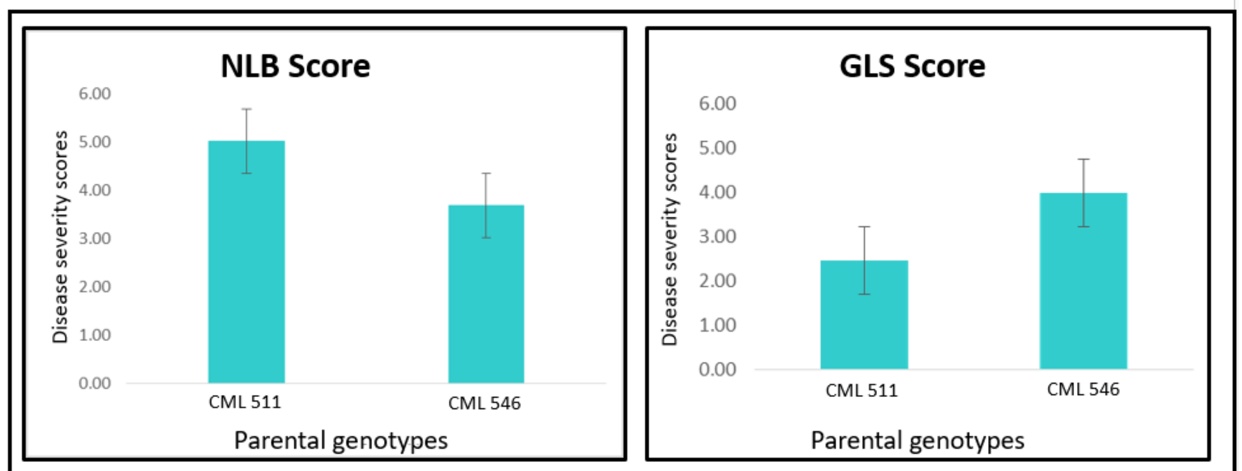


Figure 4. 7: Distribution of disease severity scores for the two parental genotypes

In Kabianga CML511 had an average score of 4.69 at the final score while CML546 had an average score of 3.85 at the final average score for TLB disease severity (Appendix B). In Maseno 2018 CML511 had an average score of 5.01 at the final score while CML546 had an average score of 4.13 at the final score. In Maseno 2019 CML511 had an average score of 5.11 at the final score while CML546 had an average score of 3.93 at the final score. This shows that CML546 is slightly more resistant than CML511 to TLB and CML511 is moderately

susceptible to TLB, the results have been demonstrated in Figure 4.7. The breeder's attributes for the two parental genotypes are presented in the materials section.

There was a great variability in disease scores for all the 230 genotypes evaluated. For gray leaf spot, the scores ranged between 1.03 and 6.9 while for Turcicum leaf blight, the scores ranged between 2.48 and 8 based on best linear unbiased predictions. A large portion of the double haploid population were extensively blighted by TLB (Figure 4.6). There was evidence supporting transgressive segregation in the population for GLS BLUPs, TLB BLUPs and DTA BLUPs, as some of the genotypes were more resistant or susceptible compared to the parental lines. The resistant genotypes of the DH population were characterized by either constant or a slight increase in disease severity index, while susceptible maize genotypes displayed a conspicuous increase in disease severity as shown in Figure 4.8 for gray leaf spot and *Turcicum* leaf blight.

From the field trials, the resistant genotypes exhibited the typical resistant chlorotic-necrotic lesions, the moderately resistant genotypes were characterized by slightly chlorotic-necrotic lesions while the susceptible lines suffered severe necrosis.

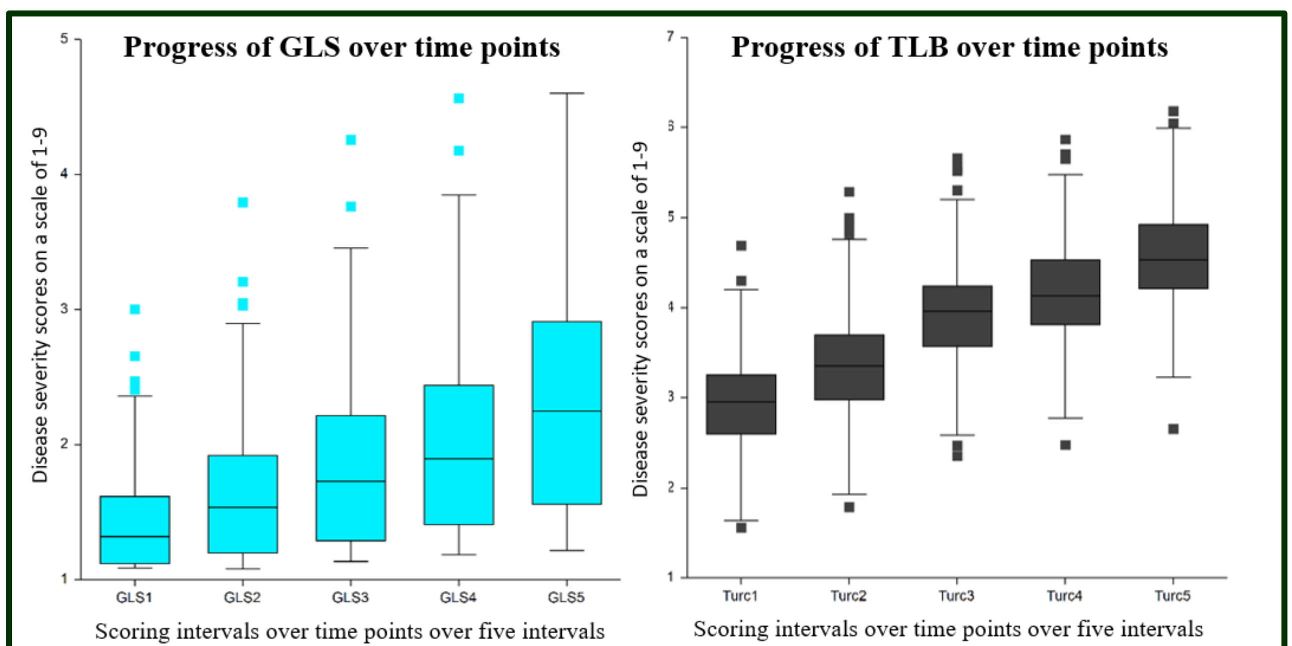
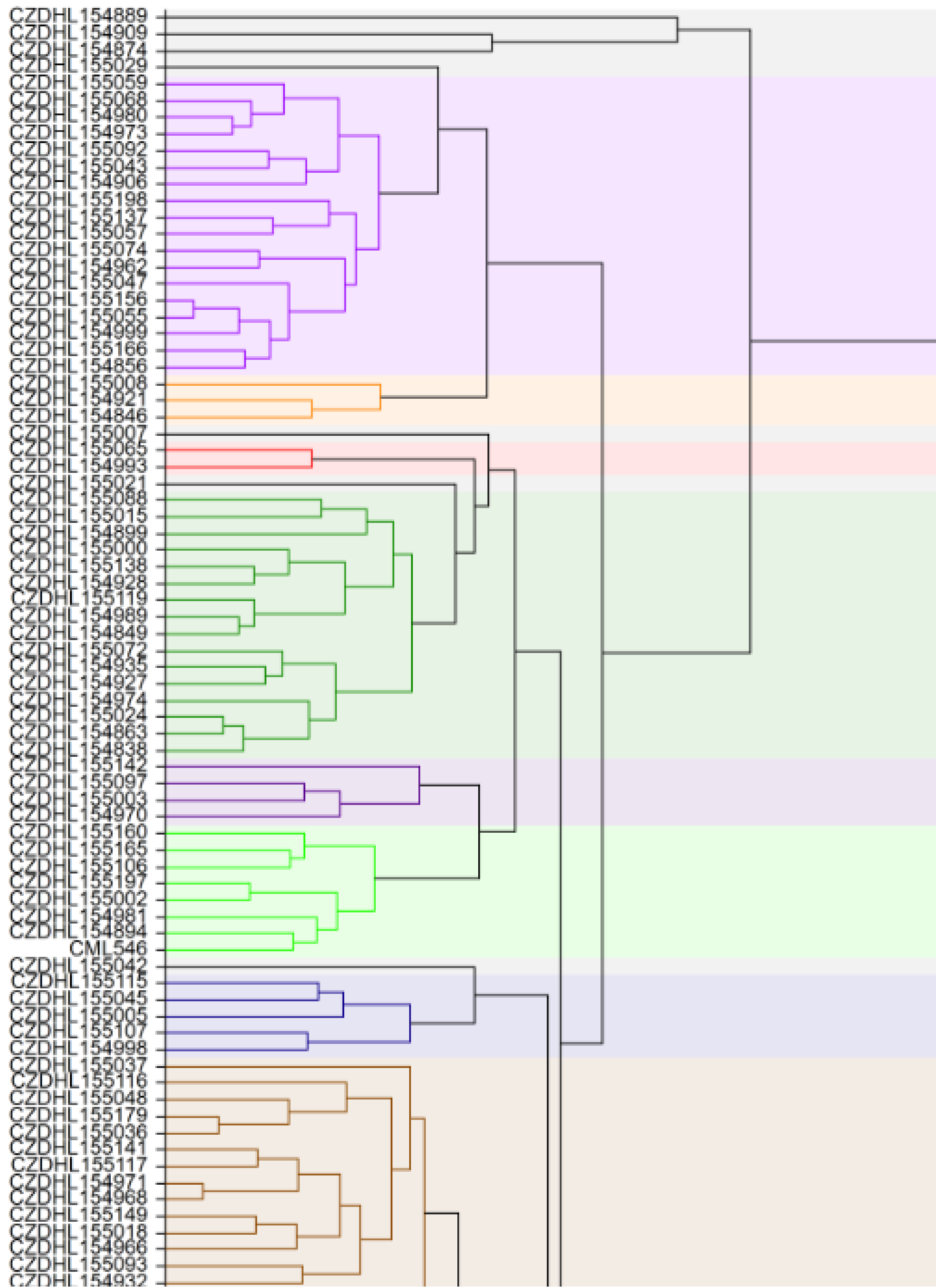


Figure 4. 8: Box plot showing progress of TLB and GLS combined across environments

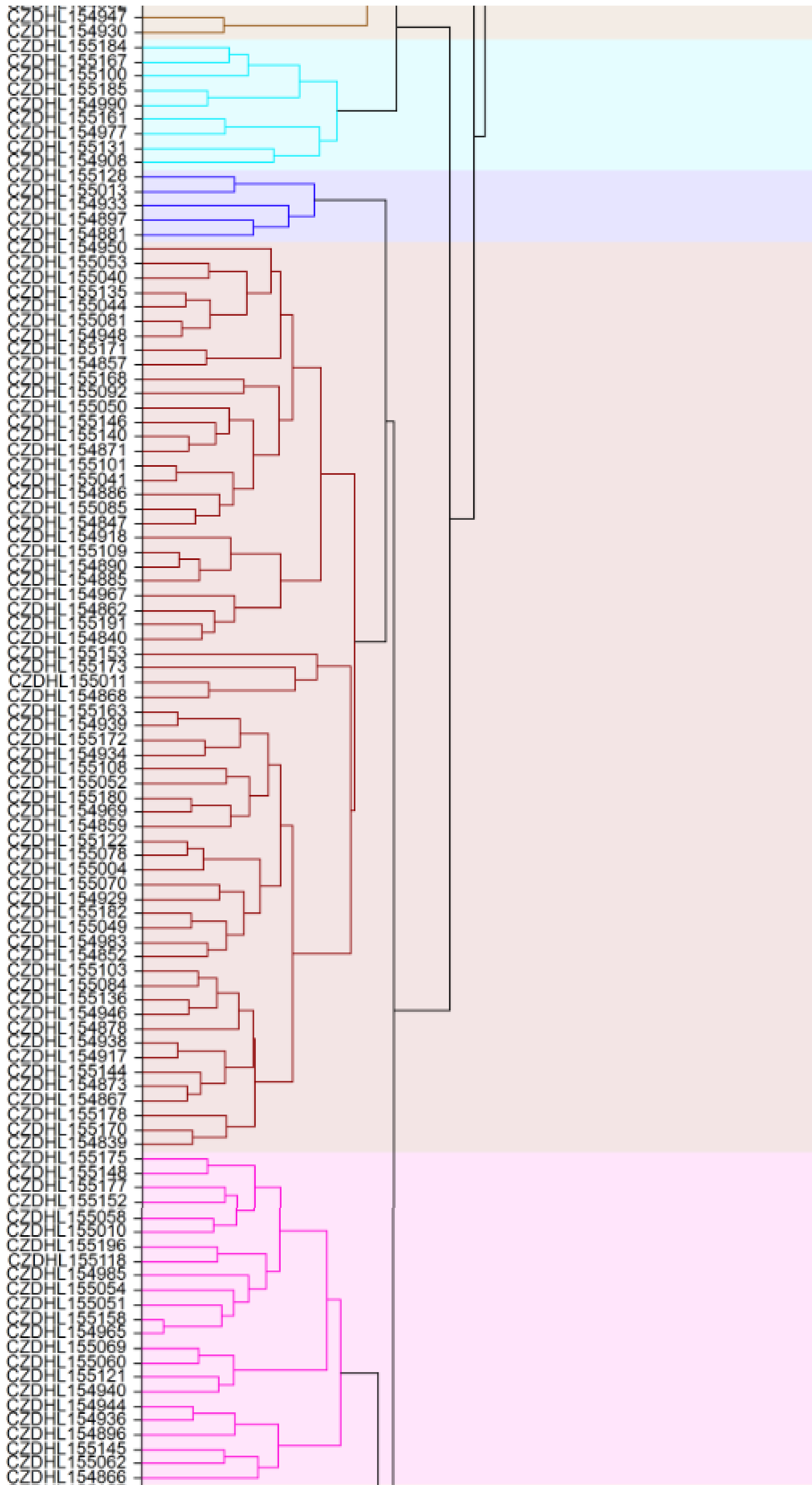
The coverage of the box plot appears wider at the final score for GLS (Figure 4.8), this could be attributed to the fact that some genotypes were expressing resistance and maintaining the low disease severity scores while some were highly susceptible. Overall there was increase in disease severity for the two diseases over scoring intervals.

4.3.1.2 Genetic diversity among the Genotypes

The hierarchical clustering dendogram constructed using unweighted group method (NCSS 2021 v21.0.3) revealed that the double haploid population was clustered into 97 groups. The cophenetic correlation coefficient was quite high at 0.6953 ($\alpha = 0.2346$) but still below the recommended value of 0.75 that is felt to be ideal for the goodness of fit. The genetic distance ranged from 0.1 to 2.1 within the population (Figure 4.9).



Diversity Dendrogram
CZDHL154889
CZDHL154909
CZDHL154874
CZDHL155029
CZDHL155059
CZDHL155068
CZDHL154980
CZDHL154973
CZDHL155092
CZDHL155043
CZDHL154906
CZDHL155198
CZDHL155137
CZDHL155057
CZDHL155074
CZDHL154962
CZDHL155047
CZDHL155156
CZDHL155055
CZDHL154999
CZDHL155166
CZDHL154856
CZDHL155008
CZDHL154921
CZDHL154846
CZDHL155007
CZDHL155065
CZDHL154993
CZDHL155021
CZDHL155088
CZDHL155015
CZDHL154899
CZDHL155000
CZDHL155138
CZDHL154928
CZDHL155119
CZDHL154989
CZDHL154849
CZDHL155072
CZDHL154935
CZDHL154927
CZDHL154974
CZDHL155024
CZDHL154863
CZDHL154838
CZDHL155142
CZDHL155097
CZDHL155003
CZDHL154970
CZDHL155160
CZDHL155165
CZDHL155106
CZDHL155197
CZDHL155002
CZDHL154981
CZDHL154894
CML546
CZDHL155042
CZDHL155115
CZDHL155045
CZDHL155005
CZDHL155107
CZDHL154998
CZDHL155037
CZDHL155116
CZDHL155048
CZDHL155179
CZDHL155036
CZDHL155141
CZDHL155117
CZDHL154971
CZDHL154968
CZDHL155149
CZDHL155018
CZDHL154966
CZDHL155093
CZDHL154932


Genotype
CZDHL154947
CZDHL154930
CZDHL155184
CZDHL155167
CZDHL155100
CZDHL155185
CZDHL154990
CZDHL155161
CZDHL154977
CZDHL155131
CZDHL154908
CZDHL155128
CZDHL155013
CZDHL154933
CZDHL154897
CZDHL154881
CZDHL154950
CZDHL155053
CZDHL155040
CZDHL155135
CZDHL155044
CZDHL155081
CZDHL154948
CZDHL155171
CZDHL154857
CZDHL155168
CZDHL155092
CZDHL155050
CZDHL155146
CZDHL155140
CZDHL154871
CZDHL155101
CZDHL155041
CZDHL154886
CZDHL155085
CZDHL154847
CZDHL154918
CZDHL155109
CZDHL154890
CZDHL154885
CZDHL154967
CZDHL154862
CZDHL155191
CZDHL154840
CZDHL155153
CZDHL155173
CZDHL155011
CZDHL154868
CZDHL155163
CZDHL154939
CZDHL155172
CZDHL154934
CZDHL155108
CZDHL155052
CZDHL155180
CZDHL154969
CZDHL154859
CZDHL155122
CZDHL155078
CZDHL155004
CZDHL155070
CZDHL154929
CZDHL155182
CZDHL155049
CZDHL154983
CZDHL154852
CZDHL155103
CZDHL155084
CZDHL155136
CZDHL154946
CZDHL154878
CZDHL154938
CZDHL154917
CZDHL155144
CZDHL154873
CZDHL154867
CZDHL155178
CZDHL155170
CZDHL154839
CZDHL155175
CZDHL155148
CZDHL155177
CZDHL155152
CZDHL155058
CZDHL155010
CZDHL155196
CZDHL155118
CZDHL154985
CZDHL155054
CZDHL155051
CZDHL155158
CZDHL154965
CZDHL155069
CZDHL155060
CZDHL155121
CZDHL154940
CZDHL154944
CZDHL154936
CZDHL154896
CZDHL155145
CZDHL155062
CZDHL154866

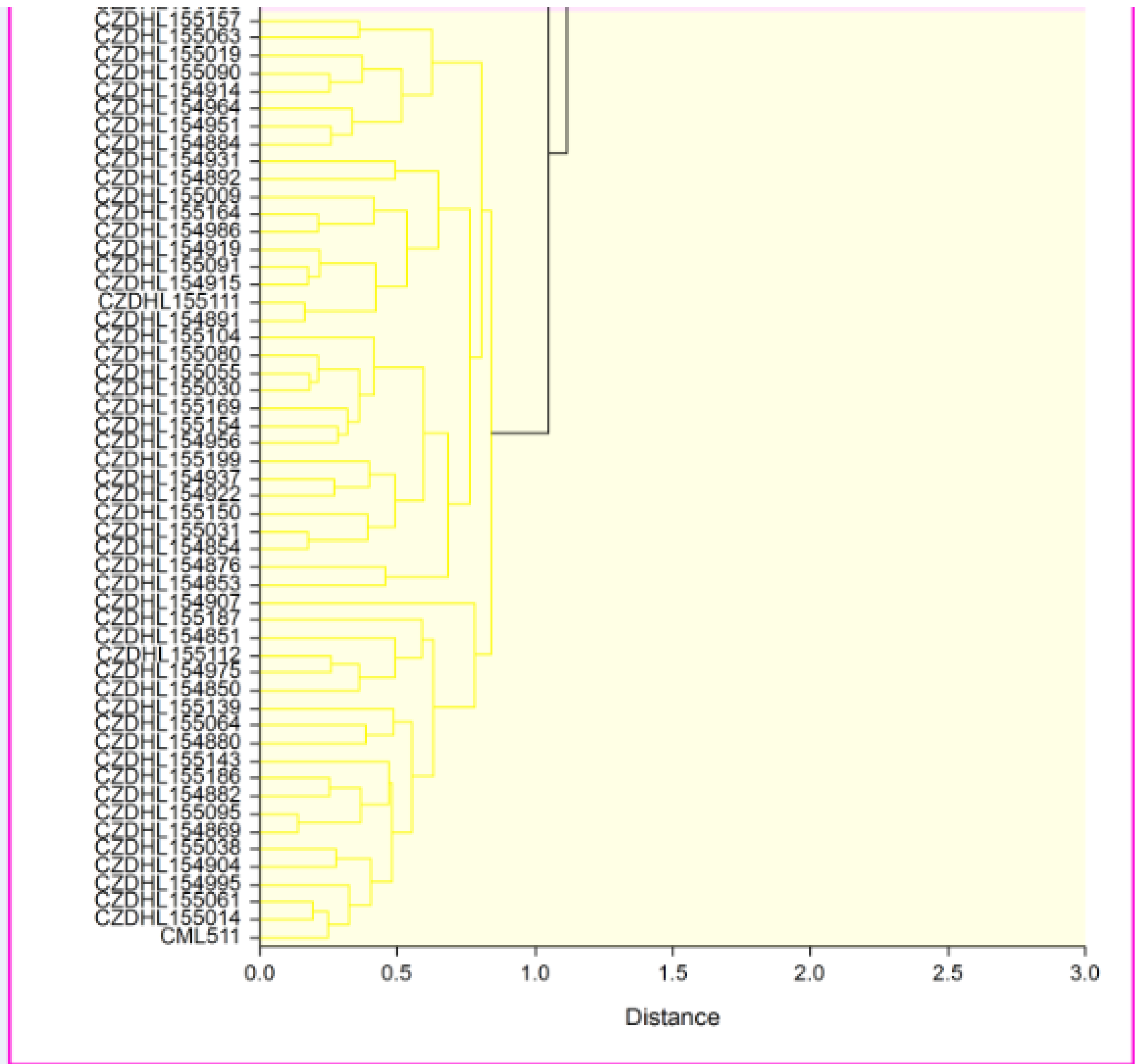


Figure 4. 9: Dendogram showing the genetic diversity among the 230 genotypes

From the diversity dendogram, numerous outliers were reported for CZDHL154889, CZDHL155029, CZDHL155007, CZDHL155021, CZDHL155142, CZDHL155042, CZDHL155037, CZDHL154907 and CZDHL155153. The genotypes that were closer together displayed small dissimilarity between them.

4.3.1.3 Distribution of disease severity scores

In this study, 228 entries of the double haploid population were evaluated for resistance to TLB and GLS over three trial environments and scoring was conducted for five scoring intervals in each trial. BLUEs and BLUPs were then determined from the disease scores. Frequency

distribution of GLS disease severity scores at the three trial environments were fairly skewed towards resistance as shown in Figure 4.10. Most of the 230 entries in the double haploid population were resistant to GLS, hence the distribution was more skewed towards resistance.

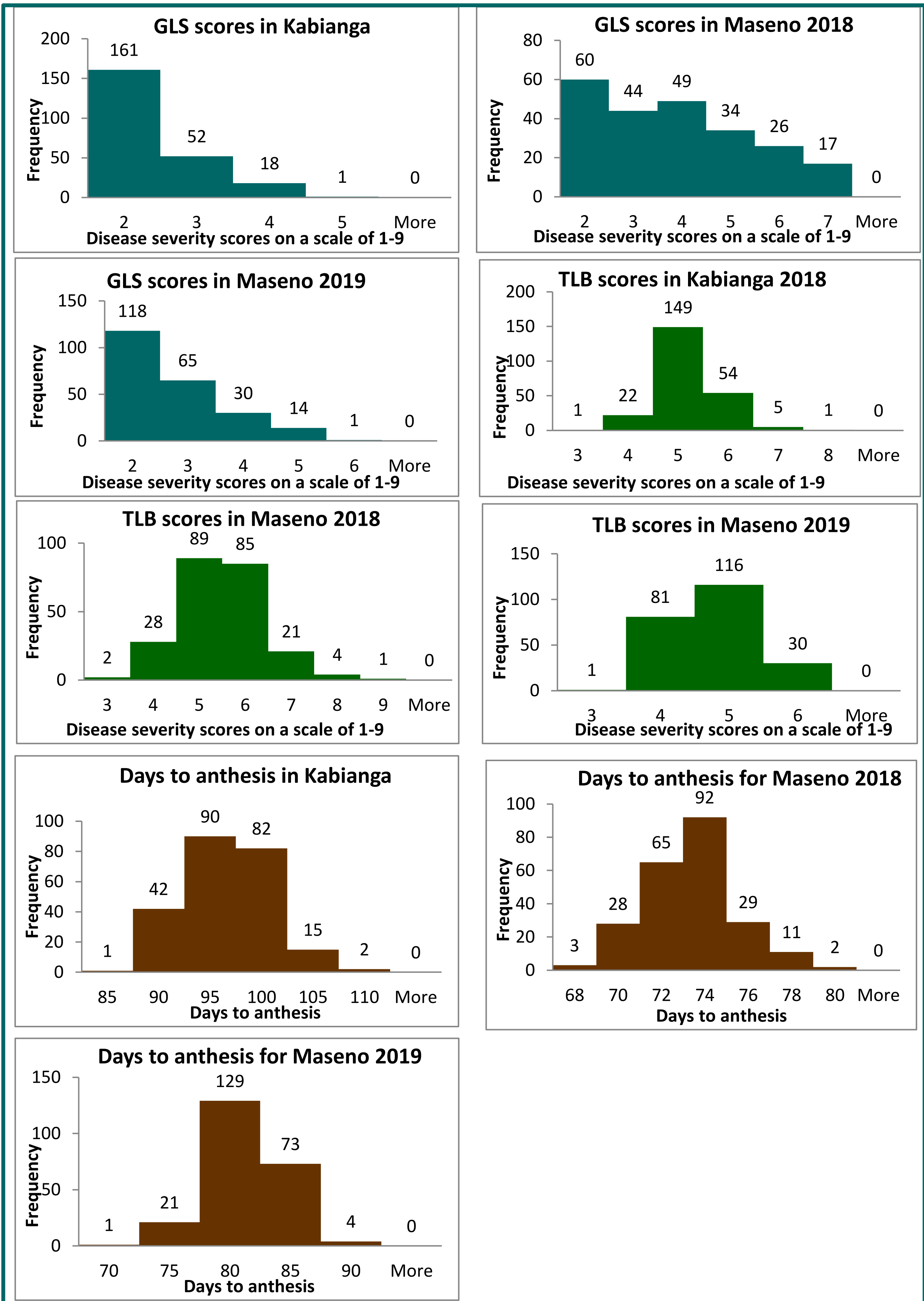


Figure 4. 10: Frequency distribution for disease severity data and days to anthesis

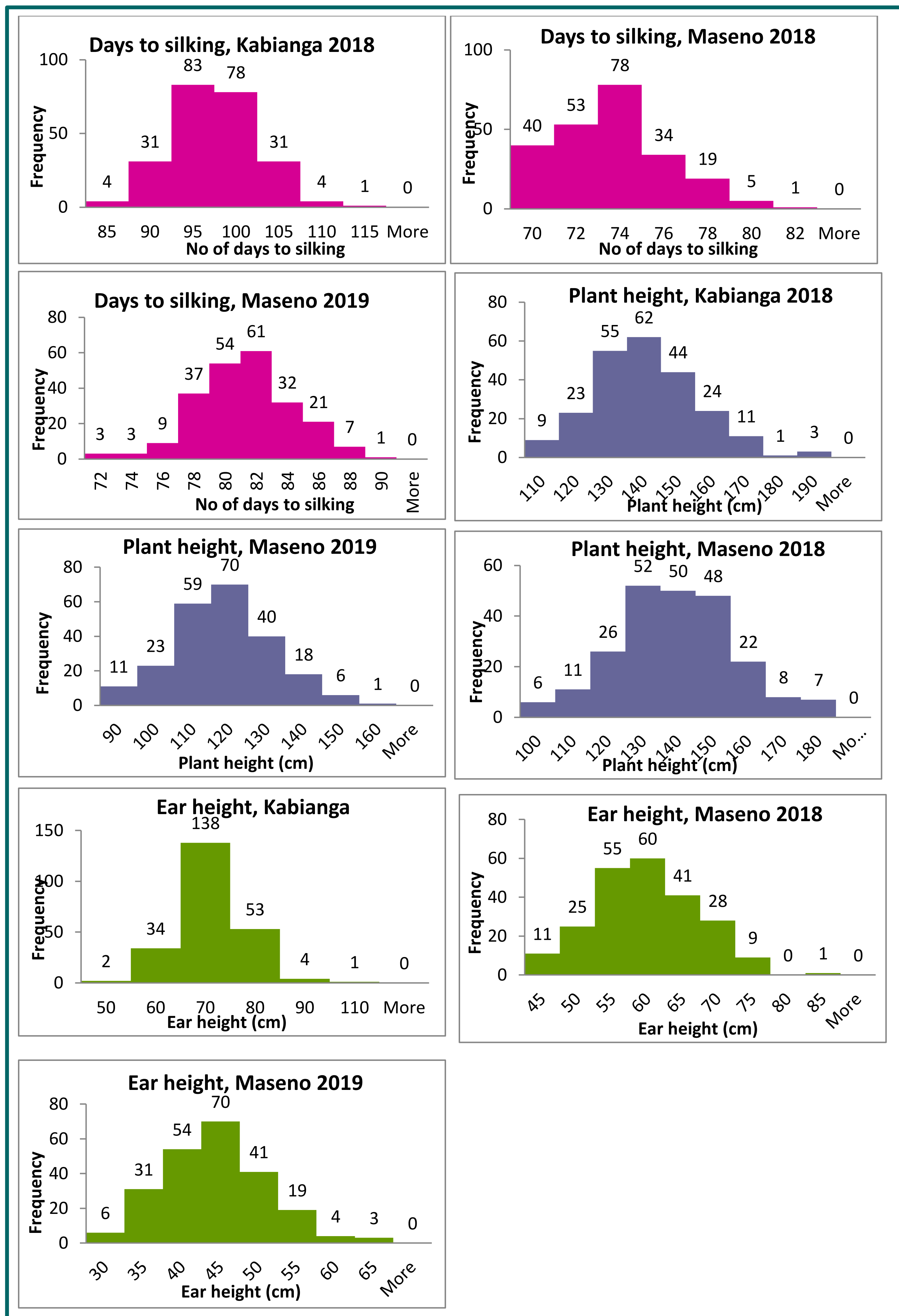


Figure 4. 11: Frequency distribution for the different agronomic traits

The frequency distribution of the disease severity scores for TLB in the double haploid population followed an approximately normal distribution as shown in Figure 4.10. This suggested that resistance to TLB is quantitatively inherited. The wide segregation of disease scores for TLB provided more evidence for quantitative resistance. The number of genotypes in each bin provided more evidence for the distribution. The DH population exhibited continuous distribution for the days to anthesis across the three environments as shown in Figure 4.10.

The frequency distribution for area under the disease progress for GLS and TLB are also provided (Appendix E). The area under the disease progress curve for GLS exhibits near-normal distribution. The area under the disease progress curve for TLB is characterized by a continuous distribution (Appendix E).

4.3.1.4 Agronomic data

The mean days to anthesis was 94 days, 72 days and 78.6 days in Kabianga (2018), Maseno long rains (2018) and Maseno long rains (2019) respectively. The variation in maturity time between the three environments was a result of the growth degree days/ heat units accumulated. It is worth noting that CML546 (DTA=87.02) flowered after CML511 (DTA=81.61) in these trials. Interestingly, CML511 was also shorter than CML546 in plant height.

The agronomic traits such as days to silking, plant height and ear height were characterized by continuous distribution of the phenotypic data (Figure 4.11). This is a clear indication that the agronomic traits are quantitative in nature.

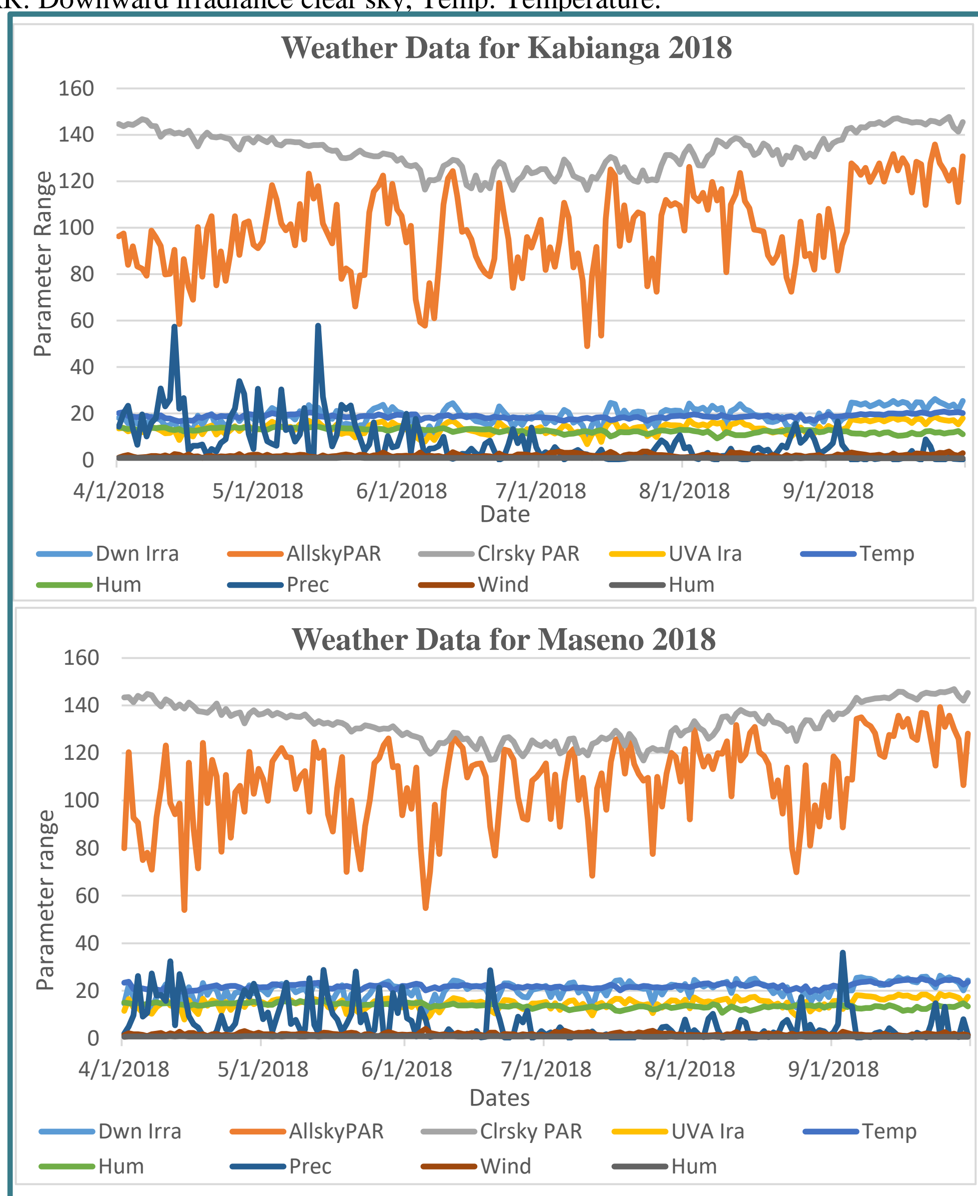
4.3.1.5 The Weather data

Differences in time to maturity could be attributed to differences in weather events across locations that affect heat accumulation. The maximum and minimum temperatures experienced in a location were important in achieving the heat units and the resultant crop days to maturity.

Table 4. 7: Averages of the weather data for the three different environments

Location	Allsky PAR (W/m²)	Temp (°C)	Specific humidity (g/kg)	Precipitation (mm/day)	Wind speed (m/s)
Kabianga 2018	99.23	18.78	12.62	7.76	2
Maseno 2018	108.31	21.97	13.72	6.28	1.76
Maseno 2019	110.5	22.68	14.27	6.198	2.76

Allsky PAR: Downward irradiance clear sky, Temp: Temperature.



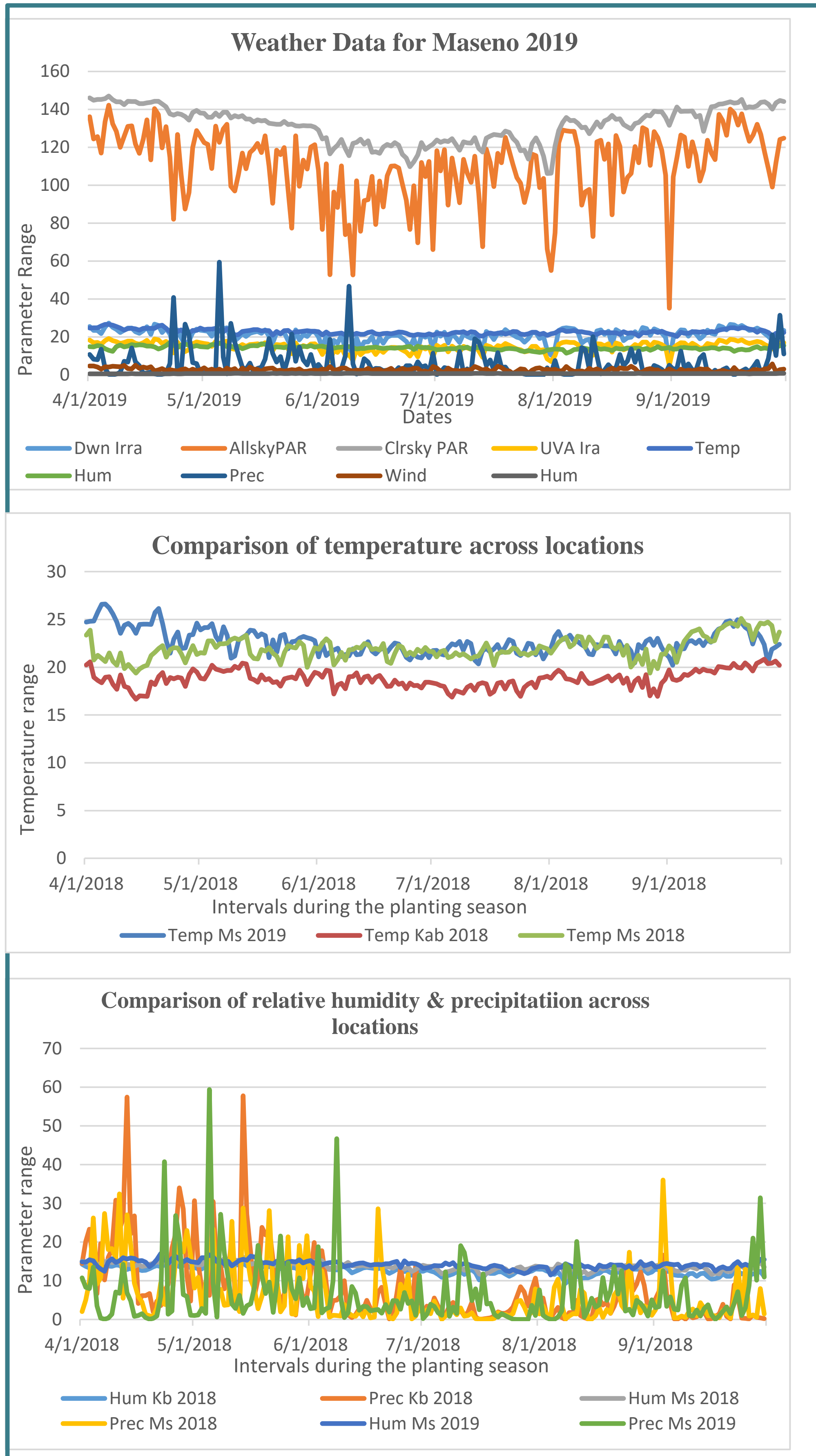


Figure 4. 12: Weather data across locations during the planting season

Dwn Irra: Downward irradiance for all sky, Allsky PAR: Downward irradiance clear sky, Allsky PAR: Active radiation, UVA Ira: UVA irradiance, Temp: Temperature at 2 metres, Hum: Specific humidity, Prec: Precipitation, Wind: Wind speed, Temp Ms 2019: Temperature for Maseno 2019.

Temperature and specific humidity during the growing season was relatively higher in Maseno 2018 and Maseno 2019 compared to Kabianga 2018 (Table 4.7). Precipitation was relatively higher in Kabianga 2018 compared to Maseno 2018 and 2019 (Table 4.7). Figure 4.12 shows a sharp difference in temperature especially between Maseno 2018, Maseno 2019 and Kabianga 2018. All the locations experienced a peak at the onset of the planting season. During the growing season, there was a drop in the amount of rainfall received followed by another increase towards the end of the growing season. Moisture stress during the flowering time affected the anthesis silking interval in Kabianga for specific genotypes that displayed a longer anthesis silking interval.

4.3.1.6 Correlation between environments

The correlation coefficient between environments for GLS disease severity scores was characterized by highly significant positive correlation at $p < 0.001$. Moderately high correlation coefficients were reported between environments for TLB disease severity scores that were significantly positive at $p < 0.001$. These shows that resistance to GLS and TLB were characterized by moderate but highly significant correlation coefficients between environments (Table 4.8).

Table 4. 8: Correlation coefficients between environments for GLS and TLB

	Gray leaf spot scores			Northern leaf blight scores		
Environment	Kabianga	Maseno LR 2018	Maseno LR 2019	Kabianga	Maseno LR 2018	Maseno LR 2019
Kabianga	1.0000***	0.5792***	0.5709***	1.0000***	0.6067***	0.4644***
MasenoLR2018	0.5792***	1.0000***	0.8248***	0.6067***	1.0000***	0.5638***
MasenoLR2019	0.5709***	0.8248***	1.0000***	0.4644***	0.5638***	1.0000***

*** implies that the correlation coefficient was significant at $p < 0.001$

The correlation coefficients across environments for days to anthesis were also highly significant at $p < 0.001$ (Table 4.8). Higher correlation coefficients were observed for days to silking that were highly significant at $p < 0.001$. This implies significant correlation between

environments for flowering time (Table 4.9). The higher correlation coefficient between Maseno 2018 and Maseno 2019 could be attributed to similar weather conditions (Temperature, specific humidity and precipitation) between Maseno 2018 and Maseno 2019. Weather parameters were slightly divergent between field sites in Maseno and Kabianga hence the moderate levels of correlation between the respective geographical locations.

Table 4. 9: Correlation coefficients between environments for flowering time

	Days to anthesis			**Days to silking**		
Environment	**Kabianga**	**Maseno LR 2018**	**Maseno LR 2019**	**Kabianga**	**Maseno LR 2018**	**Maseno LR 2019**
Kabianga	1.0000***	0.6808***	0.6229***	1.0000***	0.7156***	0.6504***
MasenoLR2018	0.6808***	1.0000***	0.7299***	0.7156***	1.0000***	0.7418***
MasenoLR2019	0.6229***	0.7299***	1.0000***	0.6504***	0.7418***	1.0000***

*** implies that the correlation coefficient was significant at $p < 0.001$, LR implies Long rains

4.3.1.7 Correlation between variables

The relationship between disease severity scores and AUDPC for GLS and flowering time (days to anthesis and days to silking) were tested. Significant but negative correlation between GLS disease severity scores, AUDPC and flowering time data were observed. (r^2 = -0.2807; α = 0.05; r^2 = -0.2697; α = 0.05; r^2 = -0.3288; α = 0.05 and r^2 = -0.3055; α =0.05) (Table 4.10).

Table 4. 10: Correlation coefficients between variables for GLS and TLB

Variables	DTA	DTS	PH	EH	GLS5	Turc5	AUDPC GLS	AUDPC TLB
DTA	1*	0.9295*	0.0897	0.1181	-0.2807*	-0.2323*	-0.3288*	-0.2075*
DTS	0.9295*	1*	0.167*	0.1521*	-0.2697*	-0.2126*	-0.3055*	-0.1725*
PH	0.0897	0.167*	1*	0.8309*	0.0552	0.2571*	0.0677*	0.2948*
EH	0.1181	0.1521*	0.8309*	1*	0.0481	0.3047*	0.0549*	0.3165*
GLS5	-0.2807*	-0.2697*	0.0552	0.0481	1*	0.1523*	0.9716*	0.1464*
Turc5	-0.2323*	-0.2126*	0.2571*	0.3047*	0.1523*	1*	0.1373*	0.9497*
AUDPC GLS	-0.3288*	-0.3055*	0.0677*	0.0549*	0.9716*	0.1373*	1*	0.1563*
AUDPC TLB	-0.2075*	-0.1725*	0.2948*	0.3165*	0.1464*	0.9497*	0.1563*	1*

***, implies significance at *α*=0.05, DTA:** Days to anthesis, DTS: days to silking, PH: Plant height, EH: Ear height, GLS5: Gray leaf spot scores, AUDPC GLS: Area under disease progress curve for GLS, AUDPC GLS: Area under disease progress curve for TLB.

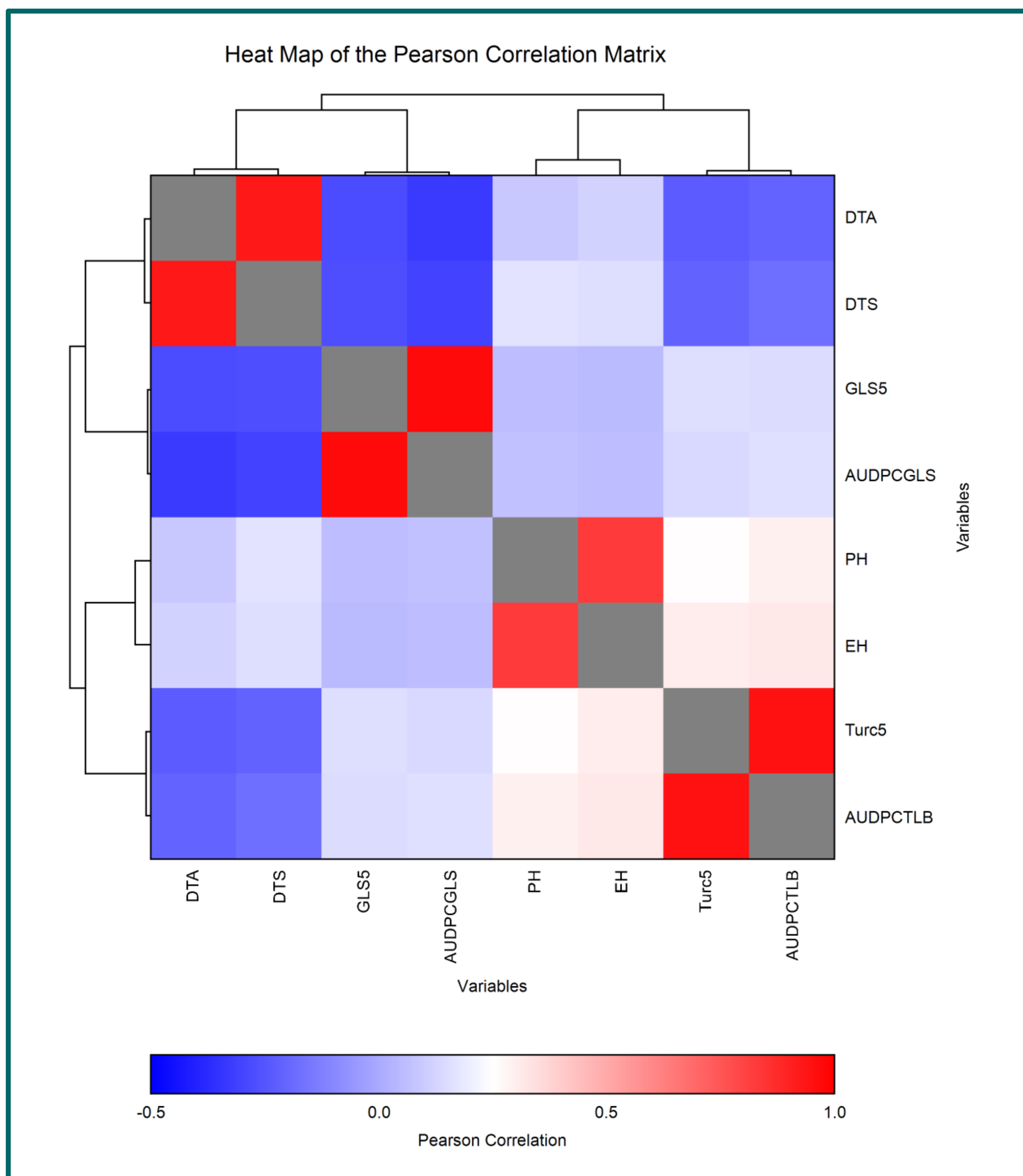


Figure 4. 13: Heat map of the Pearson correlation matrix between the different variables

DTA: Days to anthesis, DTS: days to silking, PH: Plant height, EH: Ear height, GLS5: Gray leaf spot scores, AUDPC GLS: Area under disease progress curve for GLS, AUDPC GLS: Area under disease progress curve for TLB.

The relationship between disease severity scores (AUDPC) and days to anthesis were also tested for TLB. Significant but negative correlation between disease severity scores and

flowering time were also observed (r^2 =-0.2323 at α =0.05; r^2 =-0.2126 at α =0.05; and r^2 = -0.2075 at α =0.05 and r^2 =-0.1725 at α =0.05) (Table 4.10). This indicates that there was no relationship between TLB/GLS and flowering time. The relationship between *Turcicum* leaf blight AUDPC and gray leaf spot AUDPC were further tested across the three environments, significantly positive correlation was reported (r^2 = 0.1563) at α = 0.05 (Table 4.10). Same positive correlation was reported between GLS disease severity scores and TLB disease severity scores (r^2 = 0.1523) at α = 0.05.

Further exploration of the agronomic data revealed positive but weak correlation between days to anthesis and plant height together with ear height (r^2 = 0.0897; r^2 = 0.1181). To the contrary there was significant correlation between days to silking and plant height together with ear height (r^2 = 0.167; r^2 = 0.1521). Significantly positive correlation were reported between disease severity parameters and area under disease progress curve in relation to plant height and ear height as indicated in table 4.10. The Pearson correlation coefficient between days to anthesis and days to silking was significantly high (r^2 = 0.9295). There was similarly high and significant correlation coefficient between plant height and ear height (r^2 = 0.8309) as indicated in table 4.10.

The Pearson correlation matrix between data variables was further visualized using the Heat map as illustrated in Figure 4.13. The unweighted pair group revealed clusters in the correlation matrix as indicated in Figure 4.13.

Table 4. 11: Estimates of means, ranges, genetic variance components (σ^2G) and broad sense heritability (h^2)

	AD[a]	SD[b]	Pht[c]	Eht[d]	GLS 1	GLS 2	GLS 3	GLS 4	GLS 5	AUDPC	TLB 1	TLB 2	TLB 3	TLB 4	TLB 5	AUDPC
Across location																
Mean	81.74	82.69	127.89	55.02	1.43	1.64	1.82	2.03	2.36	52.27	2.91	3.34	3.92	4.15	4.58	108.46
σ^2_G[e]	11.07	14.37	282.87	67.79	0.18	0.33	0.45	0.63	0.94	382.44	0.38	0.42	0.39	0.41	0.42	311.71
σ^2_{GxE}[f]	2.37	3.63	16.66	5.94	0.13	0.17	0.26	0.39	0.49	213.07	0.23	0.14	0.17	0.18	0.19	156.61
σ^2_e[g]	11.59	12.60	219.57	101.49	0.17	0.26	0.29	0.35	0.52	175.63	0.33	0.30	0.37	0.38	0.45	216.91
h^2_h	0.84	0.85	0.90	0.84	0.74	0.80	0.79	0.79	0.81	0.81	0.77	0.84	0.80	0.80	0.79	0.80
LSD[i]	2.69	3.04	11.21	7.07	0.42	0.52	0.61	0.72	0.84	17.31	0.59	0.52	0.56	0.58	0.60	15.95
CV[j]	4.17	4.29	11.59	18.31	29.12	31.1	29.6	29.0	30.5	25.36	19.79	16.54	15.52	14.80	14.73	13.58

AD[a] - days to anthesis, SD[b]-days to silking, Pht[c] -Plant height, Eht[d]-Ear height, GLS1-5; gray leaf spot disease scores at intervals of 7 days, TLB1-5; Northern leaf blight disease scores at 7 days intervals; CV[j]- Coefficient of variation, LSD[i]- least significant difference.

σ^2_G[e] variance attributed to the genotypic effect

σ^2_{GxE}[f] the variance attributable to genotype × environment interaction

σ^2_e[g] environmental variance

h^2_h is the heritability on entry mean basis.

4.3.1.8 Variance analysis

The AOV functionality (analysis of variance for multi-environment trials) in QTL IciMapping software was used to compute heritability estimates, components of genetic variance, genotype by environment interactions in the double haploid population. Genotype effect (σ^2_G) and the environment effect were significant for GLS and TLB area under the disease progress curve, anthesis date and silking date, while the genotype by environment interaction was low. Heritability estimates on an entry mean basis were $h^2 = 0.81$, $h^2 = 0.8$, and $h^2 = 0.84$ (Table 4.11) for GLS area under progress curve, TLB area under progress curve and for days to anthesis respectively.

The genotype × environment interaction and the genotypic components of variance were significant for the CML511*CML546 double haploid population. Genotype × environment interaction contributed to a small but significant percentage of the whole variance for flowering and disease severity. The G×E values of 2.37% and 3.63% for anthesis date and for silking date, 0.13% to 0.52% for GLS scores and 0.14% to 0.23% for TLB scores were relatively small (Table 4.11). This coupled with the high heritability estimates for GLS and TLB, indicate that much of the phenotypic variance for resistance to GLS and TLB in the double haploid population is majorly controlled by genetic factors. Combined analysis of variance identified genotypic variance, genotype by environment interaction variance and environmental variance as significant contributors to the overall variance (Table 4.11). The environment variance was remarkably high because more diverse locations were involved.

4.3.2 Construction of the genetic linkage map

The genetic linkage map was constructed using the MAP functionality in QTL IciMapping software v4.1. The linkage map was enriched with a total of 1250 high quality SNP markers. The constructed genetic linkage map comprising of 10 linkage groups spanned a total map

length of 3344.9 cM with 6.689 as the average distance between two adjacent markers. The genetic linkage map as shown in Figure 4.14, covered most of the maize genome.

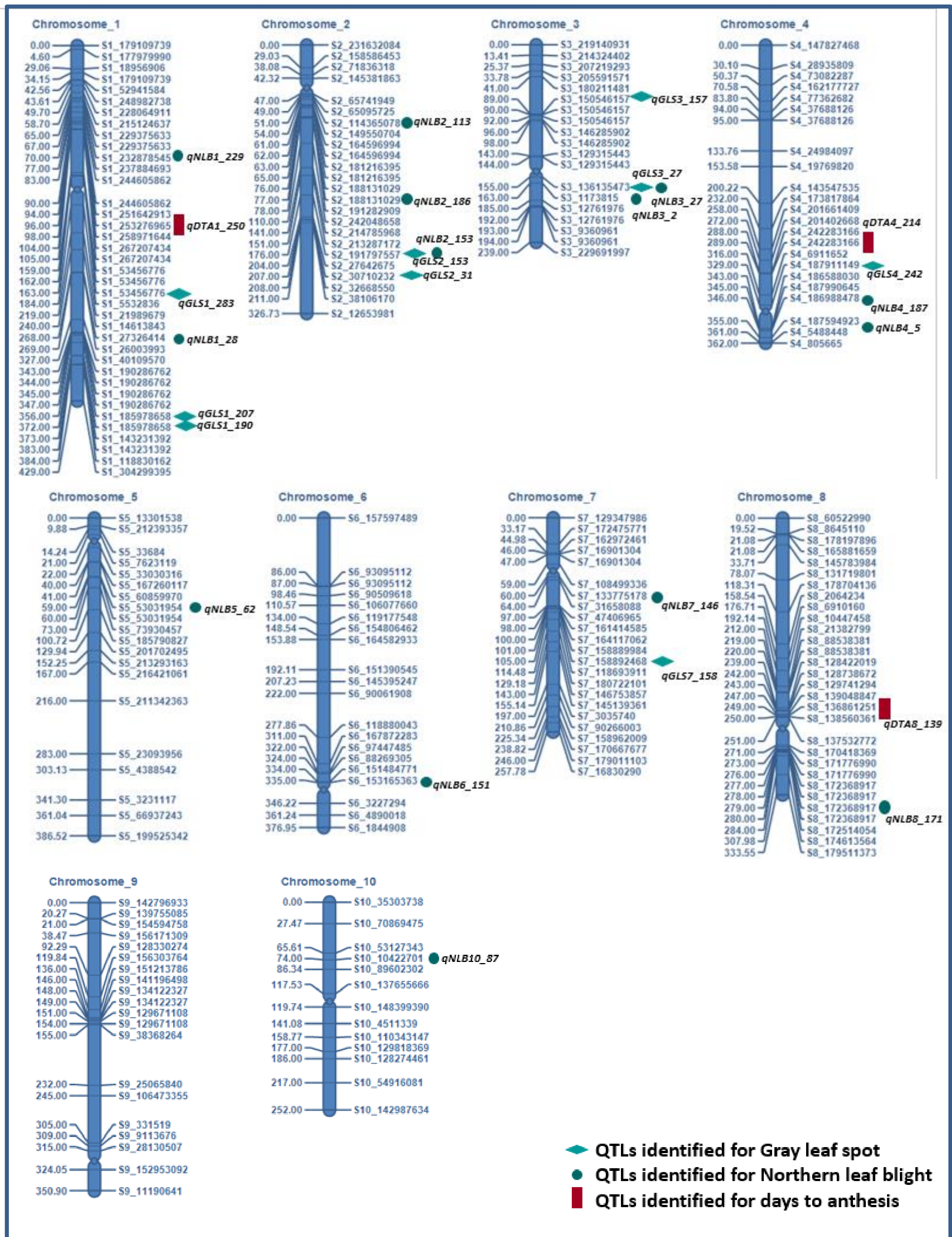


Figure 4. 14: Genetic linkage map for the DH population

4.3.3 QTL analysis

Table 4. 12. Quantitative trait loci detected in all the three environments

QTL Name	Chr[a]	QTL bin[b]	QTL Location		Position[c] (cM)	Additive effect[d]	$R^{2\,e}$	LOD score[f]
			Left Marker	**Right Marker**				
qGLS1_190	1	1.06	S1_190286762	S1_185978658	372	-0.59	16.60	25.6
qGLS1_283	1	1.11	S1_283894617	S1_53456776	163	0.44	4.79	8.80
qTLB2_164	2	2.06	S2_164596994	S2_181216395	65	-0.27	6.27	5.16
qTLB3_65	3	3.04	S3_27134253	S3_136135473	155	-0.43	12.27	4.03

qGLS quantitative trait loci for GLS resistance, *qTLB* quantitative trait loci for Turcicum leaf blight resistance
[a] the maize chromosome containing the QTL
[b] the chromosomal bin location where the QTL peaked
[c] the position of the QTL peak as defined by the LOD interval
[d] the additive effect of the QTL.
[e] represents percentage of the phenotypic variance explained by the detected QTL (%).
[f] the Logarithm of odds at the position where the QTL peaked.

Several QTLs associated with distinct levels of resistance to GLS and TLB and with small additive effects were detected through inclusive composite interval mapping. A total of 6, 11, 10, 28, 27, and 36 QTLs were detected in individual environment QTL mapping analysis for anthesis date, silking date, plant height, ear height, GLS and TLB respectively. These QTLs were distributed across all 10 chromosomes of maize cumulatively accounting for 38.75 to 64.94% of the phenotypic variation. Fourteen QTLs associated with resistance to TLB, nine QTLs associated with resistance to GLS and three QTLs significantly associated with flowering time were detected in the DH population CML511*CML546. These QTLs were detected in at least two environments out of the three, namely Maseno long rains 2018, Kabianga long rains 2018 and Maseno long rains 2019. Some QTLs could not be repeatedly identified over the same site in distinct seasons possibly due to changing climatic conditions from one season to another (Table 4.7 and Fig. 4.12). The QTLs identified in all the three environments are presented (Table 4.12). *qTLB3_65* on the chromosomal bin 3.04 was detected for both GLS and TLB.

Table 4. 13: QTL for resistance to gray leaf spot, Turcicum leaf blight and flowering time

QTL Name	Chr[a]	QTL bin[b]	QTL Location		Position (cM)[c]	Additive effect[d]	R^2 [e]	LOD score[f]
			Left Marker	**Right Marker**				
qGLS1_190	1	1.06	S1_190286762	S1_185978658	372	-0.59	**16.60**	25.6
qGLS1_207	1	1.07	S1_207695200	S1_190286762	347	0.34	**16.14**	9.30
qGLS1_283	1	1.11	S1_283894617	S1_53456776	163	0.44	4.79	8.80
qGLS2_31	2	2.04	S2_31111676	S2_30710232	207	0.24	4.19	6.00
qGLS2_153	2	2.06	S2_153787894	S2_191797557	176	0.50	8.22	6.10
qGLS3_27	3	3.04	S3_27134253	S3_136135473	155	0.38	10.1329	6.11
qGLS3_157	3	3.05	S3_157562360	S3_150546157	89	0.34	8.02	11.17
qGLS4_242	4	4.1	S4_242295766	S4_187911149	329	0.39	7.23	30.17
qGLS7_158	7	7.04	S7_158889984	S7_158892468	105	-0.19	5.40	5.40
qTLB1_28	1	1.02	S1_28106472	S1_27326414	268	-0.06	6.36	3.25
qTLB1_229	1	1.08	S1_229375633	S1_232878545	70	0.17	9.42	8.81
qTLB2_113	2	2.05	S2_113711349	S2_114365078	51	0.20	9.60	8.42
qTLB2_153	2	2.06	S2_153787894	S2_191797557	176	-0.59	6.16	3.11
qTLB2_186	2	2.07	S2_186201459	S2_188131029	77	0.31	12.50	9.39
qTLB3_2	3	3.01	S3_2734515	S3_1173815	163	0.16	7.40	6.03
qTLB3_27	3	3.04	S3_27134253	S3_136135473	155	-0.43	**12.27**	4.03
qTLB4_5	4	4.02	S4_5143260	S4_5488448	361	-0.2122	**12.77**	-0.21
qTLB4_187	4	4.08	S4_187990645	S4_186988478	346	-0.1909	8.85	5.3709
qTLB5_62	5	5.03	S5_62077939	S5_53031954	59	-0.1985	10.61	6.77
qTLB6_151	6	6.05	S6_151834390	S6_153165363	335	-0.49	8.61	5.62
qTLB7_146	7	7.03	S7_146647930	S7_133775178	60	0.14	5.60	4.09
qTLB8_171	8	8.08	S8_171776990	S8_172368917	279	0.38	**13.65**	10.48
qTLB10_87	10	10.04	S10_87874180	S10_10422701	74	-0.05	5.19	3.69
qDTA1_250	1	1.09	S1_250341978	S1_253276965	96	0.84	5.74	5.99
qDTA4_214	4	4.09	S4_214489216	S4_242283166	289	-0.59	5.93	5.73
qDTA8_139	8	8.05	S8_139048847	S8_136861251	249	2.11	**17.34**	20.85

qGLS quantitative trait loci for GLS resistance, *qTLB* quantitative trait loci for Turcicum leaf blight resistance, [a]maize chromosome, [b] the chromosomal bin, [c] the position of the QTL peak as defined by the LOD interval, [d] the additive effect of the QTL, [e] represents percentage of the phenotypic variance (%),[f] the Logarithm of odds.

Table 4. 14: The level of phenotypic variance explained in the various traits examined

Trait ID	Trait Name	Total PVE[a] (%)
1	BP_DTA[b]	63.73
3	BP_DTS[c]	60.19
5	BP_PH[d]	52.75
7	BP_EH[e]	56.12
9	BP_GLS1[f]	53.20
11	BP_GLS2	60.76
13	BP_GLS3	64.19
15	BP_GLS4	59.83
17	BP_GLS5	61.30
19	BP_AUDPC1[g]	64.49
21	BP_Turc1[h]	51.44
23	BP_Turc2	46.44
25	BP_Turc3	64.94
27	BP_Turc4	54.89
29	BP_Turc5	38.75
31	BP_AUDPC2	45.79

PVE[a]- percentage of phenotypic variance explained by the QTLs (%), DTA[b] -days to anthesis, DTS[c] days to silking, PH[d] –plant height, EH[e]- ear height, GLS[f] gray leaf spot, Turc[h]- Turcicum leaf blight at the different scoring intervals and AUDPC[g] - Area under disease progress curve.

From the analysis run, the position on the maize chromosome, the chromosomal bin, the leftmost marker, the rightmost marker, logarithm of odds (LOD), phenotypic variance explained (PVE) and the additive effects are reported for each QTL (Table 4.12 and 4.13). Phenotyping was properly done using an appropriate design of field trials and scoring procedures. In addition a large population size of 230 entries was used in combination with a good genetic linkage map.

4.3.3.1 Quantitative trait loci and their genetic effects for GLS resistance

The 27 QTLs identified for GLS were consolidated into nine QTLs that were detected in at least two environments and had significant phenotypic variance explained. The significant QTLs were found in the chromosome bin 1.06, bin 1.07, bin 1.11, bin 2.04, 2.06, bin 3.04, bin 3.05, bin 4.1 and bin 7.04 for resistance to GLS (Table 4.13). The QTL on chromosome 1 (Bin 1.06) conditioned the highest percentage of the phenotypic variance (16.60%). Six QTLs *qGLS1_207*, *qGLS1_283*, *qGLS2_31* *qGLS3_157*, *qGLS3_27* and *qGLS4_242* exhibited

positive additive effects while three QTLs *qGLS1_190, qGLS2_170* and *qGLS7_158* exhibited negative additive effects.

For GLS, the identified QTLs explained 0.79% - 16.60% of the genetic variance from the individual environments, while all the QTLs explained 61.30% of the genetic variance for GLS (Table 4.13). The chromosomal bin 1.06 was detected in all the 3 environments and explained up to 16.60% of the phenotypic variance. Most of these QTLs were identified in at least two experimental conditions. Some QTLs had large effects on the phenotypic variance explained but could only be detected in a single environment. The GLS QTL explained different percentages of the phenotypic variance at different scoring intervals.

4.3.3.2 Quantitative trait loci for resistance to TLB.

Fourteen QTLs significantly associated with resistance to *Turcicum* leaf blight were detected in this study. The following chromosomal bins were found to condition resistance to TLB namely, 1.02, 1.08, 2.05, 2.06, 2.07, 3.01, 3.04, 4.02, 4.08, 5.03, 6.05, 7.03, 8.08 and 10.04 (Table 4.13). These QTLs were reported in at least two environments indicating their possible utility in selection decisions across environments. Of the identified QTLs, the QTL on chromosome 8 (Bin 8.08) explained the largest percentage of the phenotypic variance (13.65%). Seven QTLs *qTLB1_229*, *qTLB2_113*, *qTLB2_186*, *qTLB3_2*, *qTLB7_146*, *qTLB8_170* and *qTLB10_125* displayed positive additive effects while 7 QTLs *qTLB1_17/18*, *qTLB2_164, qTLB3_27*, *qTLB4_5*, *qTLB4_187*, *qTLB5_62* and *qTLB6_151* exhibited negative additive effects.

For TLB the identified QTLs individually explained 3.19%-13.65% of the genetic variance whereas all the QTLs together explained 38.75% of the genetic variance for TLB (Table 4.14).

4.3.3.3 Flowering time QTLs

Three QTL were detected for flowering time in bins 1.09, 4.09 and 8.05 and were reported in at least two environments (Table 4.13). The three QTL showed additive effects from -0.97 to

3.77 and explained 5.74% to 17.34% of the phenotypic variation in flowering time. Out of the three QTLs, the QTL on chromosome 8 (chromosomal bin 8.05) accounted for the largest percentage of the phenotypic variance (17.34%) with a LOD score of 20.85. Other QTLs associated with flowering time included *qDTA1_250* (chromosomal bin 1.09) and *qDTA1_214* (chromosomal bin 4.09). Two QTLs namely, *qDTA1_250* and *qDTA8_139* displayed positive additive effects while *qDTA1_214* exhibited negative additive effects. Flowering time QTLs cumulatively conditioned 63.73% of the genetic variance.

4.3.3.4 Individual Location QTL

The level of phenotypic variance explained by the different traits (Table 4.14) was generated from the QTL IciMapping software v4.1. Quite a number of single environment QTL were identified in this study. However these QTLs were not considered for further action as they were not stable across environments. Fourteen QTLs associated with ear height were reported on chromosomes 1, 2, 5, 6, 7, 8, 9 and 10. For gray leaf spot area under disease progress curve eleven QTLs were reported on chromosomes 1, 2, 3, 5, 7 and 9 (Appendix F). These QTLs contributed to the 64.49% phenotypic variation reported (Table 4.14). From the gray leaf spot evaluation trials in Kabianga long rains 2018, 20 QTLs were reported on chromosomes 1, 2, 3, 4, 5, 6, 7 and 9. In Maseno long rains 2018, 14 QTLs were reported on chromosomes 1, 2, 3, 7, 9 and 10 while in Maseno long rains 2019, 14 QTLs were detected on chromosomes 1, 2, 3, 5, 6, 9 and 10 (Appendix F).

From the *Turcicum* leaf blight evaluation trials, 12 QTLs were detected for resistance to TLB on chromosomes 2, 3, 4, 5, 7 and 8 in Maseno long rains of 2018, 22 QTLs were mapped on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9. Furthermore, ten QTLs were detected for TLB evaluation in Maseno long rains of 2019. In addition, 19 QTLs were identified on chromosomes 1, 2, 3, 4, 6, 7, 8 and 10 in Kabianga 2018 (Appendix F).

4.3.3.5 Overlapping QTL

To determine whether there were correlated allele effects between flowering time QTLs and any GLS and TLB QTLs a comparison of the QTL locations was conducted. Interestingly, none of the three QTL significantly associated with flowering time were mapped on the same positions with the QTL for resistance to GLS or TLB. This suggests that the three QTL were not likely at the same chromosomal location and did not correspond to both GLS and TLB resistance QTLs. This was exacerbated by the significantly negative correlation between the two traits. However, bin 8.05 must have been pleiotropic since it was implicated in plant height, ear height and days to silking in this study (Appendix F).

4.3.3.6 Gene action

Inclusive composite interval mapping revealed that predominantly additive gene effect defined the gene action on the trait of interest for resistance to GLS and TLB. No evidence for epistatic effect for the trait of interest was detected among these loci. Gene action at the QTL for AUDPC in the combined analysis was purely additive (Appendix F).

The additive effect indicated which parent was contributing the favourable alleles. If negative it meant the allele was coming from the donor parent and if positive it was donated from the recurrent parent. Therefore the positive and negative additive effects imply that resistance effects were either from CML511 or CML546.

CHAPTER FIVE

DISCUSSION

5.1 Characterization of the genetic diversity of *Cercospora zeina* in Kenya

5.1.1 Identifying the causal pathogen of GLS in Kenya

The PCR amplification products that were visualized on the gel gave one band per isolate and all these bands were of the same size. The *CTB7* diagnostic protocol thus reveals that only *C. zeina* is present in the Kenyan population since the *CTB7* del primer pair produced amplicons of 618 bp (Figure 4.1). In case there were isolates of *C. zeae maydis* within the population, this could have resulted in amplicons of approximately 900 base pairs in the gel (Swart et al., 2017). All the 129 isolates collected and analyzed in the current study can be confidently classified as *C. zeina*. Such correct identification of the causal pathogen is an important arsenal in the management of GLS using either chemicals or host plant resistance in Kenya and Sub Saharan Africa (Albu et al., 2016).

Kinyua et al. (2010) mentioned that a combination of both the morphological and molecular characteristics should be considered before assigning an isolate to a particular species. Besides the *CTB7* diagnostic test, other phenotypic characteristics were used to confirm that *C. zeina* was the isolate cultured. For instance morphological characteristics such as growth rate (*C. zeina* took 7-10 days from inoculation under constant darkness to produce conidia in culture) (Bluhm et al., 2008).

This study confirms the observations made by Kinyua et al. (2010) that *Cercospora zeina* is the predominant pathogen causing gray leaf spot in Kenya. Furthermore these results are congruent with the conclusions made by Crous et al. (2006); (Dunkle & Levy, 2000; Nsibo et al., 2021; Okori et al., 2003). This study further provides the evidence that *C. zeae maydis* is not known to occur in Kenya, although Kibe et al. (2020a) reported that the pathogen is prevalent in East Africa but did not provide a reference for this statement.

5.1.2 Genetic diversity of *Cercospora zeina* in the four counties of Kenya

This research focused on *Cercospora zeina* isolates collected from four counties namely, Meru, Kiambu, Tharaka Nithi and Nakuru. Coincidentally, Okori et al. (2003) had previously made collections from Nakuru as well but from a different location. A total of 129 *C. zeina* isolates were collected in this study from four different counties in Kenya. Following single spore isolations and extraction of the genomic DNA, the isolates were subjected to molecular analysis of SSR markers using previously designed primer pairs. The SSR markers used in this study had previously been characterized and genetically mapped in the genome of *C. zeina*. The 11 SSR markers used in this study (Table 4.2) were sufficient to ascertain the genotypic diversity of *C. zeina* in Kenya (Muller et al., 2016).

This analysis revealed high levels of genetic diversity among the *C. zeina* isolates collected from Kenya and possible evidence of gene flow and sexual recombination. Nei's unbiased gene diversity was 0.445 for the non-clone corrected data set and 0.456 after removing clones (Table 4.3). This variation is attributed to the high number of unique haplotypes obtained from SSR analysis. Among the 129 isolates analysed, 107 unique haplotypes were obtained. This is consistent with earlier studies that have reported high levels of genetic diversity for *C. zeina* and other members of *Cercospora* species. Muller et al. (2016) reported Nei's unbiased gene diversity of 0.35 for commercial farms, while Nsibo et al. (2019) reported Nei's gene diversity of 0.45 for smallholder farms. Similarly, Okori et al. (2003) had also reported high gene diversity of *C. zeina* isolates collected from within East Africa of about 0.34. A high level of genotypic diversity was also found in *Cercospora kikuchii* by Cai and Schneider (2005). The Shannon-Wiener index of diversity (Shannon, 2001) was also relatively high for the Kenyan population at 0.763 (Table 4.3) compared to the South African population at 0.69 (smallholder) and 0.52 (commercial) (Nsibo et al., 2019). The null hypothesis that the Kenyan population is not genetically diverse is hereby rejected.

Since gray leaf spot disease of maize was reported in Kenya in the year 1995 (Kinyua et al., 2010). The high level of genetic diversity recorded in this study is a clear indication that the pathogen has been established and could be at its peak in the country.

The samples used in the current study were collected from smallholder farmers' field who in most cases practice minimal or no use of fungicides (Nsibo et al., 2019) this allows for the build-up of the inoculum for subsequent infection hence the high genetic diversity observed. In addition, most maize cultivars grown by small-scale farmers in East Africa are susceptible to GLS (Okori et al., 2003). The populations of *C. zeina* analyzed in this study were gathered from maize fields approaching maturity and the leaves exhibited high levels of GLS lesions occasioning the peak of an outbreak of GLS. The genetic diversity could also be attributed to sexual recombination within the population of *C. zeina* in Kenya and also due to mutation events (Bolton et al., 2012).

Owing to its potential benefits to soil nutrition and plant health, conservation agriculture is increasingly getting adopted in Kenya (Mkomwa et al., 2017). This allows fungal inoculum to accumulate on plant residues that are left on the field (Berger et al., 2014; Zwonitzer et al., 2010). This could also potentially lead to the high level of genetic diversity observed among *C. zeina* isolates.

5.1.3 Partial population differentiation of *Cercospora zeina* in Kenya

The Kenyan population of *C. zeina*, exhibited a relatively small but significant level of genetic differentiation for both clone corrected (PhiPT=0.12) and non-clone corrected data sets (PhiPT = 0.15) coupled with relatively high levels of gene flow (Nm= 3.85; Table 4.5). This is a clear indication that some level of migration had taken place in the population. Muller et al. (2016) reported that South African isolates of *C. zeina* exhibited low levels of genetic differentiation (G_{ST}=0.08) and high values of gene flow (Nm=5.51) among commercial farm isolates. In

addition Shrestha et al. (2017) had also reported lack of genetic differentiation among *C. sojina* isolates from Tennessee.

The partial population differentiation reported in this study was consistent with the observations made by Nsibo et al. (2019) who also reported significant genetic differentiation (PhiPT = 0.15) between commercial and smallholder farming systems in the republic of South Africa. This suggests that both migration and gene flow are contributing to the high genotypic diversity reported in the Kenyan population of *C. zeina* collected from the four counties (Burdon & Silk, 1997).

5.1.4 Existence of partial population structure among *Cercospora zeina* populations

The analysis of the multilocus genotype data using the program STRUCTURE v2.3.2 revealed that the Kenyan population was clustered into 4 subpopulations based on the Bayesian clustering algorithm (Figure 4.3). The four subgroups were also supported by the principle coordinate analysis. However the four clusters are not that discrete hence the existence of partial population structure within the population. AMOVA showed that 88% of the variance was contributed by variations from within the counties while among counties source of variation had a variance of 12% for the four counties analyzed (Table 4.5). From the AMOVA analysis it is evident that high level of gene flow contributes to the partial population structure observed. Similarly, Naegele et al. (2014) reported 13% of variation among states compared to 69% within isolates on populations of *Phytophthora capsici* from different states in the US.

Gene flow prevents the development of population structures, promotes migration of the conidia between populations hence mixing of the clones and resulting into a homogenized population (McDermott & McDonald, 2003). In most of the previous studies, it is apparent that the populations characterized by low levels of genetic differentiation exhibited high rates of migration (gene flow).

In this study within population source of variation contributed to the greater proportion of genetic variation (88%) compared to among population source of variation (12%). This is similar to the results reported by Okori et al. (2003) where most variation was attributable to within population (99%) relative to among population sources of variation (0.35%).

The presence of population subdivision, slightly high level of gene flow and lack of shared haplotypes between different geographical locations indicates that there was clear cut geographic boundaries among the isolates. This is unusual because there is exchange of the host material between the different counties in Kenya due to their close proximity.

5.1.5 Further dissecting the partial population structure

Based on the Multi locus genotypic (MLG) data similar haplotypes could not be observed among isolates from different geographical locations. In addition to the slightly high levels of gene flow reported across the four counties in the population of *C. zeina* (Table 4.5), the high genotypic diversity reported within the Kenyan population could also be attributed to sexual recombination (Milgroom, 1996).

The slightly high level of gene flow reported in this study demonstrates the existence of slight dispersal of the conidia over long distances. This transmission of the conidia could either be caused by man or may involve seed-borne transmission (Moretti et al., 2006), although seed borne transmission has not been reported for *C. zeina*. In Kenya there is a lot of trade in green maize, it is possible that the pathogen could have migrated from one location to another after infecting the ear husks or leaf sheaths (Dunkle & Levy, 2000).

These multiple introductions could be attributed to agents of migration such as the dispersal of conidia by means of wind or human intervention through trade, travel and machinery (Sommerhalder et al., 2010). Ward et al. (1999) reported that *Cercospora zeina* can move up to an estimated distance of 40 km, whereas the distance recorded in this study between various geographical locations is approximately 100 kms and the disease is spreading yearly at a scale

of 80 to 160 km. This shows the potential for the disease to migrate between different geographical locations in Kenya.

Breeding programs in Kenya produce maize varieties that can tolerate and give maximum yield under different agro ecological conditions. Given the diverse range of agro ecological conditions from which isolates were sampled in this study, it is apparent that small-scale farmers in different geographical regions select for different maize genotypes. However it is not known whether different genotypes of maize display selection for different race types of *C. zeina* (Berger et al., 2014).

5.2 Occurrence of sexual recombination in the population

This work investigated the ability of *Cercospora zeina* populations collected from the four counties in Kenya undergoing sexual reproduction. This was conducted by determining the frequency of the MAT 1-1/ MAT 1-2 mating type idiomorphs within the Kenyan population (Figure 4.5). Approximately equal frequencies of the two *C. zeina* mating types were found in Kenya. Screening for MAT genes in *C. zeina* isolates obtained from the four counties in Kenya, revealed that one of the mating types was slightly higher in number than the other. The MAT 1-1 idiomorph was slightly high in three locations except Tharaka Nithi that exhibited equal frequencies of both MAT genes (Table 4.6).

Tharaka Nithi ($\chi^2 = 1$) had a relatively smaller sample size. Chi-square test revealed that these populations did not significantly deviate from the expected 1:1 ratio of the two MAT idiomorphs. Previous studies have exhibited a uniform distribution of mating type genes among isolates within a small population indicating high probability for sexual reproduction (Groenewald et al., 2006). Presence of mating type genes alone does not qualify a pathogen to be reproducing sexually, since mating type genes have also been reported in asexual Fusarium species (Kerényi et al., 2004). Asexual reproduction could still be taking place in the population.

Previous studies have reported that recombination could be taking place in populations of *C. zeina* (Muller et al., 2016; Nsibo et al., 2019). This study shows that *C. zeina* population in Kenya is heterothallic since it displays both MAT genes at the MAT1 locus. The results further indicate that there is a potential for sexual recombination within the *C. zeina* population in Kenya. In addition, the clonal fractions reported in this study were relatively low as compared to other studies.

When a population is in linkage disequilibrium, then there is nonrandom association of alleles at different loci. $P>0.001$ indicates that the population is not in linkage disequilibrium therefore

randomly mating. Based on the multilocus analysis of index of association (I_A) and standardized index of association (rBarD) values it can be inferred that the population exhibits no linkage disequilibrium even when populations were clone corrected (Agapow & Burt, 2001). Among the populations collected from Kenya, only one location exhibited significant linkage disequilibrium (Meru) for the non-clone corrected data sets, while the rest had low levels of gametic disequilibrium for both data sets (Table 4.6). Consistent with these results, low index of association values were reported in *C. beticola* populations undergoing sexual reproduction (Groenewald et al., 2008). The slight occurrence of sexual recombination could just be sufficient to maintain high levels of genotypic diversity.

The Kenyan population was characterized by high genetic variability, low linkage disequilibrium, low clonal fraction and nearly equal distribution of the mating type genes. Similar characteristics were reported for *M. graminicola* and *C. sojina* isolates from the US (Gurung et al., 2011; Kim et al., 2013). The ability to undergo sexual reproduction is therefore important in fungi for genomic reassortments and increasing genetic diversity (Halliday & Carter, 2003). In addition, sexual reproduction enhances adaptation of the pathogen resulting in more fit individuals that could be able to break host resistance mechanism and even develop fungicide resistance (Chen & McDonald, 1996; Kim et al., 2013; McDonald & Linde, 2002).

5.2.1 Importance of this study

The results obtained from this study will be very instrumental for management of GLS through host plant resistance in breeding programmes conducted in Kenya since the use of fungicide applications in Kenya is marginal. In addition, resistance to fungicidal sprays have been reported in other pathogens within the genus *Cercospora*. For instance *Cercospora* leaf blight was reported to condition resistance to a range of soybean fungicides (Price et al., 2015).

Information on the mating type distribution across the four counties in Kenya provides an understanding of the mode of reproduction of the pathogen (McDonald & Linde, 2002), since

recombining pathogen populations tend to be more difficult to control in economically important crops. Therefore breeding programs geared towards producing GLS tolerant maize varieties should consider the high levels of diversity and genetic structure of *C. zeina* in order to achieve durable disease resistance as reported in *C. sojina* (Kim et al., 2013).

5.2.2 Limitations of this study

Bolton et al. (2012); (Human et al., 2016) reported that for sexual recombination to occur, the isolates from both mating types should be in close proximity (single leaf) to allow for genetic exchange. However, this study did not test whether both mating types could be picked up from a single leaf or from a single lesion. But then it has been hypothesized that genetic exchange could be taking place at the end of the season when lesions coalesce to allow for sexual hybridization (Bolton et al., 2012; Human et al., 2016). In addition, this study did not look at the evolutionary history of the pathogen species and the potential of mutation in driving the high genetic diversity.

5.3 Mapping the QTL conferring resistance to gray leaf spot and *Turcicum* leaf blight

5.3.1 Heritability estimates

From this study, it can be submitted that resistance to GLS (h^2 =0.81) is closely as heritable as resistance to TLB (h^2 =0.80), given the high levels of heritability estimates recorded for both diseases (Table 4.11). In short, GLS was slightly more heritable than TLB. The heritability estimates for GLS and TLB were considerably high in all environments despite the fact that genotype by environment interaction was high and could have significantly affected heritability (Flint-Garcia et al., 2005). This implies stability of GLS and TLB resistant genotypes across diverse environments and the potential for accurate mapping of GLS and TLB resistance in the population (Almeida et al., 2013; Kump et al., 2011). Other studies that have also documented high levels of heritability for GLS include Benson et al. (2015); (Wisser et al., 2011). This suggests that genetic variation conditions most of the variance in the population (Wisser et al., 2011). Similarly high levels of heritability have been reported for other foliar diseases as well for example in the IBM population to study resistance to SLB and GLS (Balint-Kurti et al., 2008; Balint-Kurti et al., 2007). On the contrary, an initial report by Lehmensiek et al. (2001) indicated that GLS exhibited relatively low heritability of resistance.

The CML511*CML546 double haploid population was highly diverse with regards to the different traits analysed. The diversity dendogram revealed high cophenetic correlation coefficient at 0.6953. The two parents differed slightly in terms of resistance to gray leaf spot. For *Turcicum* leaf blight, CML 546 was slightly more resistant than CML 511, while for gray leaf spot, CML511 was slightly more resistant than CML 546 (Figure 4.7). The population was also highly diverse (Figure 4.9). Growing seasons were characterized by weather conditions suitable for disease development. The temperatures recorded (Table 4.7) were within the ranges reported by Wathaneeyawech et al. (2015) as being suitable for infection and colonization of the host. Kabianga 2018 was occasioned by low radiance and high precipitation compared to

Maseno 2018 and Maseno 2019, which are also the predisposing conditions for infection development.

Nyanapah et al. (2020) reported that in studying the molecular basis of quantitative disease resistance, disease severity scores recorded at the fourth to fifth reproductive stage (http://extension.entm.purdue.edu/fieldcropsipm/corn-stages.php) are nearly as effective as repeated disease assessment measures like area under the disease progress curve. This study reports a relatively continuous distribution for days to anthesis and disease severity scores for TLB at the final scoring point, plant height, ear height and days to silking. Such a distribution is associated with the quantitative nature of many minor genes acting in an additive manner that is characteristic of TLB and GLS resistance (Nyanapah et al., 2020). Most of the genotypes in the double haploid population revealed a level of resistance that was skewed towards low disease severity rates for gray leaf spot. This indicates that the hybridization of the two parental lines enhances their level of resistance to GLS (Barilli et al., 2018). The expression of low disease incidence for GLS could be in agreement with the assertions made in Wang et al. (1998), that increased GLS infections is expected in genotypes resistant to other foliar diseases such as Turcicum leaf blight, due to the reduced availability of tissues for colonization. The best linear unbiased predictions for disease severity ratings, flowering time data and other traits evaluated were utilized in this study as it reduced the estimated variance (Benson et al., 2015). BLUPs were also important to control for non-genetic factors.

5.3.2 Effect of planting season on agronomic traits

This study was mainly conducted during the long rainy season as this was the period when high disease pressure was expected from gray leaf spot and *Turcicum* leaf blight. Maseno field demonstration site lies exactly on the equator a factor that is crucial in maize production systems, the country being characterized by two maize production seasons this include the March-May and September-November due to its proximity to the equator (Hassan, 1996). The

March long rains depict the major growing season in Kenya (Hassan, 1996). The season is relatively longer (March-July) in the high altitude areas of Kenya and it is the main season during which 99% of all the maize is produced (De Groote et al., 2020). The days to anthesis was longer for a genotype planted in Kabianga (high altitude area) and the same genotype exhibited shorter days to anthesis in the slightly warmer climate of Maseno consistent with the report of Hassan (1996). Temperatures recorded during the planting season were higher in Maseno compared to Kabianga (Table 4.7).

5.3.3 Relationship between agronomic traits and disease resistance

Flowering time data was collected from both the double haploid population and the parental lines in this study. The correlation coefficient between GLS AUDPC and flowering time data was r^2 = -0.3288 at α =0.05) across environments, while the correlation between TLB AUDPC and days to anthesis data was r^2 = -0.2075 at α =0.05 across the trial environments (Table 4.10). This indicates a significantly negative correlation between disease severity data and flowering time. Lower values for area under the disease progress curve (implying or associated with higher levels of disease resistance) corresponded with longer days to anthesis, hence the late-maturing genotypes were more resistant.

Such negative correlations have previously been reported in other populations as well (Asea et al., 2009; Kolkman et al., 2020; Wisser et al., 2011). On the contrary, quite a lot of previous work have reported a positive correlation between GLS resistance and flowering time (Balint-Kurti et al., 2008; Benson et al., 2015; Liu et al., 2016; Mammadov et al., 2015; Zwonitzer et al., 2010). Using a set of 1487 inbred lines and a combination of 359 SSR markers and 8244 SNP markers, Van Inghelandt et al. (2012) reported a significantly positive correlation (r = 0.53, α = 0.05) between TLB resistance and flowering time.

All the QTLs associated with flowering time in this work did not overlap with the NLB resistance QTLs and GLS resistance QTLs. The only exception was chromosome 3 (bin 3.04),

but then this was detected only in a single environment as flowering time QTL. Such a result was consistent with the findings of Berger et al. (2014) who reported that the QTLs for GLS resistance did not correspond to the flowering time QTLs. Similarly, none of the northern corn leaf blight resistance QTLs colocalized with the QTLs controlling flowering time as also reported by Chen et al. (2015).

The positive Pearson correlation coefficient between plant height and TLB AUDPC (indicated on Table 4.10) indicates that tall genotypes are more susceptible to TLB. This could be a result of the strong correlation between height and biomass yield and planting density (Berke & Rocheford, 1995). Contrarily, Galiano-Carneiro et al. (2021) reported negative correlations between plant height and NCLB.

5.3.4 Relationship between GLS and TLB

The correlation coefficient between GLS AUDPC and TLB AUDPC BLUPs was $r^2 = 0.1563$ at $\alpha = 0.05$ across locations, indicating a significant correlation between the two diseases (Figure 4.13). Two QTL region co-localized both for TLB and GLS in bin 2.06 and bin 3.04 (Table 4.13), qTLB2_153 and *qGLS2_153* overlapped with flanking markers S2_153787894 and S2_191797557. *qGLS3_27* and *qNLB3_27* were positioned on flanking markers S3_27134253 and S3_136135473. Bin 2.06 previously recognized as a consensus QTL for resistance to gray leaf spot (Shi et al., 2007; Wisser et al., 2006), was identified in the present study to condition resistance to both GLS and TLB explaining 6.16% and 8.22% of the phenotypic variance in that order. This implies the existence of slight correlation between resistances to the two diseases. The discovery of the two QTL regions could imply that *Cercospora zeina* and *E. turcicum* could be exhibiting shared aspects of pathogenesis (Kotze et al., 2019).

Danson et al. (2008) using a RIL population of 41 genotypes with the aid of SSR markers was able to detect chromosomal bin 2.06 with strong effects on resistance to gray leaf spot in Kenya.

Liu et al. (2016) detected the QTL bin 2.06 for GLS disease scores from trials conducted in Baoshan using an $F_{2:3}$ population created by crossing YML32 with Ye478. Bin 2.06 was previously identified as conditioning resistance to TLB in a population of 220 F_3 families derived from the cross D32×D145, whereby the chromosomal bin was discovered in one disease severity rating (Welz et al., 1999).

The common QTL that are consistently reported across different genetic backgrounds point to the high reliability and importance of the corresponding genomic region towards genetic improvement programs (Cui et al., 2014). To reveal whether the QTLs detected in this study, colocalized with previously reported QTLs from other studies, a comparative analysis of the chromosome positions was conducted as detailed in Mammadov et al. (2015).

5.3.5 Quantitative Trait Loci associated with resistance to gray leaf spot

This QTL mapping study over three environments revealed nine QTLs significantly associated with resistance to gray leaf spot. A comparison of the chromosome bins reported in this study with those reported previously, revealed overlap in the disease resistance gene intervals. Two QTLs identified in this study for resistance to GLS namely 1.06 and 2.06 were part of the consensus QTLs reported in Shi et al. (2007).

The QTL for GLS resistance with the largest effect *qGLS1_190* was located in bin 1.06 and was detected across the three environments where the field trials were conducted (Appendix F). In addition, this major effect QTL bin 1.06 was also identified as a QTL for GLS AUDPC in this study. Bin 1.06 has previously been described as a QTL hotspot in that the resistance genes were observed to cluster in this bin (Shi et al., 2007). Wisser and Lauter (2018) reported that there are some regions within the genome of maize containing clusters of QTL that condition resistance to different fungal diseases. The chromosome bin 1.06 has been implicated to condition resistance to common rust, southern leaf blight (SLB), stalk and ear rot besides

GLS (Balint-Kurti et al., 2008; Chung et al., 2010b; He et al., 2018; Lehmensiek et al., 2001; Lopez-Zuniga et al., 2019; Wisser et al., 2006; Zwonitzer et al., 2010).

Bin 1.06 is an important genomic region for resistance to TLB as well. The chromosomal bin 1.06 has been documented by several studies for over a decade as conferring resistance to Northern leaf blight (Chung et al., 2010b; Freymark et al., 1993; Poland et al., 2011; Zwonitzer et al., 2010). The chromosomal region has also been associated with effects on diverse traits such as grain yield and its components, anthesis silking interval and root and shoot traits under both water stress and optimal water environments (Landi et al., 2010; Ribaut et al., 1996; Tuberosa et al., 2002). The striking importance of this QTL bin to a wide range of traits led to the fine mapping of this genomic region. The region has been sufficiently narrowed and can be utilized for marker-assisted selection in maize breeding programs (Jamann et al., 2015). This provides breeders and geneticists with markers flanking the resistance QTL and conditioning the disease resistance phenotype (Benson et al., 2015).

qGLS1_207 on chromosomal bin 1.07 associated with resistance to GLS was detected in two environments; Kabianga long rains 2018 and Maseno long rains 2019 and explained up top to 16.14% of the phenotypic variance. This QTL region overlapped with markers flanking the QTL interval associated with GLS in bins 1.05-1.07 that was previously reported by Pozar et al. (2009) and He et al. (2018). The chromosomal bin 1.07 has also been identified to be significantly associated with flowering time QTL, as it overlapped with the flowering time QTL in bin 1.07 (Berger et al., 2014; Mammadov et al., 2015). Van Inghelandt et al. (2012) postulated that this gene could play a significant role in the regulation of flowering time in maize. *qGLS1_283* on the chromosome bin 1.11 was detected in all three environments where the evaluation trials were conducted and explained up to 4.79% of the phenotypic variance and high LOD score of 8.80 (Table 16).

qGLS2_31 on chromosomal bin 2.04 was detected in two environments; Kabianga 2018 and Maseno long rains 2019 and was responsible for 4.19% of the phenotypic variance and LOD score of 6.0. Previous studies have identified this QTL as conferring resistance to GLS and Southern leaf blight using different mapping populations (Balint-Kurti et al., 2008; Lennon et al., 2017).

qGLS3_157 on the chromosomal bin 3.05 was detected in two locations namely, Maseno long rains 2018 and Maseno long rains 2019. Bin 3.05 has previously been identified as conditioning resistance to two diseases namely, southern leaf blight and gray leaf spot (Kump et al., 2011; Zwonitzer et al., 2010). A number of studies have been able to position the QTL for GLS resistance with varying levels of phenotypic variance explained (He et al., 2018; Kuki et al., 2018; Lennon et al., 2017).

qGLS3_27 on chromosomal bin 3.04 was detected in two environments and explained up to 10.13% of the phenotypic variance. *qGLS3_27* with flanking markers S3_27134253 and S3_136135473 overlapped with qGLS3-26 reported in Kibe et al. (2020a) with flanking markers S3_33059091 and S3_26022906. *qGLS4_242* on chromosome bin 4.1 flanked with S4_242295766 and S4_187911149 markers also overlapped with qGLS_204 reported in Kibe et al. (2020a) that flanked S4_203557175 and S4_224911596 markers. The confirmation of these previously reported QTLs in the present study using a different set of mapping population, suggest that these could be major QTLs for resistance to gray leaf spot. *qGLS7_158* on chromosomal bin 7.04 was detected in two environments; Kabianga 2018 and Maseno long rains 2019 and accounted for 5.4% of the phenotypic variance and LOD score of 5.4.

5.3.6 Quantitative trait loci associated with resistance to *Turcicum* leaf blight

The phenotypic distribution for TLB disease severity scores was characterized by a continuous distribution, which suggests that inheritance of resistance to TLB is quantitative in nature (Barilli et al., 2018). The current study identified 14 QTLs that were significantly associated

with resistance to Turcicum leaf blight, observed in at least two environments and conditioned significant percentage of the phenotypic variance (Table 4.13). *qTLB1_28* on the maize chromosomal bin 1.02 was detected in two environments; in the long rains of Maseno 2018 and long rains of Maseno 2019 and accounted for 6.36% of the phenotypic variation. Bin 1.02 has also been associated with resistance to three fungal diseases of maize namely, gray leaf spot, Stewart's wilt and common rust (Chung et al., 2010a; Kuki et al., 2018; Mammadov et al., 2015) Chung et al. (2010a) while characterizing the developmental stages of *E. turcicum* in maize, discovered that the chromosomal bin 1.02 conferred resistance to TLB by enhancing the induction of host defence response in the cells neighbouring the site of infection, reducing the growth of the hyphae into the adjacent bundle sheath cells and inhibiting subsequent colonization in the leaves. This shows the importance of this QTL region.

A major QTL for TLB resistance *qTLB2_186* on bin 2.07 associated with 12.5% phenotypic variance and LOD of 9.39 was detected in two environments Maseno long rains 2018 and Maseno long rains 2019. The chromosomal bin 2.07 has been implicated to confer resistance to multiple diseases namely, gray leaf spot and southern leaf blight (Kuki et al., 2018; Mammadov et al., 2015).

Another major effect QTL for TLB resistance *qTLB3_27* on the chromosomal bin 3.04 was detected in all three environments where the field trials were conducted and explained up to 12.27% of the phenotypic variance. Previous research work and articles have identified bin 3.04 as a QTL hotspot conditioning resistance to multiple diseases (Martins et al., 2019; Shi et al., 2007; Wisser et al., 2006). Apart from Northern leaf blight, significant QTLs for resistance to Southern leaf blight and gray leaf spot has been identified in this chromosomal region (Kump et al., 2011; Lehmensiek et al., 2001; Lennon et al., 2017; Liu et al., 2016; Zwonitzer et al., 2010).

qTLB6_151 on the chromosomal bin 6.05 was detected in two environments namely, Maseno long rains 2018 and Maseno long rains 2019 and explained up to 8.61 of the phenotypic variance, this bin 6.05 was also reported in Chen et al. (2015); He et al. (2018); Van Inghelandt et al. (2012); Welz et al. (1999); Zwonitzer et al. (2010). This QTL has been defined as a hotspot for resistance to TLB (Miedaner et al., 2020).

qTLB7_146 on the chromosomal bin 7.03 was discovered in two environments in this study and accounted for 4.09% of the phenotypic variance. Bin 7.03 has been reported in Chen et al. (2015) in a RIL population derived from K22×By 815. Bin 7.03 was also found to be a stress adaptive QTL significantly associated with anthesis silking interval, grain yield and was detected majorly under water stress environments (Almeida et al., 2013). *qTLB10_87* on maize chromosome bin 10.04 was reported in the present study to condition resistance to TLB in at least two environments (Maseno long rains 2018 and Maseno long rains 2019) and accounted for 5.19% of the phenotypic variation. Bin 10.04 has also been reported to have a strong constitutive effect on grain yield under water stress conditions (Almeida et al., 2013).

None of the TLB QTLs in this study was found in the same position with chromosomal bins associated with the qualitative *Ht* genes. The major genes were reported in bins 2.08, 8.06, 7.04, 8.07, 8.05 and 3.06 (Galiano-Carneiro & Miedaner, 2017) that were not identified in the current study.

5.3.7 Quantitative trait loci associated with Flowering time

Experimental trials over three environments in addition to the genotypic data revealed three QTLs for the flowering time that were identified in multiple environments (days to anthesis QTL in maize bin 1.09, bin 4.08/4.09, bin 8.05) whereas two QTL (bin 3.04 and bin 2.06) were only obtained in a single environment. The chromosomal bin 3.04 was also reported by Zwonitzer et al. (2010) as a flowering time QTL. According to Zwonitzer et al. (2010) the chromosomal bin 1.09, was reported to be involved in resistance to multiple diseases namely,

Southern leaf blight (SLB) and TLB but was not significant as a flowering time QTL. The flowering time QTL bin 1.09 detected in the current study conditioned up to 5.74% of the phenotypic variance in flowering time. The maize chromosomal bin 4.09 identified in this study as a flowering time QTL was previously documented by Berger et al. (2014) in the CML444*SC Malawi population and Balint-Kurti et al. (2008).

According to the results of this study, bin 8.05 is a pleiotropic QTL and was significantly associated with different traits such as anthesis date, silking date, ear height and plant height. Previous studies have recognized chromosomal bin 8.05 as a hotspot for flowering time QTLs and genes (Balint-Kurti et al., 2008; Buckler et al., 2009; Van Inghelandt et al., 2012). *Vegetative to generative transition 1* (*vgt1*) gene which is a flowering time QTL for floral transition in maize has been mapped in bin 8.05 (Salvi et al., 2002). Bin 8.05 has also been implicated to have a role in Auxin-responsive GH3 family protein and Protein phosphatase (Kolkman et al., 2020). Interestingly, two qualitative resistance genes namely, *Ht2* and *Htn1*, were detected on the chromosomal bin 8.05. (Galiano-Carneiro & Miedaner, 2017)

Similar QTL for days to silking was discovered for days to anthesis, this is consistent with the conclusions made by Buckler et al. (2009) who reported that the genetic mechanisms of male and female flowering appear to be under the control of the same set of genes. The genetic mechanisms underlying flowering time in this study were largely characterized by additive gene action. These results are in agreement with the findings of Buckler et al. (2009) who reported that variations in days to flowering are not as a result of a few genes with large effect but due to the joint effect of many minor QTLs with additive effect on the trait of interest.

A major QTL has been defined as a QTL associated with more than 10% of the phenotypic variance (Collard et al., 2005). Some of the large effect QTLs detected in this study such as *qGLS1_190* could be introgressed into elite susceptible lines through marker-assisted backcrossing for increased resistance.

5.3.8 Quantitative trait loci affecting plant height and ear height

Poland et al. (2011) hypothesized that genes significantly associated with growth, development and plant architectural attributes could have pleiotropic effects on resistance to pathogenic infections. In this study, ear height QTLs were reported in bins 1.02, 6.01, 8.05 and 9.06. Plant height QTLs were also reported on bin 1.02, bin 6.01, bin 8.03 and bin 8.05. Bin 8.05 explained up to 14% of the phenotypic variation in plant height and 14.8% of the phenotypic variation in ear height (Appendix F). This study found significantly positive interaction between ear height, plant height and GLS area under disease progress curve, TLB area under disease progress curve (Table 4.10). This presents a clear evidence of the existence of an association between flowering time and plant architecture. In addition plant height and ear height have been reported to be highly correlated and tightly linked (Choi et al., 2019) hence the same QTLs were reported for both traits.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Objective 1: To analyze the genetic diversity of *Cercospora zeina* as the causal pathogen of gray leaf spot in Kenya.

This study confirms *Cercospora zeina* as the predominant cause of GLS on *Zea mays* in Kenya based on the 129 isolates collected from the four counties and analyzed using the *CTB7* diagnostic test. Microsatellite marker analysis revealed high genetic variability existing within the population of *Cercospora zeina* collected from four counties in Kenya. In addition high levels of polymorphisms coupled with intermediate levels of gene flow were also detected in the population. The isolates of *C. zeina* collected from Kenya also exhibited partial population structure based on the STRUCTURE analysis.

Objective 2: To determine the role sexual recombination plays in driving the genetic diversity of *Cercospora zeina*.

The population was also characterized by nearly equal distribution of the mating type genes providing evidence that sexual recombination could be responsible for the genetic structure of *C. zeina.* To a large extent, the high genetic diversity in the population from the four counties in Kenya is attributed to the occurrence of sexual recombination.

Objective 3: To identify the QTLs associated with gray leaf spot and *Turcicum* leaf blight resistance in CIMMYT DH population (CML 511×CML 546).

In the present study, the QTL analysis detected nine QTLs for resistance to gray leaf spot that was identified based on their expression in multiple environments. The QTL for GLS resistance with the largest phenotypic variance explained, *qGLS1_190* was detected in all the three environments where the field trials were conducted. In addition, there were 14 TLB resistance QTL and three QTL were detected for flowering time. Most of the QTLs explained significant

proportions of the phenotypic variance. A negative correlation between flowering time and the two diseases was reported as none of the identified QTL for flowering time overlapped with the QTL for TLB and GLS resistance. The manifold of QTL identified in the present study co-localized with QTLs previously mapped in the hotspot for GLS and TLB resistance QTL within the genome of maize. Following the initial identification of these QTLs in the present study, the region of interest may involve a large confidence interval that may be narrowed through fine mapping. The inbred lines CML511 and CML546 and the progenies of the DH population could be an excellent source of resistance to GLS and TLB in population improvement programs.

RECOMMENDATIONS

Practical recommendations based on the study

Cercospora zeae maydis is not known to occur in Kenya. Stringent sanitary and phytosanitary measures should be put in place to prevent entry of the pathogen into the country.

For subsequent monitoring studies and research aiming to identify the causal pathogen of gray leaf spot, CTB7 diagnostic test is a powerful tool that would improve identification of the species.

Disease management practices should focus on preventing the occurrence of sexual reproduction to assist in the control of gray leaf spot. Sexual reproduction takes place at the end of the season on maize residues, avoiding the use of no till practices could prevent it.

These results indicate that the inbred line CML511 and the progenies of the DH population could be an excellent source of resistance to GLS in population improvement programs.

The DNA markers that are closer to the QTLs can be characterized for their subsequent use in maize breeding programs via marker assisted selection.

Recommendations for further research

A study is needed to identify whether different maize genotypes select for different haplotypes of *Cercospora zeina* within the country.

Following the initial identification of these QTLs in this study, QTL effects associated with TLB and GLS resistance are affected by environmental factors. Further investigation should be undertaken to validate the identified QTLs and their additive effects.

Future research is needed to fine map the TLB and GLS QTLs identified in this study by improving the marker density for their subsequent utilization in maize breeding programs. The region of interest may involve a large confidence interval that may be narrowed through fine mapping.

REFERENCES

Abate, T., Mugo, S., De Groote, H., & Regassa, M. W. . (2015). Maize in Kenya: Chance for Getting Back to Former Glory. DT Maize. *(CIMMYT), 4(3), 1-3*.

Adipala, E., Lipps, P., & Madden, L. (1993). Reaction of maize cultivars from Uganda to Exserohilum turcicum. *Phytopathology*, *83*(2), 271-223.

Agapow, P.-M., & Burt, A. (2001). Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes*, *1*(1-2), 101-102. https://doi.org/https://doi.org/10.1046/j.1471-8278.2000.00014.x

Akbari, M., Wenzl, P., Caig, V., Carling, J., Xia, L., Yang, S., Uszynski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M. J., Howes, N., Sharp, P., Vaughan, P., Rathmell, B., Huttner, E., & Kilian, A. (2006). Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor Appl Genet*, *113*(8), 1409-1420. https://doi.org/10.1007/s00122-006-0365-4

Albu, S., Schneider, R. W., Price, P. P., & Doyle, V. P. (2016). *Cercospora* cf. *flagellaris* and *Cercospora* cf. *sigesbeckiae* Are Associated with *Cercospora* Leaf Blight and Purple Seed Stain on Soybean in North America. *Phytopathology*, *106*(11), 1376-1385. https://doi.org/10.1094/phyto-12-15-0332-r

Almeida, G. D., Makumbi, D., Magorokosho, C., Nair, S., Borém, A., Ribaut, J. M., Bänziger, M., Prasanna, B. M., Crossa, J., & Babu, R. (2013). QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance. *Theor Appl Genet*, *126*(3), 583-600. https://doi.org/10.1007/s00122-012-2003-7

Alvarado, G., Rodríguez, F., Pacheco, A., Burgueño, J., Crossa, J., Vargas, M., Pérez-Rodríguez, P., & Lopez-Cruz, M. (2020). META-R: A software to analyze data from multi-environment plant breeding trials. *The Crop Journal*, *8*. https://doi.org/10.1016/j.cj.2020.03.010

Asea, G., Vivek, B. S., Bigirwa, G., Lipps, P. E., & Pratt, R. C. (2009). Validation of consensus quantitative trait loci associated with resistance to multiple foliar pathogens of maize. *Phytopathology*, *99*(5), 540-547. https://doi.org/10.1094/phyto-99-5-0540

Asíns, M. J. (2002). Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding*, *121*(4), 281-291. https://doi.org/https://doi.org/10.1046/j.1439-0523.2002.730285.x

Ayala Hernández, C. C., Vivek, B., & Banziger, M. (2020). *Fieldbook2020* Version V2) CIMMYT Research Data & Software Repository Network. https://doi.org/hdl:11529/10548370

Balint-Kurti, P., & Johal, G. (2009). Handbook of maize: its biology. In.

Balint-Kurti, P., Yang, J., Esbroeck, G., Jung, J., & Smith, M. (2010). Use of a Maize Advanced Intercross Line for Mapping of QTL for Northern Leaf Blight Resistance and Multiple Disease Resistance. *Crop Science*, *50*. https://doi.org/10.2135/cropsci2009.02.0066

Balint-Kurti, P. J., Wisser, R. J., & Zwonitzer, J. C. (2008). Use of an Advanced Intercross Line Population for Precise Mapping of Quantitative Trait Loci for Gray Leaf Spot Resistance in Maize. *Crop Science*, *48*, 1696-1704.

Balint-Kurti, P. J., Zwonitzer, J. C., Wisser, R. J., Carson, M. L., Oropeza-Rosas, M. A., Holland, J. B., & Szalma, S. J. (2007). Precise mapping of quantitative trait loci for resistance to southern leaf

blight, caused by *Cochliobolus heterostrophus* race O, and flowering time using advanced intercross maize lines. *Genetics*, *176*(1), 645-657. https://doi.org/10.1534/genetics.106.067892

Barilli, E., Cobos, M. J., Carrillo, E., Kilian, A., Carling, J., & Rubiales, D. (2018). A High-Density Integrated DArTseq SNP-Based Genetic Map of *Pisum fulvum* and Identification of QTLs Controlling Rust Resistance. *Front Plant Sci*, *9*, 167. https://doi.org/10.3389/fpls.2018.00167

Beckman, P. M., & Payne, G. A. (1983). Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. *Phytopathology*, *73*(2), 286-289.

Bennetzen, J. L., & Hake, S. (2009). *Handbook of maize: Genetics and genomics*. https://doi.org/10.1007/978-0-387-77863-1

Benson, J. M., Poland, J. A., Benson, B. M., Stromberg, E. L., & Nelson, R. J. (2015). Resistance to gray leaf spot of maize: genetic architecture and mechanisms elucidated through nested association mapping and near-isogenic line analysis. *PLoS Genet*, *11*(3), e1005045. https://doi.org/10.1371/journal.pgen.1005045

Berger, D. K., Carstens, M., Korsman, J. N., Middleton, F., Kloppers, F. J., Tongoona, P., & Myburg, A. A. (2014). Mapping QTL conferring resistance in maize to gray leaf spot disease caused by Cercospora zeina. *BMC Genet*, *15*, 60. https://doi.org/10.1186/1471-2156-15-60

Berger, D. K., Mokgobu, T., de Ridder, K., Christie, N., & Aveling Theresa, A. S. (2020). Benefits of maize resistance breeding and chemical control against northern leaf blight in smallholder farms in South Africa. *South African Journal of Science*, *116*(11-12), 113-119. https://doi.org/10.17159/sajs.2020/8286

Berke, T. G., & Rocheford, T. (1995). Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. *Crop Science*, *35*, 1542-1549.

Beyene, Y., Gowda, M., Olsen, M., Robbins, K. R., Pérez-Rodríguez, P., Alvarado, G., Dreher, K., Gao, S. Y., Mugo, S., Prasanna, B. M., & Crossa, J. (2019). Empirical Comparison of Tropical Maize Hybrids Selected Through Genomic and Phenotypic Selections. *Front Plant Sci*, *10*, 1502. https://doi.org/10.3389/fpls.2019.01502

Bluhm, B. H., Dhillon, B., Lindquist, E. A., Kema, G. H., Goodwin, S. B., & Dunkle, L. D. (2008). Analyses of expressed sequence tags from the maize foliar pathogen *Cercospora zeae-maydis* identify novel genes expressed during vegetative, infectious, and reproductive growth. *BMC Genomics*, *9*, 523. https://doi.org/10.1186/1471-2164-9-523

Bolton, M., Secor, G., Rivera, V., Weiland, J., Rudolph, K., Birla, K., Rengifo, J., & Campbell, L. (2012). Evaluation of the potential for sexual reproduction in field populations of *Cercospora beticola* from USA. *Fungal biology*, *116*, 511-521. https://doi.org/10.1016/j.funbio.2012.01.011

Bolton, M. D., de Jonge, R., Inderbitzin, P., Liu, Z., Birla, K., Van de Peer, Y., Subbarao, K. V., Thomma, B. P. H. J., & Secor, G. A. (2014). The heterothallic sugarbeet pathogen *Cercospora beticola* contains exon fragments of both MAT genes that are homogenized by concerted evolution. *Fungal Genetics and Biology*, *62*, 43-54. https://doi.org/https://doi.org/10.1016/j.fgb.2013.10.011

Borchardt, D. S., Welz, H. G., & Geiger, H. H. (1998). Genetic Structure of *Setosphaeria turcica* Populations in Tropical and Temperate Climates. *Phytopathology*, *88 4*, 322-329.

Brunelli, K. R., Dunkle, L. D., Sobrinho, C. A., Fazza, A. C., & Camargo, L. E. (2008). Molecular variability in the maize grey leaf spot pathogens in Brazil. *Genetics and Molecular Biology*, *31*, 938-942.

Bubeck, D. M., Goodman, M. M., Beavis, W. D., & Grant, D. (1993). Quantitative Trait Loci Controlling Resistance to Gray Leaf Spot in Maize. *Crop Science*, *33*(4), cropsci1993.0011183X003300040041x. https://doi.org/https://doi.org/10.2135/cropsci1993.0011183X003300040041x

Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., Ersoz, E., Flint-Garcia, S., Garcia, A., Glaubitz, J. C., Goodman, M. M., Harjes, C., Guill, K., Kroon, D. E., Larsson, S., Lepak, N. K., Li, H., Mitchell, S. E., Pressoir, G., . . . McMullen, M. D. (2009). The genetic architecture of maize flowering time. *Science*, *325*(5941), 714-718. https://doi.org/10.1126/science.1174276

Bunkoed, W., Kasam, S., Chaijuckam, P., Yhamsoongnern, J., & Prathuangwong, S. (2014). Sexual reproduction of *Setosphaeria turcica* in natural corn fields in Thailand. *Kasetsart Journal - Natural Science*, *48*, 175-182.

Burdon, J. J., & Silk, J. (1997). Sources and Patterns of Diversity in Plant-Pathogenic Fungi. *Phytopathology®*, *87*(7), 664-669. https://doi.org/10.1094/phyto.1997.87.7.664

Cai, G., & Schneider, R. W. (2005). Vegetative Compatibility Groups in *Cercospora kikuchii*, the Causal Agent of *Cercospora* Leaf Blight and Purple Seed Stain in Soybean. *Phytopathology*, *95 3*, 257-261.

Carson, M. (1995). A new gene in maize conferring the" chlorotic halo" reaction to infection by *Exserohilum turcicum*.

Chen, G., Wang, X., Long, S., Jaqueth, J., Li, B., Yan, J., & Ding, J. (2015). Mapping of QTL conferring resistance to northern corn leaf blight using high-density SNPs in maize. *Molecular Breeding*, *36*(1), 4. https://doi.org/10.1007/s11032-015-0421-3

Chen, R. S., & McDonald, B. A. (1996). Sexual reproduction plays a major role in the genetic structure of populations of the fungus *Mycosphaerella graminicola*. *Genetics*, *142*(4), 1119-1127. https://doi.org/10.1093/genetics/142.4.1119

Choi, J. K., Sa, K. J., Park, D. H., Lim, S. E., Ryu, S. H., Park, J. Y., Park, K. J., Rhee, H. I., Lee, M., & Lee, J. K. (2019). Construction of genetic linkage map and identification of QTLs related to agronomic traits in DH population of maize (*Zea mays* L.) using SSR markers. *Genes Genomics*, *41*(6), 667-678. https://doi.org/10.1007/s13258-019-00813-x

Chung, C. L., Jamann, T., Longfellow, J., & Nelson, R. (2010a). Characterization and fine-mapping of a resistance locus for northern leaf blight in maize bin 8.06. *Theor Appl Genet*, *121*(2), 205-227. https://doi.org/10.1007/s00122-010-1303-z

Chung, C. L., Longfellow, J. M., Walsh, E. K., Kerdieh, Z., Van Esbroeck, G., Balint-Kurti, P., & Nelson, R. J. (2010b). Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize--*Setosphaeria turcica* pathosystem. *BMC Plant Biol*, *10*, 103. https://doi.org/10.1186/1471-2229-10-103

CIMMYT, G. M. P. (2015). *CIMMYT Maize Lines (CMLs) - Pedigree and characterization data* Version DRAFT VERSION) CIMMYT Research Data & Software Repository Network. https://doi.org/hdl:11529/10246

Clements, M. J., Dudley, J. W., & White, D. G. (2000). Quantitative trait Loci associated with resistance to gray leaf spot of corn. *Phytopathology*, *90*(9), 1018-1025. https://doi.org/10.1094/phyto.2000.90.9.1018

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci*, *363*(1491), 557-572. https://doi.org/10.1098/rstb.2007.2170

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1), 169-196. https://doi.org/10.1007/s10681-005-1681-5

Cooper, M., DeLacy, I. H., Basford, K. E., & Hammer, G. L. (1996). Relationships among analytical methods used to analyse genotypic adaptation in multi-environment trials.

Crous, P. W., Groenewald, J. Z., Groenewald, M., Caldwell, P., Braun, U., & Harrington, T. C. (2006). Species of *Cercospora* associated with grey leaf spot of maize. *Stud Mycol*, *55*, 189-197. https://doi.org/10.3114/sim.55.1.189

Cui, F., Zhao, C., Ding, A., Li, J., Wang, L., Li, X., Bao, Y., Li, J., & Wang, H. (2014). Construction of an integrative linkage map and QTL mapping of grain yield-related traits using three related wheat RIL populations. *Theor Appl Genet*, *127*(3), 659-675. https://doi.org/10.1007/s00122-013-2249-8

Danson, J., Lagat, M., Kimani, M., & Kuria, A. (2008). Quantitative trait loci (QTLs) for resistance to gray leaf spot and common rust diseases of maize. *African Journal of Biotechnology*, *7*(18).

Daub, M. E., & Ehrenshaft, M. (2000). The Photoactivated *Cercospora* Toxin Cercosporin: Contributions to Plant Disease and Fundamental Biology. *Annu Rev Phytopathol*, *38*, 461-490. https://doi.org/10.1146/annurev.phyto.38.1.461

De Groote, H., Kimenju, S. C., Munyua, B., Palmas, S., Kassie, M., & Bruce, A. (2020). Spread and impact of fall armyworm (*Spodoptera frugiperda* J.E. Smith) in maize production areas of Kenya. *Agric Ecosyst Environ*, *292*, 106804. https://doi.org/10.1016/j.agee.2019.106804

de Jonge, R., Ebert, M. K., Huitt-Roehl, C. R., Pal, P., Suttle, J. C., Spanner, R. E., Neubauer, J. D., Jurick, W. M., 2nd, Stott, K. A., Secor, G. A., Thomma, B., Van de Peer, Y., Townsend, C. A., & Bolton, M. D. (2018). Gene cluster conservation provides insight into cercosporin biosynthesis and extends production to the genus *Colletotrichum*. *Proc Natl Acad Sci U S A*, *115*(24), E5459-e5466. https://doi.org/10.1073/pnas.1712798115

Dean, A. M. (2018). Haploids, polymorphisms and fluctuating selection. *Theor Popul Biol*, *124*, 16-30. https://doi.org/10.1016/j.tpb.2018.07.003

Derera, J., Tongoona, P., Pixley, K. V., Vivek, B., Laing, M. D., & van Rij, N. C. (2008). Gene action controlling gray leaf spot resistance in southern African maize germplasm. *Crop Science*, *48*(1), 93-98.

Ding, J., Ali, F., Chen, G., Li, H., Mahuku, G., Yang, N., Narro, L., Magorokosho, C., Makumbi, D., & Yan, J. (2015). Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. *BMC Plant Biol*, *15*, 206. https://doi.org/10.1186/s12870-015-0589-z

Dingerdissen, A. L., Geiger, H. H., Lee, M., Schechert, A., & Welz, H. G. (1996). Interval mapping of genes for quantitative resistance of maize to Setosphaeria turcica, cause of northern leaf blight, in a tropical environment. *Molecular Breeding*, *2*(2), 143-156. https://doi.org/10.1007/BF00441429

Dunkle, L. D., & Levy, M. (2000). Genetic Relatedness of African and United States Populations of *Cercospora zeae-maydis*. *Phytopathology*, *90*(5), 486-490. https://doi.org/10.1094/phyto.2000.90.5.486

Dwivedi, S. L., Britt, A. B., Tripathi, L., Sharma, S., Upadhyaya, H. D., & Ortiz, R. (2015). Haploids: Constraints and opportunities in plant breeding. *Biotechnol Adv*, *33*(6 Pt 1), 812-829. https://doi.org/10.1016/j.biotechadv.2015.07.001

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359-361. https://doi.org/10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol*, *14*(8), 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, *131*(2), 479-491. https://doi.org/10.1093/genetics/131.2.479

Fan, Y., Ma, J., Gui, X., An, X., Sun, S., & Dong, J. (2007). Distribution of mating types and genetic diversity induced by sexual recombination in *Setosphaeria turcica* in northern China. *Frontiers of Agriculture in China*, *1*(4), 368-376. https://doi.org/10.1007/s11703-007-0062-3

Ferguson, L. M., & Carson, M. L. (2004). Spatial Diversity of *Setosphaeria turcica* Sampled from the Eastern United States. *Phytopathology*, *94*(8), 892-900. https://doi.org/10.1094/phyto.2004.94.8.892

Ferguson, L. M., & Carson, M. L. (2007). Temporal Variation in *Setosphaeria turcica* Between 1974 and 1994 and Origin of Races 1, 23, and 23N in the United States. *Phytopathology*, *97*(11), 1501-1511. https://doi.org/10.1094/phyto-97-11-1501

Flint-Garcia, S. A., Thuillet, A. C., Yu, J., Pressoir, G., Romero, S. M., Mitchell, S. E., Doebley, J., Kresovich, S., Goodman, M. M., & Buckler, E. S. (2005). Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant J*, *44*(6), 1054-1064. https://doi.org/10.1111/j.1365-313X.2005.02591.x

French, E., Kim, B.-S., & Iyer-Pascuzzi, A. S. (2016). Mechanisms of quantitative disease resistance in plants. *Seminars in Cell & Developmental Biology*, *56*, 201-208. https://doi.org/https://doi.org/10.1016/j.semcdb.2016.05.015

Freymark, P. J., Lee, M., Woodman, W. L., & Martinson, C. A. (1993). Quantitative and qualitative trait loci affecting host-plant response to *Exserohilum turcicum* in maize (*Zea mays* L.). *Theor Appl Genet*, *87*(5), 537-544. https://doi.org/10.1007/bf00221876

Galiano-Carneiro, A. L., Kessel, B., Presterl, T., & Miedaner, T. (2021). Intercontinental trials reveal stable QTL for Northern corn leaf blight resistance in Europe and in Brazil. *Theor Appl Genet*, *134*(1), 63-79. https://doi.org/10.1007/s00122-020-03682-1

Galiano-Carneiro, A. L., & Miedaner, T. (2017). Genetics of Resistance and Pathogenicity in the Maize/*Setosphaeria turcica* Pathosystem and Implications for Breeding. *Front Plant Sci*, *8*, 1490. https://doi.org/10.3389/fpls.2017.01490

Gevers, H., & Lake, J. (1994). GLSI-A major gene for resistance to grey leaf spot in maize. *South African Journal of Science*, *90*(7), 377-380.

Goodwin, S. B., Dunkle, L. D., & Zismann, V. L. (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. *Phytopathology*, *91*(7), 648-658. https://doi.org/10.1094/phyto.2001.91.7.648

Gordon, S. G. (2004). Linkage of molecular markers to *Cercospora zeae-maydis* resistance in maize. *Crop Science*, *v. 44*(no. 2), pp. 628-636-2004 v.2044 no.2002.

Gordon, S. G., Lipps, P. E., & Pratt, R. C. (2006). Heritability and Components of Resistance to *Cercospora zeae-maydis* Derived from Maize Inbred VO613Y. *Phytopathology*, *96*(6), 593-598. https://doi.org/10.1094/phyto-96-0593

Groenewald, M., Groenewald, J. Z., Harrington, T. C., Abeln, E. C., & Crous, P. W. (2006). Mating type gene analysis in apparently asexual *Cercospora* species is suggestive of cryptic sex. *Fungal Genet Biol*, *43*(12), 813-825. https://doi.org/10.1016/j.fgb.2006.05.008

Groenewald, M., Linde, C. C., Groenewald, J. Z., & Crous, P. W. (2008). Indirect evidence for sexual reproduction in *Cercospora beticola* populations from sugar beet [Article]. *Plant Pathology*, *2008 v.57 no.1*(no. 1), pp. 25-32. https://doi.org/10.1111/j.1365-3059.2007.01697.x

Gurung, S., Goodwin, S. B., Kabbage, M., Bockus, W. W., & Adhikari, T. B. (2011). Genetic Differentiation at Microsatellite Loci Among Populations of *Mycosphaerella graminicola* from California, Indiana, Kansas, and North Dakota [Article]. *Phytopathology*, *2011 v.101 no.10*(no. 10), pp. 1251-1259. https://doi.org/10.1094/phyto-08-10-0212

Halliday, C. L., & Carter, D. A. (2003). Clonal reproduction and limited dispersal in an environmental population of *Cryptococcus neoformans* var *gattii* isolates from Australia. *J Clin Microbiol*, *41*(2), 703-711. https://doi.org/10.1128/jcm.41.2.703-711.2003

Hassan, R. M. (1996). Planting strategies of maize farmers in Kenya: a simultaneous equations analysis in the presence of discrete dependent variables. *Agricultural Economics*, *15*(2), 137-149. https://doi.org/https://doi.org/10.1111/j.1574-0862.1996.tb00427.x

He, W., Yang, L., Leng, Y., Zhang, B., Yang, J., Li, L., Chen, Y., Kang, J., Tang, H., & Deng, L. (2018). QTL mapping for resistance of maize to grey leaf spot. *Journal of Phytopathology*, *166*(3), 167-176.

Hilu, H., & Hooker, A. (1964). HOST-PATHOGEN RELATIONSHIP OF *Helminthosporium turcicum* IN RESISTANT+ SUSCEPTIBLE CORN SEEDLINGS. *Phytopathology*, *54*(5), 570-&.

Holland, J. B., & Coles, N. D. (2011). QTL Controlling Masculinization of Ear Tips in a Maize (*Zea mays* L.) Intraspecific Cross. *G3 (Bethesda)*, *1*(5), 337-341. https://doi.org/10.1534/g3.111.000786

Hsieh, L. S. (2011). Coexistence Of Sibling Species Of *Cercospora* Causing Gray Leaf Spot On Maize In Southern New York State.

Human, M. P., Barnes, I., Craven, M., & Crampton, B. G. (2016). Lack of Population Structure and Mixed Reproduction Modes in *Exserohilum turcicum* from South Africa. *Phytopathology*, *106*(11), 1386-1392. https://doi.org/10.1094/phyto-12-15-0311-r

Human, M. P., Berger, D. K., & Crampton, B. G. (2020). Time-Course RNAseq Reveals *Exserohilum turcicum* Effectors and Pathogenicity Determinants. *Front Microbiol*, *11*, 360. https://doi.org/10.3389/fmicb.2020.00360

Hurni, S., Scheuermann, D., Krattinger, S. G., Kessel, B., Wicker, T., Herren, G., Fitze, M. N., Breen, J., Presterl, T., Ouzunova, M., & Keller, B. (2015). The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc Natl Acad Sci U S A*, *112*(28), 8780-8785. https://doi.org/10.1073/pnas.1502522112

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res*, *29*(4), E25. https://doi.org/10.1093/nar/29.4.e25

Jacquier, N. M. A., & Widiez, T. (2021). Absent daddy, but important father. *Nat Plants*, *7*(12), 1544-1545. https://doi.org/10.1038/s41477-021-01030-9

Jamann, T. M., Balint-Kurti, P. J., & Holland, J. B. (2015). QTL mapping using high-throughput sequencing. *Methods Mol Biol*, *1284*, 257-285. https://doi.org/10.1007/978-1-4939-2444-8_13

Jenco, H., & FW Jr, N. (1992). Diurnal and seasonal periodicity of *Cercospora zeae-maydis* in Iowa. *Phytopathology*, *82*, 992.

Jenns, A., Daub, M., & Upchurch, R. (1989). Regulation of cercosporin accumulation in culture by medium and temperature manipulation. *Phytopathology*, *79*(2), 213-219.

Juliatti, F. C., Pedrosa, M. G., Silva, H. D., & da Silva, J. V. C. (2009). Genetic mapping for resistance to gray leaf spot in maize. *Euphytica*, *169*(2), 227-238. https://doi.org/10.1007/s10681-009-9943-2

Kerényi, Z., Moretti, A., Waalwijk, C., Oláh, B., & Hornok, L. (2004). Mating type sequences in asexually reproducing *Fusarium* species. *Appl Environ Microbiol*, *70*(8), 4419-4423. https://doi.org/10.1128/aem.70.8.4419-4423.2004

Kibe, M., Nair, S. K., Das, B., Bright, J. M., Makumbi, D., Kinyua, J., Suresh, L. M., Beyene, Y., Olsen, M. S., Prasanna, B. M., & Gowda, M. (2020a). Genetic Dissection of Resistance to Gray Leaf Spot by Combining Genome-Wide Association, Linkage Mapping, and Genomic Prediction in Tropical Maize Germplasm. *Front Plant Sci*, *11*, 572027. https://doi.org/10.3389/fpls.2020.572027

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., Hopper, C., Aschenbrenner-Kilian, M., Evers, M., Peng, K., Cayla, C., Hok, P., & Uszynski,

G. (2012). Diversity arrays technology: a generic genome profiling technology on open platforms. *Methods Mol Biol*, *888*, 67-89. https://doi.org/10.1007/978-1-61779-870-2_5

Kim, H., Newell, A. D., Cota-Sieckmeyer, R. G., Rupe, J. C., Fakhoury, A. M., & Bluhm, B. H. (2013). Mating-type distribution and genetic diversity of *Cercospora sojina* populations on soybean from Arkansas: evidence for potential sexual reproduction. *Phytopathology*, *103*(10), 1045-1051. https://doi.org/10.1094/phyto-09-12-0229-r

Kimura, M., Anzai, H., & Yamaguchi, I. (2001). Microbial toxins in plant-pathogen interactions: Biosynthesis, resistance mechanisms, and significance. *J Gen Appl Microbiol*, *47*(4), 149-160. https://doi.org/10.2323/jgam.47.149

Kinyua, Z. M., Smith, J. J., Kibata, G. N., Simons, S. A., & Langat, B. C. (2010). Status of grey leaf spot disease in Kenyan maize production ecosystems. *African Crop Science Journal*, *18*(4), 183-194. https://www.ajol.info/index.php/acsj/article/view/68647

Kloppers, R., & Tweer, S. (2009). Northern corn leaf blight. *Northern corn leaf blight.*

Kolkman, J. M., Strable, J., Harline, K., Kroon, D. E., Wiesner-Hanks, T., Bradbury, P. J., & Nelson, R. J. (2020). Maize Introgression Library Provides Evidence for the Involvement of *liguleless1* in Resistance to Northern Leaf Blight. *G3 (Bethesda)*, *10*(10), 3611-3622. https://doi.org/10.1534/g3.120.401500

Korsman, J., Meisel, B., Kloppers, F. J., Crampton, B. G., & Berger, D. K. (2012). Quantitative phenotyping of grey leaf spot disease in maize using real-time PCR. *European Journal of Plant Pathology*, *133*(2), 461-471. https://doi.org/10.1007/s10658-011-9920-1

Koshikumo, É. S. M., Alves, E., & Pinho, R. G. V. (2014). Induction and detection of toxin in *Cercospora zeina* and *Cercospora sorghi* f. sp. *maydis*.

Kotze, R. G., van der Merwe, C. F., Crampton, B. G., & Kritzinger, Q. (2019). A histological assessment of the infection strategy of *Exserohilum turcicum* in maize. *Plant Pathology*, *68*(3), 504-512. https://doi.org/https://doi.org/10.1111/ppa.12961

Kuki, M. C., Scapim, C. A., Rossi, E. S., Mangolin, C. A., Amaral Júnior, A. T. D., & Pinto, R. J. B. (2018). Genome wide association study for gray leaf spot resistance in tropical maize core. *PLoS One*, *13*(6), e0199539. https://doi.org/10.1371/journal.pone.0199539

Kump, K. L., Bradbury, P. J., Wisser, R. J., Buckler, E. S., Belcher, A. R., Oropeza-Rosas, M. A., Zwonitzer, J. C., Kresovich, S., McMullen, M. D., Ware, D., Balint-Kurti, P. J., & Holland, J. B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat Genet*, *43*(2), 163-168. https://doi.org/10.1038/ng.747

Kurimella, R. K., Bhatia, R., Singh, K. P., & Panwar, S. (2021). Production of Haploids and Doubled Haploids in Marigold (*Tagetes* spp.) Using Anther Culture. *Methods Mol Biol*, *2289*, 271-287. https://doi.org/10.1007/978-1-0716-1331-3_18

Landi, P., Giuliani, S., Salvi, S., Ferri, M., Tuberosa, R., & Sanguineti, M. C. (2010). Characterization of root-yield-1.06, a major constitutive QTL for root and agronomic traits in maize across water regimes. *J Exp Bot*, *61*(13), 3553-3562. https://doi.org/10.1093/jxb/erq192

Latterell, F. M., & Rossi, A. E. (1983). Gray leaf spot of corn: a disease on the move. *Plant Disease*, *67*(8), 842-847.

Lehmensiek, A., Esterhuizen, A. M., van Staden, D., Nelson, S. W., & Retief, A. E. (2001). Genetic mapping of gray leaf spot (GLS) resistance genes in maize. *Theoretical and Applied Genetics*, *103*(5), 797-803. https://doi.org/10.1007/s001220100599

Lennon, J. R., Krakowsky, M., Goodman, M., Flint-Garcia, S., & Balint-Kurti, P. J. (2017). Identification of Teosinte Alleles for Resistance to Southern Leaf Blight in Near Isogenic Maize Lines. *Crop Science*, *57*(4), 1973-1983. https://doi.org/https://doi.org/10.2135/cropsci2016.12.0979

Leonard, K. J., & Suggs, E. G. (1974). *Setosphaeria prolata*, The Ascigerous State of *Exserohilum prolatum*. *Mycologia*, *66*(2), 281-297. https://doi.org/10.1080/00275514.1974.12019603

Lipps, P. (1998). Gray leaf spot: a global threat to corn production. *APSnet Features*.

Littell, R. C., Milliken, G., Stroup, W. W., Wolfinger, R., & Schabenberger, O. (2007). SAS for mixed models. In (Vol. 61).

Liu, L., Zhang, Y. D., Li, H. Y., Bi, Y. Q., Yu, L. J., Fan, X. M., Tan, J., Jeffers, D. P., & Kang, M. S. (2016). QTL Mapping for Gray Leaf Spot Resistance in a Tropical Maize Population. *Plant Dis*, *100*(2), 304-312. https://doi.org/10.1094/pdis-08-14-0825-re

Lopez-Zuniga, L. O., Wolters, P., Davis, S., Weldekidan, T., Kolkman, J. M., Nelson, R., Hooda, K. S., Rucker, E., Thomason, W., Wisser, R., & Balint-Kurti, P. (2019). Using Maize Chromosome Segment Substitution Line Populations for the Identification of Loci Associated with Multiple Disease Resistance. *G3 (Bethesda)*, *9*(1), 189-201. https://doi.org/10.1534/g3.118.200866

Luttrell, E. (1958). The perfect stage of *Helminthosporium turcicum*. *Phytopathology*, *48*(5 (1)).

Mahalingam, T., Chen, W., Rajapakse, C. S., Somachandra, K. P., & Attanayake, R. N. (2020). Genetic Diversity and Recombination in the Plant Pathogen *Sclerotinia sclerotiorum* Detected in Sri Lanka. *Pathogens*, *9*(4). https://doi.org/10.3390/pathogens9040306

Mallowa, S. O., Esker, P. D., Paul, P. A., Bradley, C. A., Chapara, V. R., Conley, S. P., & Robertson, A. E. (2015). Effect of Maize Hybrid and Foliar Fungicides on Yield Under Low Foliar Disease Severity Conditions. *Phytopathology*, *105*(8), 1080-1089. https://doi.org/10.1094/phyto-08-14-0210-r

Mammadov, J., Sun, X., Gao, Y., Ochsenfeld, C., Bakker, E., Ren, R., Flora, J., Wang, X., Kumpatla, S., Meyer, D., & Thompson, S. (2015). Combining powers of linkage and association mapping for precise dissection of QTL controlling resistance to gray leaf spot disease in maize (*Zea mays* L.). *BMC Genomics*, *16*, 916. https://doi.org/10.1186/s12864-015-2171-3

Martins, L. B., Rucker, E., Thomason, W., Wisser, R. J., Holland, J. B., & Balint-Kurti, P. (2019). Validation and Characterization of Maize Multiple Disease Resistance QTL. *G3 (Bethesda)*, *9*(9), 2905-2912. https://doi.org/10.1534/g3.119.400195

Matsuoka, Y., Mitchell, S. E., Kresovich, S., Goodman, M., & Doebley, J. (2002). Microsatellites in *Zea* - variability, patterns of mutations, and use for evolutionary studies. *Theor Appl Genet*, *104*(2-3), 436-450. https://doi.org/10.1007/s001220100694

McDermott, J. M., & McDonald, B. (2003). Gene Flow in Plant Pathosystems. *Annual Review of Phytopathology*, *31*, 353-373. https://doi.org/10.1146/annurev.py.31.090193.002033

McDonald, B. A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology*, *87*(4), 448-453. https://doi.org/10.1094/phyto.1997.87.4.448

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annu Rev Phytopathol*, *40*, 349-379. https://doi.org/10.1146/annurev.phyto.40.120501.101443

Meisel, B., Korsman, J., Kloppers, F., & Berger, D. (2009). *Cercospora zeina* is the causal agent of grey leaf spot disease of maize in southern Africa. *European Journal of Plant Pathology*, *124*, 577-583. https://doi.org/10.1007/s10658-009-9443-1

Meng, L., Li, H., Zhang, L., & Wang, J. (2015). QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*, *3*(3), 269-283.

Menkir, A., & Ayodele, M. (2005). Genetic Analysis of Resistance to Gray Leaf Spot of Midaltitude Maize Inbred Lines. *Crop Science*, *45*(1), cropsci2005.0163, Article cropsci2005.0163. https://doi.org/https://doi.org/10.2135/cropsci2005.0163a

Miedaner, T., Boeven, A. L. G., Gaikpa, D. S., Kistner, M. B., & Grote, C. P. (2020). Genomics-Assisted Breeding for Quantitative Disease Resistances in Small-Grain Cereals and Maize. *Int J Mol Sci*, *21*(24). https://doi.org/10.3390/ijms21249717

Miles, L. A., Miles, T. D., Kirk, W. W., & Schilder, A. M. C. (2012). Strobilurin (QoI) Resistance in Populations of *Erysiphe necator* on Grapes in Michigan. *Plant Dis*, *96*(11), 1621-1628. https://doi.org/10.1094/pdis-01-12-0041-re

Milgroom, M. G. (1996). Recombination and the multilocus structure of fungal populations. *Annu Rev Phytopathol*, *34*, 457-477. https://doi.org/10.1146/annurev.phyto.34.1.457

Mkomwa, S., Lugandu, S., Kuria, P. N., & Mutai, W. (2017). Empowering smallholder farmers with profitable and sustainable farming using conservation agriculture: the case of East Africa.

Muiru, W., Koopmann, B., Tiedemann, A., Mutitu, E., & Kimenju, J. (2010). Race typing and evaluation of aggressiveness of *Exserohilum turcicum* isolates of Kenyan, German and Austrian origin. *World Journal of Agricultural Sciences*, *6*(3), 277-284.

Muller, M. F., Barnes, I., Kunene, N. T., Crampton, B. G., Bluhm, B. H., Phillips, S. M., Olivier, N. A., & Berger, D. K. (2016). *Cercospora zeina* from Maize in South Africa Exhibits High Genetic Diversity and Lack of Regional Population Differentiation. *Phytopathology*, *106*(10), 1194-1205. https://doi.org/10.1094/phyto-02-16-0084-fi

Munialo, S., Dahlin, S., Onyango, C., Kosura, W., Marstorp, H., & Oborn, I. (2019). Soil and management-related factors contributing to maize yield gaps in western Kenya. *Food and Energy Security*, *9*. https://doi.org/10.1002/fes3.189

Munkvold, G. P., Martinson, C. A., Shriver, J. M., & Dixon, P. M. (2001). Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. *Phytopathology*, *91*(5), 477-484. https://doi.org/10.1094/phyto.2001.91.5.477

Naegele, R. P., Boyle, S., Quesada-Ocampo, L. M., & Hausbeck, M. K. (2014). Genetic diversity, population structure, and resistance to *Phytophthora capsici* of a worldwide collection of eggplant germplasm. *PLoS One*, *9*(5), e95930. https://doi.org/10.1371/journal.pone.0095930

Naegele, R. P., DeLong, J., Alzohairy, S. A., Saito, S., Abdelsamad, N., & Miles, T. D. (2021). Population Genetic Analyses of *Botrytis cinerea* Isolates From Michigan Vineyards Using a High-Throughput Marker System Approach. *Frontiers in Microbiology*, *12*, 660874. https://doi.org/10.3389/fmicb.2021.660874

Nasu, S., Suzuki, J., Ohta, R., Hasegawa, K., Yui, R., Kitazawa, N., Monna, L., & Minobe, Y. (2002). Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (*Oryza sativa*, O*ryza rufipogon*) and establishment of SNP markers. *DNA Res*, *9*(5), 163-171. https://doi.org/10.1093/dnares/9.5.163

NCSS. (2021). NCSS and PASS number crucher statistical systems. *https://www.ncss.com/software/ncss/ncss-documentation/*.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89*(3), 583-590. https://doi.org/10.1093/genetics/89.3.583

Ngugi, H., Julian, A., King, S., & Peacocke, B. (2000). Epidemiology of sorghum anthracnose (*Colletotrichum sublineolum*) and leaf blight (*Exserohilum turcicum*) in Kenya. *Plant Pathology*, *49*(1), 129-140. http://oar.icrisat.org/id/eprint/1918

Nieuwoudt, A., Human, M. P., Craven, M., & Crampton, B. G. (2018). Genetic differentiation in populations of *Exserohilum turcicum* from maize and sorghum in South Africa. *Plant Pathology*, *67*(7), 1483-1491. https://doi.org/https://doi.org/10.1111/ppa.12858

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative Resistance to Biotrophic Filamentous Plant Pathogens: Concepts, Misconceptions, and Mechanisms. *Annual Review of Phytopathology*, *53*(1), 445-470. https://doi.org/10.1146/annurev-phyto-080614-115928

Nsibo, D. L., Barnes, I., Kunene, N. T., & Berger, D. K. (2019). Influence of farming practices on the population genetics of the maize pathogen *Cercospora zeina* in South Africa. *Fungal Genet Biol*, *125*, 36-44. https://doi.org/10.1016/j.fgb.2019.01.005

Nsibo, D. L., Barnes, I., Omondi, D. O., Dida, M. M., & Berger, D. K. (2021). Population genetic structure and migration patterns of the maize pathogenic fungus, *Cercospora zeina* in East and Southern Africa. *Fungal Genet Biol*, *149*, 103527. https://doi.org/10.1016/j.fgb.2021.103527

Nyanapah, J. O., Ayiecho, P. O., Nyabundi, J. O., Otieno, W., & Ojiambo, P. S. (2020). Field Characterization of Partial Resistance to Gray Leaf Spot in Elite Maize Germplasm. *Phytopathology*, *110*(10), 1668-1679. https://doi.org/10.1094/phyto-12-19-0446-r

Ochatt, S. J., & Seguí-Simarro, J. M. (2021). Analysis of Ploidy in Haploids and Doubled Haploids. *Methods Mol Biol*, *2287*, 105-125. https://doi.org/10.1007/978-1-0716-1315-3_4

Oerke, E. C. (2006). Crop Losses to Pests. *The Journal of Agricultural Science*, *144*, 31-43. https://doi.org/10.1017/S0021859605005708

Ogliari, J. B., Guimarães, M. A., Geraldi, I. O., & Camargo, L. E. A. (2005). New resistance genes in the *Zea mays: Exserohilum turcicum* pathosystem. *Genetics and Molecular Biology*, *28*, 435-439.

Okori, P., Fahleson, J., Rubaihayo, P. R., Adipala, E., & Dixelius, C. (2003). Assessment Of Genetic Variation Among East African *Cercospora Zeae-maydis*. *African Crop Science Journal (ISSN: 1021-9730) Vol 11 Num 2*, *11*. https://doi.org/10.4314/acsj.v11i2.27520

Onono, P. A., Wawire, N. H. W., & Ombuki, C. N. (2013). The response of maize production in Kenya to economic incentives.

Origa, L. O. (2011). Effect of supplemental irrigation on growth, yield and economic returns of onion (*Allium cepa*) and tomato (*Solanum lycopersicum*) in Kibwezi district, Eastern Kenya.

Pataky, J. K., & Ledencan, T. (2006). Resistance Conferred by the *Ht1* Gene in Sweet Corn Infected by Mixtures of Virulent and Avirulent *Exserohilum turcicum*. *Plant Dis*, *90*(6), 771-776. https://doi.org/10.1094/pd-90-0771

Paul, P. A., & Munkvold, G. P. (2004). A model-based approach to preplanting risk assessment for gray leaf spot of maize. *Phytopathology*, *94 12*, 1350-1357.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics*, *28*(19), 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Perkins, J., & Pedersen, W. (1987). Disease development and yield losses associated with northern leaf blight on corn. *Plant Disease*, *71*(10), 940-943.

Pirofski, L.-a., & Casadevall, A. (2012). Q&A: What is a pathogen? A question that begs the point. *BMC Biology*, *10*(1), 6. https://doi.org/10.1186/1741-7007-10-6

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends Plant Sci*, *14*(1), 21-29. https://doi.org/10.1016/j.tplants.2008.10.006

Poland, J. A., Bradbury, P. J., Buckler, E. S., & Nelson, R. J. (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proc Natl Acad Sci U S A*, *108*(17), 6893-6898. https://doi.org/10.1073/pnas.1010894108

Pozar, G., Butruille, D., Silva, H. D., McCuddin, Z. P., & Penna, J. C. (2009). Mapping and validation of quantitative trait loci for resistance to *Cercospora zeae-maydis* infection in tropical maize (*Zea mays* L.). *Theor Appl Genet*, *118*(3), 553-564. https://doi.org/10.1007/s00122-008-0920-2

Prasanna, B. M., Cairns, J. E., Zaidi, P. H., Beyene, Y., Makumbi, D., Gowda, M., Magorokosho, C., Zaman-Allah, M., Olsen, M., Das, A., Worku, M., Gethi, J., Vivek, B. S., Nair, S. K., Rashid, Z., Vinayan, M. T., Issa, A. B., San Vicente, F., Dhliwayo, T., & Zhang, X. (2021). Beat the stress: breeding for climate resilience in maize for the tropical rainfed environments. *Theor Appl Genet*, *134*(6), 1729-1752. https://doi.org/10.1007/s00122-021-03773-7

Pratt, R., & Gordon, S. (2010). Breeding for Resistance to Maize Foliar Pathogens. In (pp. 119-173). https://doi.org/10.1002/9780470650349.ch3

Price, P. P., 3rd, Purvis, M. A., Cai, G., Padgett, G. B., Robertson, C. L., Schneider, R. W., & Albu, S. (2015). Fungicide Resistance in *Cercospora kikuchii*, a Soybean Pathogen. *Plant Dis*, *99*(11), 1596-1603. https://doi.org/10.1094/pdis-07-14-0782-re

Pritchard, J. K., Wen, X., & Falush, D. (2009). Documentation for structure software : Version 2 . 3.

Rafalski, A. (2002). Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol*, *5*(2), 94-100. https://doi.org/10.1016/s1369-5266(02)00240-6

Raman, R., Cowley, R., Raman, H., & Luckett, D. (2014). Analyses Using SSR and DArT Molecular Markers Reveal that Ethiopian Accessions of White Lupin (*Lupinus albus* L.) Represent a Unique Genepool. *Open Journal of Genetics*, *04*, 87-98. https://doi.org/10.4236/ojgen.2014.42012

Raymundo, A., AD, R., & AL, H. (1981). Effect of gene *HtN* on the development of northern corn leaf blight epidemics. pascal-francis.inist.fr

Ribaut, J. M., Hoisington, D. A., Deutsch, J. A., Jiang, C., & Gonzalez-de-Leon, D. (1996). Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theor Appl Genet*, *92*(7), 905-914. https://doi.org/10.1007/bf00221905

Ringer, C., & Grybauskas, A. (1995). Infection cycle components and disease progress of gray leaf spot on field corn. *Plant Disease*, *79*(1), 24-28.

Romero, L. R. (2016). *Occurrence and importance of foliar diseases on maize (Zea mays L.) in Central Europe* Georg-August-Universität Göttingen]. core.ac.uk

Rupe, J., Siegel, M., & Hartman, J. (1982). Influence of environment and plant maturity on gray leaf spot of corn caused by *Cercospora zeae-maydis*. *Phytopathology*, *72*(12), 1587-1591. cabdirect.org

Saghai, M., Yue, Y., Xiang, Z., Stromberg, E., & Rufener, G. (1996). Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. *Theoretical and Applied Genetics*, *93*(4), 539-546.

Saito, B. C., Silva, L., Andrade, J., & Goodman, M. (2018). Adaptability and stability of corn inbred lines regarding resistance to gray leaf spot and northern leaf blight. *Crop Breeding and Applied Biotechnology*, *18*, 148-154. https://doi.org/10.1590/1984-70332018v18n2a21

Sánchez-Sevilla, J. F., Horvath, A., Botella, M. A., Gaston, A., Folta, K., Kilian, A., Denoyes, B., & Amaya, I. (2015). Diversity Arrays Technology (DArT) Marker Platforms for Diversity Analysis and Linkage Mapping in a Complex Crop, the Octoploid Cultivated Strawberry (*Fragaria × ananassa*). *PLoS One*, *10*(12), e0144960. https://doi.org/10.1371/journal.pone.0144960

Sansaloni, C., Franco, J., Santos, B., Percival-Alwyn, L., Singh, S., Petroli, C., Campos, J., Dreher, K., Payne, T., Marshall, D., Kilian, B., Milne, I., Raubach, S., Shaw, P., Stephen, G., Carling, J., Pierre, C. S., Burgueño, J., Crosa, J., . . . Pixley, K. (2020). Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints. *Nat Commun*, *11*(1), 4572. https://doi.org/10.1038/s41467-020-18404-w

Sansaloni, C. P., Petroli, C. D., Carling, J., Hudson, C. J., Steane, D. A., Myburg, A. A., Grattapaglia, D., Vaillancourt, R. E., & Kilian, A. (2010). A high-density Diversity Arrays Technology (DArT) microarray for genome-wide genotyping in Eucalyptus. *Plant Methods*, *6*, 16. https://doi.org/10.1186/1746-4811-6-16

Sartori, M., Nesci, A., García, J., Passone, M. A., Montemarani, A., & Etcheverry, M. (2017). Efficacy of epiphytic bacteria to prevent northern leaf blight caused by *Exserohilum turcicum* in maize. *Rev Argent Microbiol*, *49*(1), 75-82. https://doi.org/10.1016/j.ram.2016.09.008

Schechert, A., Welz, H. G., & Geiger, H. H. (1999). QTL for Resistance to *Setosphaeria turcica* in Tropical African Maize. *Crop Science*, *39*, 514-523.

Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T. A., Minx, P., Reily, A. D., Courtney, L., Kruchowski, S. S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., . . . Wilson, R. K. (2009). The B73 maize genome: complexity, diversity, and dynamics. *Science*, *326*(5956), 1112-1115. https://doi.org/10.1126/science.1178534

Seeb, J. E., Carvalho, G., Hauser, L., Naish, K., Roberts, S., & Seeb, L. W. (2011). Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. *Molecular Ecology Resources*, *11*(s1), 1-8. https://doi.org/https://doi.org/10.1111/j.1755-0998.2010.02979.x

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecol Lett*, *9*(5), 615-629. https://doi.org/10.1111/j.1461-0248.2006.00889.x

Setyawan, B. (2016). Short Communication: Resistance of eleven new hybrid maize genotypes to Turcicum leaf blight (*Exserohilum turcicum*). *Biodiversitas, Journal of Biological Diversity*, *17*, 604-608. https://doi.org/10.13057/biodiv/d170230

Seymour, D. K., Filiault, D. L., Henry, I. M., Monson-Miller, J., Ravi, M., Pang, A., Comai, L., Chan, S. W., & Maloof, J. N. (2012). Rapid creation of Arabidopsis doubled haploid lines for quantitative trait locus mapping. *Proc Natl Acad Sci U S A*, *109*(11), 4227-4232. https://doi.org/10.1073/pnas.1117277109

Shankara, K., & Gowda, K. T. P. (2011). Assessment of yield loss due to turcicum leaf blight of maize caused by *Exserohilum turcicum Mysore journal of agricultural sciences*, *45*, 691-692.

Shi, L.-y., Li, X.-h., Hao, Z.-f., Xie, C.-x., Ji, H.-l., LÜ, X.-l., Zhang, S., & Pan, G.-t. (2007). Comparative QTL Mapping of Resistance to Gray Leaf Spot in Maize Based on Bioinformatics. *Agricultural Sciences in China*, *6*(12), 1411-1419. https://doi.org/https://doi.org/10.1016/S1671-2927(08)60002-4

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, *3*(3), 307. https://doi.org/10.1007/s12571-011-0140-5

Shrestha, S. K., Cochran, A., Mengistu, A., Lamour, K., Castro-Rocha, A., & Young-Kelly, H. (2017). Genetic diversity, QoI fungicide resistance, and mating type distribution of *Cercospora sojina*-Implications for the disease dynamics of frogeye leaf spot on soybean. *PLoS One*, *12*(5), e0177220. https://doi.org/10.1371/journal.pone.0177220

Sibiya, J., Tongoona, P., Derera, J., & van Rij, N. (2012). Genetic analysis and genotype × environment (G × E) for grey leaf spot disease resistance in elite African maize (*Zea mays* L.) germplasm. *Euphytica*, *185*(3), 349-362. https://doi.org/10.1007/s10681-011-0466-2

Simcox, K. D., & Bennetzen, J. L. (1993). Mapping the *HtN* resistance gene to the long arm of chromosome 8. *Maize Genetics Cooperation Newsletter*, *67*, 118-118.

Sommerhalder, R. J., McDonald, B. A., Mascher, F., & Zhan, J. (2010). Sexual recombinants make a significant contribution to epidemics caused by the wheat pathogen *Phaeosphaeria nodorum*. *Phytopathology*, *100*(9), 855-862. https://doi.org/10.1094/phyto-100-9-0855

Sserumaga, J. P., Makumbi, D., Assanga, S. O., Mageto, E. K., Njeri, S. G., Jumbo, B. M., & Bruce, A. Y. (2020). Identification and diversity of tropical maize inbred lines with resistance to common rust (*Puccinia sorghi* Schwein). *Crop Sci*, *60*(6), 2971-2989. https://doi.org/10.1002/csc2.20345

Stewart, C. N., Jr., & Via, L. E. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *Biotechniques*, *14*(5), 748-750.

Stoddart, J. A., & Taylor, J. F. (1988). Genotypic diversity: estimation and prediction in samples. *Genetics*, *118*(4), 705-711. https://doi.org/10.1093/genetics/118.4.705

Strable, J., & Scanlon, M. J. (2009). Maize (*Zea mays*): a model organism for basic and applied research in plant biology. *Cold Spring Harb Protoc*, *2009*(10), pdb.emo132. https://doi.org/10.1101/pdb.emo132

Stromberg, E., & Donahue, P. (1986). Hybrid performance and yield losses associated with gray leaf spot disease. Report of annual Corn and Sorghum Research Conference (USA),

Stromberg, E. L. (2009). Gray leaf spot disease of corn.

Sucher, J., Boni, R., Yang, P., Rogowsky, P., Büchner, H., Kastner, C., Kumlehn, J., Krattinger, S. G., & Keller, B. (2017). The durable wheat disease resistance gene *Lr34* confers common rust and northern corn leaf blight resistance in maize. *Plant Biotechnol J*, *15*(4), 489-496. https://doi.org/10.1111/pbi.12647

Swart, V., Crampton, B. G., Ridenour, J. B., Bluhm, B. H., Olivier, N. A., Meyer, J. J. M., & Berger, D. K. (2017). Complementation of *CTB7* in the Maize Pathogen *Cercospora zeina* Overcomes the Lack of In Vitro Cercosporin Production. *Mol Plant Microbe Interact*, *30*(9), 710-724. https://doi.org/10.1094/mpmi-03-17-0054-r

Syvänen, A. C. (2001). Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat Rev Genet*, *2*(12), 930-942. https://doi.org/10.1038/35103535

Technow, F., Bürger, A., & Melchinger, A. E. (2013). Genomic prediction of northern corn leaf blight resistance in maize with combined or separated training sets for heterotic groups. *G3 (Bethesda)*, *3*(2), 197-203. https://doi.org/10.1534/g3.112.004630

Tehon, L., & Daniels, E. (1925). Notes on the parasitic fungi of Illinois—II. *Mycologia*, *17*(6), 240-249.

Thakur, R., Leath, S., & Leonard, K. (1989). Resistance effects of temperature and light on virulence of *Exserohilum turcicum* on corn. *Phytopathology*, *79*(6), 631-635.

Tuberosa, R., Salvi, S., Sanguineti, M. C., Landi, P., Maccaferri, M., & Conti, S. (2002). Mapping QTLs regulating morpho-physiological traits and yield: case studies, shortcomings and perspectives

in drought-stressed maize. *Ann Bot*, *89 Spec No*(7), 941-963. https://doi.org/10.1093/aob/mcf134

Upchurch, R. G., Walker, D. C., Rollins, J. A., Ehrenshaft, M., & Daub, M. E. (1991). Mutants of *Cercospora kikuchii* Altered in Cercosporin Synthesis and Pathogenicity. *Appl Environ Microbiol*, *57*(10), 2940-2945. https://doi.org/10.1128/aem.57.10.2940-2945.1991

Vaghefi, N., Hay, F., Kikkert, J., & Pethybridge, S. (2016). Genotypic diversity and resistance to azoxystrobin of *Cercospora beticola* on processing table beet in New York. *Plant Disease*, *100*. https://doi.org/10.1094/PDIS-09-15-1014-RE

Van Inghelandt, D., Melchinger, A. E., Martinant, J. P., & Stich, B. (2012). Genome-wide association mapping of flowering time and northern corn leaf blight (*Setosphaeria turcica*) resistance in a vast commercial maize germplasm set. *BMC Plant Biol*, *12*, 56. https://doi.org/10.1186/1471-2229-12-56

Vavilov, N. (1928). Geographical centers of our cultivated plants. Proceedings of the 5th International Congress of Genetics, NY,

Wallace, J. G., Larsson, S. J., & Buckler, E. S. (2014). Entering the second century of maize quantitative genetics. *Heredity (Edinb)*, *112*(1), 30-38. https://doi.org/10.1038/hdy.2013.6

Wang, J., Levy, M., & Dunkle, L. D. (1998). Sibling species of cercospora associated with gray leaf spot of maize. *Phytopathology*, *88*(12), 1269-1275. https://doi.org/10.1094/phyto.1998.88.12.1269

Warburton, M. (2005). *Laboratory Protocols CIMMYT Applied Molecular Genetics Laboratory*.

Ward, J. M. (1996). Epidemiology and management of grey leaf spot : a new disease of maize in South Africa.

Ward, J. M., & Nowell, D. C. (1998). Integrated Management Practices for the Control of Maize Grey Leaf Spot. *Integrated Pest Management Reviews*, *3*, 177-188.

Ward, J. M. J., Stromberg, E. L., Nowell, D. C., & Nutter, F. W., Jr. (1999). Gray leaf Spot: A Disease of Global Importance in Maize Production. *Plant Dis*, *83*(10), 884-895. https://doi.org/10.1094/pdis.1999.83.10.884

Wathaneeyawech, S., Sirithunya, P., & Smitamana, P. (2015). Study of the host range of Northern Corn Leaf Blight disease and effect of *Exserohilum turcicum* toxin on sweet corn. *International Journal of Agricultural Technology*, *11*, 953-963.

Weems, J. D., & Bradley, C. A. (2018). *Exserohilum turcicum* Race Population Distribution in the North Central United States. *Plant Dis*, *102*(2), 292-299. https://doi.org/10.1094/pdis-01-17-0128-re

Welz, G., & Geiger, H. (2000). Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breeding*, *119*, 1-14. https://doi.org/10.1046/j.1439-0523.2000.00462.x

Welz, H. G. (1998). *Genetics and Epidemiology of the Pathosystem Zea mays/Setosphaeria turcica*.

Welz, H. G., Xia, X. C., Bassetti, P., Melchinger, A. E., & Lübberstedt, T. (1999). QTLs for resistance to *Setosphaeria turcica* in an early maturing Dent×Flint maize population. *Theor Appl Genet*, *99*(3-4), 649-655. https://doi.org/10.1007/s001220051280

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., & Kilian, A. (2004). Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proc Natl Acad Sci U S A*, *101*(26), 9915-9920. https://doi.org/10.1073/pnas.0401076101

Weyen, J. (2021). Applications of Doubled Haploids in Plant Breeding and Applied Research. *Methods Mol Biol*, *2287*, 23-39. https://doi.org/10.1007/978-1-0716-1315-3_2

Wise, K., & Mueller, D. (2011). Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn. *APSnet Feature Articles*. https://doi.org/10.1094/APSnetFeature-2011-0531

Wisser, R. J., Balint-Kurti, P. J., & Nelson, R. J. (2006). The genetic architecture of disease resistance in maize: a synthesis of published studies. *Phytopathology*, *96*(2), 120-129. https://doi.org/10.1094/phyto-96-0120

Wisser, R. J., Kolkman, J. M., Patzoldt, M. E., Holland, J. B., Yu, J., Krakowsky, M., Nelson, R. J., & Balint-Kurti, P. J. (2011). Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a GST gene. *Proc Natl Acad Sci U S A*, *108*(18), 7339-7344. https://doi.org/10.1073/pnas.1011739108

Wisser, R. J., & Lauter, N. (2018). Genomics of Fungal Disease Resistance. In J. Bennetzen, S. Flint-Garcia, C. Hirsch, & R. Tuberosa (Eds.), *The Maize Genome* (pp. 201-211). Springer International Publishing. https://doi.org/10.1007/978-3-319-97427-9_13

Xia, H., Gao, W., Qu, J., Dai, L., Gao, Y., Lu, S., Zhang, M., Wang, P., & Wang, T. (2020). Genetic mapping of northern corn leaf blight-resistant quantitative trait loci in maize. *Medicine (Baltimore)*, *99*(31), e21326. https://doi.org/10.1097/md.0000000000021326

Xu, L., Zhang, Y., Shao, S., Chen, W., Tan, J., Zhu, M., Zhong, T., Fan, X., & Xu, M. (2014). High-resolution mapping and characterization of *qRgls2*, a major quantitative trait locus involved in maize resistance to gray leaf spot. *BMC Plant Biol*, *14*, 230. https://doi.org/10.1186/s12870-014-0230-6

Yan, J., Warburton, M., & Crouch, J. (2011). Association mapping for enhancing maize (*Zea mays* L.) genetic improvement. *Crop Science*, *51*(2), 433-449.

Yang, P., Scheuermann, D., Kessel, B., Koller, T., Greenwood, J. R., Hurni, S., Herren, G., Zhou, S., Marande, W., Wicker, T., Krattinger, S. G., Ouzunova, M., & Keller, B. (2021). Alleles of a wall-associated kinase gene account for three of the major northern corn leaf blight resistance loci in maize. *Plant J*, *106*(2), 526-535. https://doi.org/10.1111/tpj.15183

Zhang, L.-H., Dong, J.-G., Wang, C.-H., & Li, Z.-P. (2007). Purification and structural analysis of a selective toxin fraction produced by the plant pathogen *Setosphaeria turcica*. *Agricultural Sciences in China*, *6*(4), 452-457.

Zhang, X., Fernandes, S. B., Kaiser, C., Adhikari, P., Brown, P. J., Mideros, S. X., & Jamann, T. M. (2020). Conserved defense responses between maize and sorghum to *Exserohilum turcicum*. *BMC Plant Biol*, *20*(1), 67. https://doi.org/10.1186/s12870-020-2275-z

Zhang, X., Yang, Q., Rucker, E., Thomason, W., & Balint-Kurti, P. (2017). Fine mapping of a quantitative resistance gene for gray leaf spot of maize (*Zea mays* L.) derived from teosinte (*Z. mays* ssp. *parviglumis*). *Theor Appl Genet*, *130*(6), 1285-1295. https://doi.org/10.1007/s00122-017-2888-2

Zhang, Y., Xu, L., Fan, X., Tan, J., Chen, W., & Xu, M. (2012). QTL mapping of resistance to gray leaf spot in maize. *Theor Appl Genet*, *125*(8), 1797-1808. https://doi.org/10.1007/s00122-012-1954-z

Zwonitzer, J. C., Coles, N. D., Krakowsky, M. D., Arellano, C., Holland, J. B., McMullen, M. D., Pratt, R. C., & Balint-Kurti, P. J. (2010). Mapping resistance quantitative trait Loci for three foliar diseases in a maize recombinant inbred line population-evidence for multiple disease resistance? *Phytopathology*, *100*(1), 72-79. https://doi.org/10.1094/phyto-100-1-0072

APPENDICES

Appendix A: Distribution of mating type genes in the Kenyan population.

Sample name *	Location of collection	Final Name	Mating type
			MAT1-1 OR MAT1-2
2018.KE.Mkushi	Control	2018.KE.Mkushi	MAT1-1
2018.KE.CHO.005	Tharaka Nithi	2018.KE.CHO.005	MAT1-1
2018.KE.CHO.006	Tharaka Nithi	2018.KE.CHO.006	MAT1-1
2018.KE.CHO.010	Tharaka Nithi	2018.KE.CHO.010	MAT1-1
2018.KE.CHO.019	Tharaka Nithi	2018.KE.CHO.019	MAT1-2
2018.KE.CHO.025	Tharaka Nithi	2018.KE.CHO.025	MAT1-1
2018.KE.CHO.031	Tharaka Nithi	2018.KE.CHO.031	MAT1-1
2018.KE.CHO.038	Tharaka Nithi	2018.KE.CHO.038	MAT1-2
2018.KE.CHO.044	Tharaka Nithi	2018.KE.CHO.044	MAT1-2
2018.KE.CHO.050	Tharaka Nithi	2018.KE.CHO.050	MAT1-2
2018.KE.CHO.085	Tharaka Nithi	2018.KE.CHO.085	NOT KNOWN
2018.KE.CHO.230	Tharaka Nithi	2018.KE.CHO.230	MAT1-2
2018.KE.CHO.283	Tharaka Nithi	2018.KE.CHO.283	MAT1-1
2018.KE.MER.001	Meru	2018.KE.MER.001	MAT1-1
2018.KE.MER.002	Meru	2018.KE.MER.002	NOT KNOWN
2018.KE.MER.004	Meru	2018.KE.MER.004	MAT1-1
2018.KE.MER.005	Meru	2018.KE.MER.005	MAT1-1
2018.KE.MER.006	Meru	2018.KE.MER.006	MAT1-2
2018.KE.MER.009	Meru	2018.KE.MER.009	MAT1-2
2018.KE.MER.023	Meru	2018.KE.MER.023	MAT1-1
2018.KE.MER.027	Meru	2018.KE.MER.027	MAT1-2
2018.KE.MER.028	Meru	2018.KE.MER.053	MAT1-2
2018.KE.MER.030	Meru	2018.KE.MER.029	MAT1-2
2018.KE.MER.030.2	Meru	2018.KE.MER.036	MAT1-2
2018.KE.MER.033	Meru	2018.KE.MER.033	MAT1-2
2018.KE.MER.S033	Meru	2018.KE.MER.046	NOT KNOWN
2018.KE.MER.033.2	Meru	2018.KE.MER.030	NOT KNOWN
2018.KE.MER.S033.2	Meru	2018.KE.MER.047	MAT1-2
2018.KE.MER.035	Meru	2018.KE.MER.035	MAT1-2
2018.KE.MER.S035	Meru	2018.KE.MER.288	MAT1-1
2018.KE.MER.036	Meru	2018.KE.MER.036	MAT1-2
2018.KE.MER.039	Meru	2018.KE.MER.039	MAT1-1
2018.KE.MER.040	Meru	2018.KE.MER.040	MAT1-1
2018.KE.MER.041	Meru	2018.KE.MER.041	MAT1-2
2018.KE.MER.043	Meru	2018.KE.MER.043	MAT1-1
2018.KE.MER.044	Meru	2018.KE.MER.044	MAT1-1
2018.KE.MER.045	Meru	2018.KE.MER.045	MAT1-2
2018.KE.MER.046	Meru	2018.KE.MER.046	MAT1-1
2018.KE.MER.047	Meru	2018.KE.MER.047	MAT1-1

2018.KE.MER.048	Meru	2018.KE.MER.048	MAT1-1
2018.KE.MER.049	Meru	2018.KE.MER.049	MAT1-1
2018.KE.MER.051	Meru	2018.KE.MER.051	MAT1-2
2018.KE.MER.052	Meru	2018.KE.MER.052	MAT1-2
2018.KE.MER.054	Meru	2018.KE.MER.054	MAT1-1
2018.KE.MER.055	Meru	2018.KE.MER.055	MAT1-1
2018.KE.MER.056	Meru	2018.KE.MER.056	MAT1-2
2018.KE.MER.057	Meru	2018.KE.MER.057	NOT KNOWN
2018.KE.MER.058	Meru	2018.KE.MER.058	MAT1-2
2018.KE.MER.061	Meru	2018.KE.MER.061	NOT KNOWN
2018.KE.MER.062	Meru	2018.KE.MER.062	MAT1-2
2018.KE.MER.086	Meru	2018.KE.MER.086	MAT1-1
2018.KE.MER.107	Meru	2018.KE.MER.107	MAT1-2
2018.KE.MER.135	Meru	2018.KE.MER.135	MAT1-1
2018.KE.MER.140	Meru	2018.KE.MER.140	MAT1-1
2018.KE.MER.146	Meru	2018.KE.MER.146	MAT1-1
2018.KE.MER.153	Meru	2018.KE.MER.153	MAT1-2
2018.KE.MER.154	Meru	2018.KE.MER.154	MAT1-2
2018.KE.MER.156	Meru	2018.KE.MER.156	MAT1-2
2018.KE.MER.157	Meru	2018.KE.MER.157	MAT1-2
2018.KE.MER.158	Meru	2018.KE.MER.158	MAT1-2
2018.KE.MER.177	Meru	2018.KE.MER.177	MAT1-2
2018.KE.MER.178	Meru	2018.KE.MER.178	MAT1-2
2018.KE.MER.180	Meru	2018.KE.MER.180	MAT1-1
2018.KE.MER.181	Meru	2018.KE.MER.181	MAT1-1
2018.KE.MER.184	Meru	2018.KE.MER.184	MAT1-1
2018.KE.MER.190	Meru	2018.KE.MER.190	MAT1-2
2018.KE.MER.196	Meru	2018.KE.MER.196	NOT KNOWN
2018.KE.MER.198	Meru	2018.KE.MER.198	NOT KNOWN
2018.KE.MER.199	Meru	2018.KE.MER.199	MAT1-1
2018.KE.MER.200	Meru	2018.KE.MER.200	MAT1-1
2018.KE.MER.202	Meru	2018.KE.MER.202	MAT1-1
2018.KE.MER.203	Meru	2018.KE.MER.203	MAT1-1
2018.KE.MER.204	Meru	2018.KE.MER.204	MAT1-1
2018.KE.MER.207	Meru	2018.KE.MER.207	MAT1-2
2018.KE.MER.208	Meru	2018.KE.MER.208	MAT1-2
2018.KE.MER.221	Meru	2018.KE.MER.221	NOT KNOWN
2018.KE.MER.222	Meru	2018.KE.MER.222	MAT1-1
2018.KE.MER.223	Meru	2018.KE.MER.223	NOT KNOWN
2018.KE.MER.229	Meru	2018.KE.MER.229	MAT1-1
2018.KE.MER.231	Meru	2018.KE.MER.231	MAT1-1
2018.KE.MER.233	Meru	2018.KE.MER.233	MAT1-1
2018.KE.MER.240	Meru	2018.KE.MER.240	MAT1-1
2018.KE.MER.250	Meru	2018.KE.MER.250	MAT1-1
2018.KE.MER.264	Meru	2018.KE.MER.264	MAT1-2
2018.KE.MER.286	Meru	2018.KE.MER.286	NOT KNOWN

2018.KE.MER.287	Meru	2018.KE.MER.287	MAT1-1
2018.KE.MER.298	Meru	2018.KE.MER.298	MAT1-1
2018.KE.MER.308	Meru	2018.KE.MER.308	MAT1-2
2018.KE.MER.309	Meru	2018.KE.MER.309	MAT1-2
2018.KE.NAK.002	Nakuru	2018.KE.NAK.002	MAT1-2
2018.KE.SUB.004	Subukia	2018.KE.SUB.004	MAT1-1
2018.KE.SUB.013	Subukia	2018.KE.SUB.013	NOT KNOWN
2018.KE.SUB.S014	Subukia	2018.KE.SUB.014	MAT1-1
2018.KE.SUB.024	Subukia	2018.KE.SUB.024	MAT1-2
2018.KE.SUB.024.2	Subukia	2018.KE.SUB.025	MAT1-2
2018.KE.SUB.027	Subukia	2018.KE.SUB.027	MAT1-1
2018.KE.SUB.014	Subukia	2018.KE.SUB.014	MAT1-1
2018.KE.SUB.029	Subukia	2018.KE.SUB.029	MAT1-1
2018.KE.VIC.004	Vicar	2018.KE.VIC.004	MAT1-2
2018.KE.VIC.008	Vicar	2018.KE.VIC.008	MAT1-1
2018.KE.KIA.068	Kiambu	2018.KE.KIA.068	MAT1-2
2018.KE.KIA.087	Kiambu	2018.KE.KIA.087	MAT1-2
2018.KE.KIA.088	Kiambu	2018.KE.KIA.088	NOT KNOWN
2018.KE.KIA.101	Kiambu	2018.KE.KIA.101	MAT1-1
2018.KE.KIA.133	Kiambu	2018.KE.KIA.133	MAT1-2
2018.KE.KIA.134	Kiambu	2018.KE.KIA.134	MAT1-2
2018.KE.KIA.141	Kiambu	2018.KE.KIA.141	MAT1-2
2018.KE.KIA.145	Kiambu	2018.KE.KIA.145	MAT1-2
2018.KE.KIA.149	Kiambu	2018.KE.KIA.149	NOT KNOWN
2018.KE.KIA.150	Kiambu	2018.KE.KIA.150	NOT KNOWN
2018.KE.KIA.152	Kiambu	2018.KE.KIA.152	NOT KNOWN
2018.KE.KIA.152.2	Kiambu	2018.KE.KIA.153	MAT1-2
2018.KE.KIA.166	Kiambu	2018.KE.KIA.166	NOT KNOWN
2018.KE.KIA.167	Kiambu	2018.KE.KIA.167	MAT1-2
2018.KE.KIA.168	Kiambu	2018.KE.KIA.168	MAT1-1
2018.KE.KIA.174	Kiambu	2018.KE.KIA.174	MAT1-1
2018.KE.KIA.176	Kiambu	2018.KE.KIA.176	MAT1-1
2018.KE.KIA.182	Kiambu	2018.KE.KIA.182	MAT1-1
2018.KE.KIA.183	Kiambu	2018.KE.KIA.183	MAT1-1
2018.KE.KIA.187	Kiambu	2018.KE.KIA.187	NOT KNOWN
2018.KE.KIA.188	Kiambu	2018.KE.KIA.188	MAT1-1
2018.KE.KIA.189	Kiambu	2018.KE.KIA.189	MAT1-1
2018.KE.KIA.191	Kiambu	2018.KE.KIA.191	MAT1-1
2018.KE.KIA.193	Kiambu	2018.KE.KIA.193	MAT1-2
2018.KE.KIA.194	Kiambu	2018.KE.KIA.194	MAT1-2
2018.KE.KIA.209	Kiambu	2018.KE.KIA.209	MAT1-1
2018.KE.KIA.211	Kiambu	2018.KE.KIA.211	MAT1-1
2018.KE.KIA.216	Kiambu	2018.KE.KIA.216	MAT1-1
2018.KE.KIA.217	Kiambu	2018.KE.KIA.217	NOT KNOWN
2018.KE.KIA.218	Kiambu	2018.KE.KIA.218	MAT1-1
2018.KE.KIA.224	Kiambu	2018.KE.KIA.224	MAT1-2

2018.KE.KIA.248	Kiambu	2018.KE.KIA.248	MAT1-2
2018.KE.KIA.256	Kiambu	2018.KE.KIA.256	MAT1-2
2018.KE.KIA.290	Kiambu	2018.KE.KIA.290	MAT1-1
2018.KE.KIA.291	Kiambu	2018.KE.KIA.291	MAT1-1
2018.KE.KIA.292	Kiambu	2018.KE.KIA.292	MAT1-1
2018.KE.KIA.305	Kiambu	2018.KE.KIA.305	NOT KNOWN
2018.KE.KIA.306	Kiambu	2018.KE.KIA.306	MAT1-1
2018.KE.KIA.999	Kiambu	2018.KE.KIA.999	MAT1-2

Appendix B: Averages for the different response variables across environments.

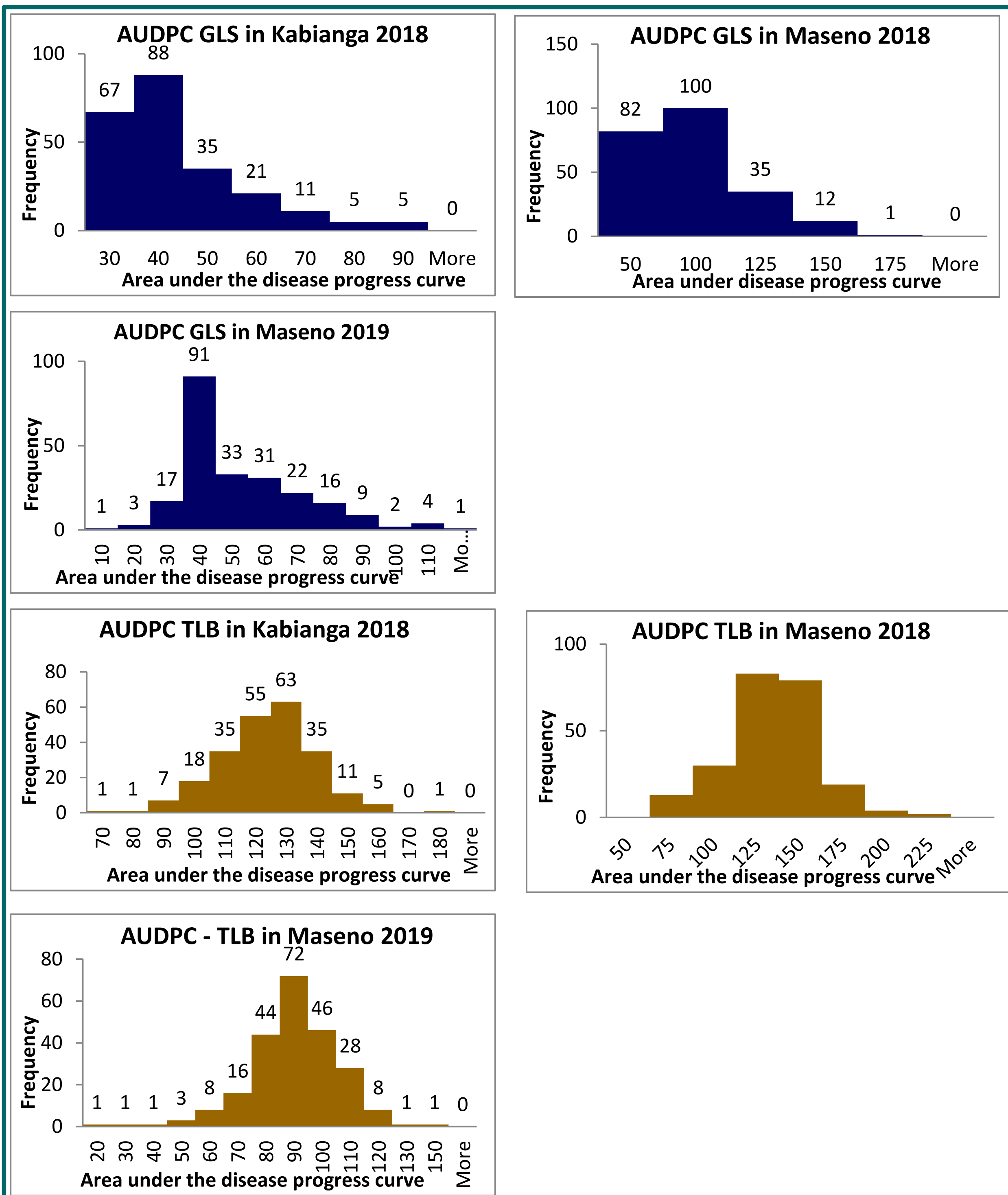
Genotype	CML511	CML546
BP_Ke_DTA	94.10429349	100.6547657
BP_Ke_DTS	94.63760181	102.8311145
BP_Ke_PH	133.9315266	133.8352981
BP_Ke_EH	67.10557274	60.15386917
BP_Ke_GLS1	1.056137762	1.048188756
BP_Ke_GLS2	1.298417797	1.047744115
BP_Ke_GLS3	1.316071803	1.316071803
BP_Ke_GLS4	1.901677257	1.617967997
BP_Ke_GLS5	1.91693763	1.91693763
BP_Ke_GLS6	2.532734826	2.811151914
BP_Ke_AUDPC1	49.76225205	47.74577798
BP_Ke_Turc1	3.249325062	2.982781534
BP_Ke_Turc2	3.590005447	3.022093038
BP_Ke_Turc3	4.321499603	3.278913655
BP_Ke_Turc4	4.304521671	3.307151493
BP_Ke_Turc5	4.602914122	3.636916212
BP_Ke_Turc6	4.687190223	3.845639828
BP_Ke_AUDPC2	121.7742071	93.41246548
BP_M2_DTA	72.17889129	75.59455448
BP_M2_DTS	72.08874848	76.63185021
BP_M2_PH	119.710513	134.5474557
BP_M2_EH	54.23809871	52.09033114
BP_M2_GLS1	2.014430708	2.522538268
BP_M2_GLS2	2.393346219	3.11396371

BP_M2_GLS3	2.167265068	3.742468702
BP_M2_GLS4	3.657016409	4.711973262
BP_M2_GLS5	3.084186919	5.986494668
BP_M2_AUDPC1	73.33463771	111.3664653
BP_M2_Turc1	1.949834378	2.509425374
BP_M2_Turc2	3.873557362	3.495435817
BP_M2_Turc3	3.825895215	3.560947894
BP_M2_Turc4	4.404491248	3.830444717
BP_M2_Turc5	5.014610156	4.138228398
BP_M2_AUDPC2	103.8555094	98.61818163
BP_M3_DTA	78.49558256	80.49463735
BLUE_DTA	78.40782426	80.89724445
BP_M3_DTS	78.87774366	81.82510192
BP_M3_PH	114.9431193	112.4815771
BP_M3_EH	45.71034127	34.57308177
BP_M3_GLS1	1.325473509	1.877618666
BP_M3_GLS2	1.890150643	2.246776702
BP_M3_GLS3	1.966244201	2.530497851
BP_M3_GLS4	2.007985386	2.895183646
BP_M3_GLS5	2.309297984	3.477476506
BP_M3_AUDPC1	54.45206895	73.89294647
BP_M3_Turc1	2.171220852	1.87773355
BP_M3_Turc2	3.45233192	2.330277405
BP_M3_Turc3	3.636299894	3.542316631
BP_M3_Turc4	4.70378326	3.496606571
BP_M3_Turc5	5.110488939	3.935261678
BP_M3_AUDPC2	110.2800841	86.50794471

Appendix C: An overview of the experimental field in Kabianga 2018.

Appendix D: Diseased maize crop at early stages of infection.

Appendix E: Frequency distribution graphs for area under the disease progress curve, GLS and TLB.



Appendix F: Physical locations and genetic map positions for the identified QTL.

Trait Name	QTL name	[a]Chr	Position (cM)	[b]LOD	[c]PVE (%)	[d]Add	[e]LeftMarker	[e]RightMarker	LeftCI	RightCI	Bins
CML511/CML546											
DTA	*qDTA2_153*	2	176	3.11	5.93	3.77	S2_153787894	S2_191797557	175.5	176.5	2.06
	qDTA3_28	3	143	4.57	3.21	-0.97	S3_28258001	S3_129315443	142.5	144.5	3.04
	qDTA4_192	4	343	3.32	2.46	0.80	S4_192869349	S4_186588030	341.5	345.5	4.08
	qDTA8_135	8	247	10.81	10.37	-1.63	S8_135475467	S8_139048847	246.5	247.5	8.05
	qDTA8_139	8	249	20.85	17.34	2.11	S8_139048847	S8_136861251	248.5	249.5	8.05
DTS	*qDTS1_230*	1	65	4.95	5.37	-1.17	S1_225916227	S1_229375633	62.5	68.5	1.07
	qDTS4_192	4	343	3.53	3.90	1.00	S4_192869349	S4_186588030	340.5	345.5	4.08
	qDTS8_139	8	249	23.79	30.24	2.77	S8_139048847	S8_136861251	248.5	249.5	8.05
	qDTS9_136	9	151	4.27	7.06	1.34	S9_135788881	S9_129671108	150.5	151.5	9.05
PH	*qPH1_28*	1	268	4.37	7.67	-5.14	S1_28106472	S1_27326414	267.5	269.5	1.02
	qPH8_129	8	243	21.53	23.39	8.98	S8_128738672	S8_129741294	241.5	243.5	8.05
	qPH9_154	9	21	5.41	10.65	-8.61	S9_139755085	S9_154594758	20.5	21.5	9.06
	qPH10_144	10	252	3.34	2.90	-3.16	S10_144194648	S10_142987634	248.5	259	10.07
EH	*qEH2_135*	2	54	3.23	3.73	-1.56	S2_135043270	S2_149550704	52.5	54.5	2.05
	qEH5_216	5	167	3.41	4.56	-1.72	S5_215584397	S5_216421061	165.5	167.5	5.09
	qEH8_128	8	242	16.76	20.78	3.68	S8_128422019	S8_128738672	241.5	243.5	8.05
	qEH9_139	9	21	5.22	11.24	-3.87	S9_139755085	S9_154594758	20.5	21.5	9.06
GLS Location 1	*qGLS1_283*	1	159	7.33	2.10	0.08	S1_283894617	S1_53456776	154.5	162.5	1.11
	qGLS1_207	1	343	12.13	3.70	0.17	S1_207695200	S1_190286762	342.5	343.5	1.07
	qGLS1_190	1	372	4.42	0.79	-0.04	S1_190286762	S1_185978658	367.5	374.5	1.06
	qGLS4_242	4	329	30.17	7.23	0.39	S4_242295766	S4_187911149	328.5	329.5	4.1
	qGLS5_211	5	216	3.27	1.65	0.16	S5_14468483	S5_211342363	215.5	216.5	5.02
	qGLS7_158	7	105	5.77	1.05	-0.05	S7_158889984	S7_158892468	103.5	105.5	7.04
	qGLS7_172	7	246	8.55	3.24	0.17	S7_172306746	S7_179011103	245.5	246.5	7.05

qGLS9_106	9	305	6.48	2.15	0.26	S9_106473355	S9_331519	302.5	306.5	9.04
qGLS1_207	1	344	11.88	6.69	0.28	S1_207695200	S1_190286762	342.5	344.5	1.07
qGLS2_188	2	78	5.00	1.53	0.08	S2_188131095	S2_191282909	77.5	78.5	2.07
qGLS3_27	3	155	8.14	6.15	0.28	S3_27134253	S3_136135473	154.5	155.5	3.04
qGLS4_177	4	355	6.31	5.68	0.20	S4_177968538	S4_187594923	354.5	355.5	4.07
qGLS6_128	6	222	4.01	5.81	0.31	S6_128146941	S6_90061908	219.5	222.5	6.05
qGLS7_158	7	105	6.13	1.84	-0.09	S7_158889984	S7_158892468	104.5	105.5	7.04
qGLS7_172	7	246	5.22	4.66	0.30	S7_172306746	S7_179011103	245.5	246.5	
qGLS8_123	8	239	7.45	4.25	-0.22	S8_123293333	S8_128422019	238.5	239.5	
qGLS9_106	9	305	5.63	3.30	0.40	S9_106473355	S9_331519	303.5	306.5	9.04
qGLS1_207	1	344	3.33	8.38	0.25	S1_207695200	S1_190286762	343.5	345.5	1.07
qGLS2_30	2	208	4.27	2.40	0.08	S2_30710232	S2_32668550	207.5	208.5	2.04
qGLS3_27	3	155	3.16	8.40	0.28	S3_27134253	S3_136135473	154.5	155.5	3.04
qGLS5_213	5	40	3.59	2.59	-0.09	S5_213500351	S5_167260117	38.5	41.5	
qGLS7_158	7	105	5.20	2.90	-0.09	S7_158889984	S7_158892468	104.5	105.5	7.04
qGLS7_2	7	143	3.02	6.05	0.15	S7_2664764	S7_146753857	140.5	143.5	
qGLS1_207	1	343	3.20	7.94	0.37	S1_207695200	S1_190286762	342.5	345.5	1.07
qGLS2_153	2	176	6.10	8.22	0.50	S2_153787894	S2_191797557	175.5	176.5	2.06
qGLS2_30	2	208	6.21	3.75	0.13	S2_30710232	S2_32668550	207.5	208.5	2.04
qGLS3_27	3	155	6.11	10.13	0.38	S3_27134253	S3_136135473	154.5	155.5	3.04
qGLS5_167	5	41	3.03	1.75	-0.09	S5_167260117	S5_60859970	38.5	41.5	5.04
qGLS7_164	7	46	6.92	4.61	-0.16	S7_164331844	S7_16901304	45.5	47.5	7.04
qGLS1_207	1	343	4.21	8.10	0.50	S1_207695200	S1_190286762	342.5	344.5	1.07
qGLS2_153	2	176	4.66	8.04	0.59	S2_153787894	S2_191797557	175.5	176.5	2.06
qGLS2_30	2	208	7.20	3.95	0.17	S2_30710232	S2_32668550	207.5	208.5	2.04
qGLS3_27	3	155	4.00	8.62	0.45	S3_27134253	S3_136135473	154.5	155.5	3.04
qGLS5_167	5	41	3.75	1.99	-0.13	S5_167260117	S5_60859970	39.5	41.5	5.04
qGLS7_168	7	47	6.14	3.38	-0.17	S7_164331844	S7_16901304	45.5	47.5	7.04
qGLS1_169	1	162	3.96	4.34	0.23	S1_283894617	S1_53456776	154.5	163.5	1.11
qGLS1_170	1	372	9.10	9.31	-0.25	S1_190286762	S1_185978658	369.5	373.5	1.06

	qGLS1_171	1	383	6.43	7.09	0.22	S1_185978658	S1_143231392	382.5	383.5	
	qGLS2_172	2	208	3.32	3.25	0.15	S2_30710232	S2_32668550	207.5	208.5	2.04
	qGLS7_173	7	105	5.40	5.40	-0.19	S7_158889984	S7_158892468	104.5	105.5	7.04
AUDPC GLS	*qAUDC1_207*	1	343	4.44	8.31	10.99	S1_207695200	S1_190286762	342.5	345.5	1.07
	qAUDC2_153	2	176	4.84	7.53	14.77	S2_153787894	S2_191797557	175.5	176.5	2.06
	qAUDC2_30	2	208	5.65	3.17	3.56	S2_30710232	S2_32668550	207.5	208.5	2.04
	qAUDC3_27	3	155	7.08	10.59	11.10	S3_27134253	S3_136135473	154.5	155.5	3.04
	qAUDC5_167	5	41	4.49	2.49	-3.34	S5_167260117	S5_60859970	39.5	41.5	5.04
	qAUDC7_164	7	47	6.59	3.77	-4.27	S7_164331844	S7_16901304	45.5	47.5	7.04
TLB Location 1	*qTLB2_113*	2	51	8.42	9.60	0.20	S2_113711349	S2_114365078	50.5	51.5	2.05
	qTLB3_2	3	163	4.63	5.36	0.15	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB4_200	4	272	4.29	8.54	-0.19	S4_200040593	S4_201402668	271.5	272.5	4.08
	qTLB4_5	4	362	4.47	4.97	-0.15	S4_5488448	S4_805665	361.5	369.5	4.02
	qTLB5_62	5	60	6.37	7.75	-0.18	S5_62077939	S5_53031954	58.5	61.5	5.03
	qTLB8_171	8	280	8.69	10.59	0.21	S8_171776990	S8_172368917	276.5	283.5	8.08
	qTLB2_65	2	49	6.14	7.60	0.16	S2_65741949	S2_65095725	48.5	50.5	2.04
	qTLB3_65	3	192	4.38	7.03	-0.16	S3_65853211	S3_12761976	189.5	194.5	3.04
	qTLB4_169	4	232	5.74	7.39	-0.17	S4_169986782	S4_173817864	230.5	234.5	4.06
	qTLB5_62	5	59	6.77	10.61	-0.20	S5_62077939	S5_53031954	57.5	60.5	5.03
	qTLB8_171	8	278	3.55	4.47	0.13	S8_171776990	S8_172368917	273.5	281.5	8.08
	qTLB2_170	2	62	5.09	6.19	0.16	S2_170427602	S2_164596994	61.5	62.5	2.06
	qTLB2_164	2	65	3.18	4.91	-0.14	S2_164596994	S2_181216395	64.5	65.5	2.06
	qTLB3_2	3	163	4.80	5.89	0.16	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB4_187	4	346	5.37	8.85	-0.19	S4_187990645	S4_186988478	343.5	347.5	4.08
	qTLB4_5	4	362	5.92	7.10	-0.17	S4_5488448	S4_805665	361.5	368.5	4.02
	qTLB5_62	5	60	3.00	3.89	-0.13	S5_62077939	S5_53031954	57.5	61.5	5.03
	qTLB2_113	2	51	5.11	3.96	0.15	S2_113711349	S2_114365078	50.5	51.5	2.05
	qTLB2_153	2	176	3.11	6.16	-0.59	S2_153787894	S2_191797557	175.5	176.5	2.06
	qTLB3_27	3	155	4.03	12.27	-0.43	S3_27134253	S3_136135473	154.5	155.5	3.04

	qTLB3_2	3	163	4.18	3.35	0.14	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB4_205	4	258	3.43	3.92	0.15	S4_205737072	S4_201661409	257.5	265.5	4.09
	qTLB4_5	4	362	3.88	2.99	-0.14	S4_5488448	S4_805665	360.5	368.5	4.02
	qTLB5_62	5	60	6.31	5.71	-0.18	S5_62077939	S5_53031954	58.5	60.5	5.03
	qTLB2_135	2	54	5.57	7.09	-0.16	S2_135043270	S2_149550704	53.5	54.5	2.05
	qTLB3_2	3	163	6.03	7.40	0.16	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB4_186	4	345	3.15	3.64	-0.11	S4_186588030	S4_187990645	343.5	347.5	4.08
	qTLB4_5	4	361	4.86	12.77	-0.21	S4_5143260	S4_5488448	360.5	366.5	4.02
	qTLB7_146	7	60	4.09	5.60	0.14	S7_146647930	S7_133775178	59.5	61.5	7.03
	qTLB5_113	2	51	7.27	7.98	0.18	S2_113711349	S2_114365078	50.5	51.5	2.05
	qTLB3_2	3	163	5.59	6.28	0.16	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB4_205	4	258	5.95	9.35	0.20	S4_205737072	S4_201661409	257.5	258.5	4.09
	qTLB4_5	4	361	3.74	9.16	-0.20	S4_5143260	S4_5488448	360.5	368.5	4.02
	qTLB5_62	5	60	3.49	4.10	-0.13	S5_62077939	S5_53031954	57.5	61.5	5.03
AUDPC TLB	*qAUDC2_65*	2	49	8.06	8.03	5.28	S2_65741949	S2_65095725	48.5	50.5	2.05
	qAUDC3_2	3	163	5.33	5.39	4.34	S3_2734515	S3_1173815	162.5	163.5	3.01
	qAUDC4_205	4	258	4.47	6.32	4.75	S4_205737072	S4_201661409	257.5	264.5	4.09
	qAUDC4_5	4	362	3.95	3.82	-3.72	S4_5488448	S4_805665	360.5	367.5	4.08
	qAUDC5_62	5	60	11.93	13.94	-6.99	S5_62077939	S5_53031954	58.5	60.5	5.03
	qAUDC5_158	5	283	4.73	4.77	-4.14	S5_15869219	S5_23093956	262.5	287.5	4.03
	qAUDC7_96	7	59	3.29	3.97	3.72	S7_96120864	S7_108499336	57.5	59.5	7.02
DTA	*qDTA1_250*	1	96	7.04	9.28	0.66	S1_250341978	S1_253276965	95.5	96.5	1.09
	qDTA1_214	4	288	5.06	7.38	-0.59	S4_214489216	S4_242283166	279.5	294.5	4.09
	qDTA8_139	8	249	9.00	12.11	0.75	S8_139048847	S8_136861251	248.5	249.5	8.05
DTS	*qDTS1_250*	1	96	6.62	9.67	0.78	S1_250341978	S1_253276965	95.5	96.5	1.09
	qDTS4_214	4	288	3.61	5.65	-0.59	S4_214489216	S4_242283166	277.5	295.5	4.09
	qDTS8_136	8	250	11.15	16.70	1.01	S8_136861251	S8_138560361	249.5	250.5	8.05
PH	*qPH1_253*	1	98	3.89	6.43	-4.37	S1_253276965	S1_258971644	95.5	99.5	1.09
	qPH1_19	1	219	5.44	6.94	-4.54	S1_19920008	S1_21989679	217.5	220.5	1.02

	qPH5_62	5	60	6.09	8.06	-4.88	S5_62077939	S5_53031954	57.5	61.5	5.03
	qPH6_34	6	86	3.19	4.18	3.51	S6_34950594	S6_93095112	79.5	92.5	6.01
	qPH8_19	8	212	3.22	6.03	-4.23	S8_19657881	S8_21382799	210.5	212.5	8.02
	qPH8_135	8	247	10.21	14.05	-6.42	S8_135475467	S8_139048847	246.5	247.5	8.05
EH	*qEH1_28*	1	268	3.19	5.16	-2.02	S1_28106472	S1_27326414	267.5	269.5	1.02
	qEH2_233	2	110	4.03	5.09	-2.04	S2_233445021	S2_242048658	107.5	111.5	2.09
	qEH6_34	6	86	5.27	4.99	1.99	S6_34950594	S6_93095112	84.5	91.5	6.01
	qEH8_128	8	243	14.26	14.82	3.42	S8_128738672	S8_129741294	241.5	243.5	8.05
	qEH9_139	9	21	5.17	11.36	-4.19	S9_139755085	S9_154594758	20.5	21.5	9.06
GLS Location 2	*qGLS1_283*	1	162	4.79	3.17	0.25	S1_283894617	S1_53456776	154.5	163.5	1.11
	qGLS1_190	1	356	4.93	4.18	0.22	S1_190286762	S1_185978658	355.5	356.5	1.06
	qGLS1_190	1	372	18.15	12.47	-0.37	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS1_185	1	383	11.33	8.60	0.31	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS7_158	7	105	3.55	2.10	-0.15	S7_158889984	S7_158892468	103.5	105.5	7.04
	qGLS9_25	9	232	4.80	5.22	-0.24	S9_25854304	S9_25065840	231.5	233.5	9.03
	qGLS1_283	1	163	5.70	3.48	0.31	S1_283894617	S1_53456776	155.5	163.5	1.11
	qGLS1_190	1	356	5.13	4.78	0.28	S1_190286762	S1_185978658	355.5	356.5	1.06
	qGLS1_190	1	372	21.36	15.67	-0.49	S1_190286762	S1_185978658	371.5	372.5	1.06
	qGLS2_185	1	383	12.66	10.43	0.40	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS3_157	3	90	5.80	3.74	0.24	S3_157562360	S3_150546157	88.5	93.5	3.05
	qGLS4_158	7	105	4.76	2.93	-0.21	S7_158889984	S7_158892468	104.5	105.5	7.04
	qGLS9_129	9	155	3.12	1.95	-0.17	S9_129671108	S9_38368264	153.5	161.5	9.05
	qGLS1_283	1	163	8.80	4.79	0.44	S1_283894617	S1_53456776	158.5	163.5	1.11
	qGLS1_190	1	356	5.55	4.82	0.33	S1_190286762	S1_185978658	355.5	356.5	1.06
	qGLS1_190	1	372	25.06	16.60	-0.60	S1_190286762	S1_185978658	371.5	372.5	1.06
	qGLS1_185	1	383	11.67	9.09	0.44	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS2_213	2	151	4.05	2.28	-0.22	S2_213714960	S2_213287172	149.5	152.5	2.08
	qGLS2_30	2	208	5.03	2.70	0.24	S2_30710232	S2_32668550	207.5	208.5	2.04
	qGLS3_157	3	92	8.03	4.65	0.32	S3_157562360	S3_150546157	88.5	95.5	3.05

	qGLS7_158	7	105	4.89	2.61	-0.24	S7_158889984	S7_158892468	104.5	105.5	7.04
	qGLS8_135	9	154	3.57	2.83	-0.25	S9_135788881	S9_129671108	152.5	160.5	9.05
	qGLS10_43	10	217	4.08	6.87	0.49	S10_43765534	S10_54916081	216.5	217.5	10.01
	qGLS1_232	1	77	3.99	3.14	-0.31	S1_232878545	S1_237884693	74.5	77.5	1.08
	qGLS1_283	1	163	5.31	3.48	0.44	S1_283894617	S1_53456776	157.5	163.5	1.11
	qGLS1_190	1	356	5.18	5.09	0.40	S1_190286762	S1_185978658	355.5	356.5	1.06
	qGLS1_190	1	372	20.28	15.83	-0.69	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS1_185	1	383	13.26	11.52	0.59	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS3_157	3	89	7.01	4.67	0.38	S3_157562360	S3_150546157	88.5	94.5	3.05
	qGLS7_158	7	105	3.76	2.45	-0.27	S7_158889984	S7_158892468	103.5	105.5	7.04
	qGLS1_244	1	94	3.47	3.09	0.34	S1_244605862	S1_251642913	93.5	94.5	1.08
	qGLS1_283	1	163	5.91	3.88	0.52	S1_283894617	S1_53456776	158.5	163.5	1.11
	qGLS1_185	1	373	15.84	15.21	-0.75	S1_185978658	S1_143231392	372.5	373.5	1.06
	qGLS5_185	1	383	13.12	11.31	0.64	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS3_157	3	90	8.23	5.83	0.48	S3_157562360	S3_150546157	88.5	94.5	3.05
	qGLS9_25	9	232	3.60	4.34	-0.40	S9_25854304	S9_25065840	226.5	233.5	9.03
AUDPC	*qAUDC1_283*	1	163	6.40	4.01	11.70	S1_283894617	S1_53456776	157.5	163.5	1.11
	qAUDC1_190	1	356	5.29	4.97	9.89	S1_190286762	S1_185978658	355.5	356.5	1.06
	qAUDC1_190	1	372	20.94	15.57	-16.96	S1_190286762	S1_185978658	371.5	372.5	1.06
	qAUDC1_185	1	383	13.20	10.91	14.13	S1_185978658	S1_143231392	382.5	383.5	1.06
	qAUDC3_157	3	90	6.54	4.28	9.08	S3_157562360	S3_150546157	88.5	95.5	3.05
	qAUDC7_158	7	105	4.54	2.84	-7.27	S7_158889984	S7_158892468	104.5	105.5	7.04
	qAUDC9_129	9	155	3.94	2.53	-6.80	S9_129671108	S9_38368264	153.5	160.5	9.05
TLB	*qTLB2_105*	2	47	3.82	4.43	0.21	S2_105059499	S2_65741949	45.5	48.5	2.05
	qTLB3_65	3	185	3.90	5.68	0.25	S3_65853211	S3_12761976	184.5	185.5	3.04
	qTLB3_12	3	193	5.26	6.95	-0.27	S3_12761976	S3_9360961	191.5	194.5	3.03
	qTLB4_118	4	316	3.04	3.97	-0.25	S4_118326581	S4_6911652	315.5	316.5	4.05
	qTLB5_69	5	73	3.65	6.26	-0.26	S5_69767339	S5_73930457	72.5	73.5	5.03
	qTLB8_85	8	220	3.59	4.11	-0.21	S8_85069149	S8_88538381	218.5	220.5	8.03

qTLB8_171	8	279	10.48	13.65	0.38	S8_171776990	S8_172368917	276.5	282.5	8.08
qTLB9_141	9	146	4.83	5.54	-0.24	S9_141487185	S9_141196498	145.5	146.5	9.06
qTLB1_17	1	240	3.26	6.34	-0.24	S1_17979298	S1_14613843	239.5	240.5	1.02
qTLB1_303	1	429	3.13	3.06	0.16	S1_303106691	S1_304299395	423.5	429	1.12
qTLB2_113	2	51	4.56	4.62	0.20	S2_113711349	S2_114365078	50.5	51.5	2.05
qTLB2_186	2	77	3.89	4.05	0.18	S2_186201459	S2_188131029	74.5	77.5	2.07
qTLB3_28	3	144	4.83	5.12	-0.22	S3_28258001	S3_129315443	142.5	144.5	3.04
qTLB5_62	5	60	6.46	7.06	-0.24	S5_62077939	S5_53031954	57.5	61.5	5.03
qTLB6_151	6	335	4.01	5.24	-0.37	S6_151834390	S6_153165363	334.5	335.5	6.05
qTLB7_164	7	101	3.77	6.56	-0.24	S7_164117062	S7_158889984	98.5	101.5	7.04
qTLB8_171	8	277	8.54	9.25	0.28	S8_171776990	S8_172368917	274.5	280.5	7.05
qTLB9_141	9	149	6.87	7.28	-0.25	S9_141817547	S9_134122327	147.5	150.5	9.06
qTLB2_186	2	77	9.39	12.50	0.31	S2_186201459	S2_188131029	75.5	77.5	2.07
qTLB3_28	3	144	4.98	6.55	-0.24	S3_28258001	S3_129315443	142.5	144.5	3.04
qTLB5_167	5	41	3.86	4.69	0.20	S5_167260117	S5_60859970	40.5	41.5	5.04
qTLB6_88	6	334	5.11	6.54	-0.22	S6_88269305	S6_151484771	330.5	334.5	6.02
qTLB7_160	7	98	3.51	4.28	-0.18	S7_160006332	S7_161414585	97.5	98.5	7.04
qTLB8_172	8	284	4.65	6.03	0.21	S8_172368917	S8_172514054	282.5	286.5	8.08
qTLB9_141	9	149	4.90	6.20	-0.22	S9_141817547	S9_134122327	146.5	149.5	9.06
qTLB9_154	9	315	3.11	3.77	-0.30	S9_154817518	S9_28130507	310.5	315.5	9.08
qTLB2_186	2	77	9.45	11.92	0.32	S2_186201459	S2_188131029	74.5	77.5	2.07
qTLB6_118	6	134	3.20	3.66	-0.18	S6_118742764	S6_119177548	132.5	134.5	6.04
qTLB6_151	6	335	5.62	8.61	-0.49	S6_151834390	S6_153165363	334.5	335.5	6.05
qTLB7_121	7	97	5.56	6.56	-0.24	S7_121214712	S7_47406965	91.5	97.5	7.02
qTLB8_170	8	276	6.17	7.37	0.26	S8_170418369	S8_171776990	273.5	279.5	8.08
qTLB2_170	2	62	10.22	9.87	0.34	S2_170427602	S2_164596994	61.5	62.5	2.07
qTLB2_164	2	65	5.16	6.27	-0.27	S2_164596994	S2_181216395	64.5	65.5	2.06
qTLB3_12	3	194	4.68	7.26	-0.29	S3_12761976	S3_9360961	193.5	195.5	3.03
qTLB6_88	6	334	4.03	3.67	-0.21	S6_88269305	S6_151484771	331.5	334.5	6.02
qTLB7_161	7	100	5.40	4.90	-0.24	S7_161414585	S7_164117062	98.5	101.5	7.04

	qTLB8_171	8	279	4.80	4.34	0.23	S8_171776990	S8_172368917	276.5	285.5	8.08
	qTLB10_125	10	177	3.69	3.19	0.19	S10_125383403	S10_129818369	174.5	177.5	10.04
AUDPC	*qAUDC2_186*	2	76	8.89	12.62	9.41	S2_186201459	S2_188131029	74.5	77.5	2.06
	qAUDC3_150	3	98	4.18	4.91	5.97	S3_150546157	S3_146285902	95.5	98.5	3.05
	qAUDC4_77	4	95	3.49	5.65	6.47	S4_77362682	S4_37688126	85.5	105.5	4.05
	qAUDC6_151	6	335	3.08	5.02	-10.67	S6_151834390	S6_153165363	334.5	335.5	6.05
	qAUDC7_121	7	97	6.03	7.32	-7.21	S7_121214712	S7_47406965	92.5	97.5	7.02
	qAUDC8_172	8	284	6.41	8.11	7.52	S8_172368917	S8_172514054	282.5	286.5	7.05
	qAUDC9_141	9	148	4.58	5.68	-6.30	S9_141817547	S9_134122327	146.5	149.5	9.06
DTA	*qDTA1_250*	1	96	5.99	5.74	0.84	S1_250341978	S1_253276965	95.5	96.5	1.09
	qDTA1_258	1	104	5.97	6.62	0.89	S1_258971644	S1_267207434	101.5	107.5	1.09
	qDTA4_214	4	289	5.73	5.93	-0.85	S4_214489216	S4_242283166	281.5	294.5	4.09
	qDTA8_138	8	251	5.28	7.37	0.94	S8_138560361	S8_137532772	249.5	252.5	8.05
DTS	*qDTS1_225*	1	67	6.88	4.52	-0.92	S1_225916227	S1_229375633	61.5	69.5	1.07
	qDTS1_258	1	105	3.81	3.15	0.77	S1_258971644	S1_267207434	103.5	109.5	1.09
	qDTS6_101	6	324	3.73	5.18	1.85	S6_101722172	S6_88269305	323.5	326.5	6.03
	qDTS8_138	8	251	10.90	10.18	1.39	S8_138560361	S8_137532772	250.5	251.5	8.05
PH	*qPH1_253*	1	269	7.67	10.57	-4.28	S1_27326414	S1_26003993	268.5	269.5	1.02
	qPH2_170	2	62	4.68	6.05	3.24	S2_170427602	S2_164596994	61.5	62.5	2.06
	qPH5_7	5	21	5.47	7.28	3.58	S5_33684	S5_7623119	18.5	21.5	5
	qPH6_34	6	87	4.48	6.02	3.24	S6_34950594	S6_93095112	80.5	92.5	6.01
	qPH8_85	8	219	3.44	4.22	-2.74	S8_85069149	S8_88538381	218.5	220.5	8.03
	qPH8_135	8	247	6.29	8.41	-3.82	S8_135475467	S8_139048847	246.5	247.5	8.05
	qPH9_25	9	232	3.76	7.67	3.66	S9_25854304	S9_25065840	231.5	232.5	8.03
EH	*qEH1_2*	1	184	3.73	6.75	-1.95	S1_2881853	S1_5532836	179.5	185.5	1.01
	qEH1_19	1	219	6.27	4.71	-1.63	S1_19920008	S1_21989679	217.5	220.5	1.02
	qEH1_143	1	384	6.84	5.07	-1.68	S1_143231392	S1_118830162	383.5	387.5	1.05
	qEH2_16	2	141	9.48	8.31	-2.18	S2_16409599	S2_214785968	130.5	146.5	2.03
	qEH2_33	2	211	5.14	5.53	-1.76	S2_33774554	S2_38106170	210.5	211.5	2.04

	qEH6_34	6	87	5.95	4.63	1.61	S6_34950594	S6_93095112	84.5	91.5	6.01
	qEH7_29	7	64	8.38	7.91	3.84	S7_29157407	S7_31658088	63.5	64.5	7.02
	qEH7_3	7	197	4.74	4.16	1.52	S7_3326900	S7_3035740	183.5	204.5	7
	qEH8_135	8	247	12.73	10.76	-2.45	S8_135475467	S8_139048847	246.5	247.5	7.03
GLS Location 3	*qGLS1_207*	1	345	15.22	11.24	0.30	S1_207695200	S1_190286762	344.5	346.5	1.07
	qGLS1_190	1	372	4.44	1.72	-0.09	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS3_157	3	89	3.14	1.12	0.07	S3_157562360	S3_150546157	88.5	95.5	3.05
	qGLS5_197	5	22	3.12	6.57	0.22	S5_197790379	S5_33030316	21.5	22.5	5.06
	qGLS6_151	6	322	8.59	3.44	0.54	S6_151390569	S6_97447485	321.5	322.5	6.06
	qGLS9_129	9	155	3.73	1.42	-0.08	S9_129671108	S9_38368264	153.5	160.5	9.05
	qGLS9_98	9	245	4.07	7.27	-0.30	S9_98198252	S9_106473355	241.5	248.5	9.03
	qGLS9_19	9	309	3.36	7.11	0.21	S9_19998599	S9_9113676	308.5	309.5	9.01
	qGLS1_207	1	347	9.30	16.14	0.34	S1_207695200	S1_190286762	345.5	349.5	1.07
	qGLS1_190	1	372	5.70	3.68	-0.15	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS5_197	5	22	3.20	10.90	0.30	S5_197790379	S5_33030316	21.5	22.5	5.06
	qGLS6_151	6	322	3.57	2.40	0.49	S6_151390569	S6_97447485	321.5	322.5	6.06
	qGLS1_283	1	163	5.56	4.35	0.22	S1_283894617	S1_53456776	157.5	163.5	1.11
	qGLS1_190	1	356	3.41	3.49	0.15	S1_190286762	S1_185978658	355.5	357.5	1.06
	qGLS1_190	1	372	12.35	10.55	-0.24	S1_190286762	S1_185978658	370.5	373.5	1.06
	qGLS1_185	1	383	6.07	5.77	0.18	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS9_141	9	146	4.48	3.58	-0.14	S9_141487185	S9_141196498	145.5	146.5	9.06
	qGLS1_283	1	163	5.24	4.49	0.26	S1_283894617	S1_53456776	158.5	163.5	1.11
	qGLS1_190	1	372	12.91	12.16	-0.31	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS1_185	1	383	6.97	7.21	0.24	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS3_157	3	89	4.13	3.50	0.17	S3_157562360	S3_150546157	88.5	93.5	3.05
	qGLS5_197	5	22	3.12	12.35	0.38	S5_197790379	S5_33030316	21.5	22.5	5.06
	qGLS9_141	9	146	4.18	3.55	-0.17	S9_141487185	S9_141196498	145.5	146.5	9.06
	qGLS10_135	10	186	3.15	5.96	0.23	S10_135292723	S10_128274461	185.5	186.5	10.05
	qGLS1_283	1	163	5.84	3.95	0.32	S1_283894617	S1_53456776	158.5	163.5	1.11

	qGLS1_190	1	356	3.97	3.63	0.23	S1_190286762	S1_185978658	355.5	356.5	1.06
	qGLS1_190	1	372	15.04	11.37	-0.39	S1_190286762	S1_185978658	370.5	372.5	1.06
	qGLS1_185	1	383	6.44	5.51	0.27	S1_185978658	S1_143231392	382.5	383.5	1.06
	qGLS2_170	2	61	3.00	3.28	-0.21	S2_170427602	S2_164596994	59.5	61.5	2.06
	qGLS2_31	2	207	6.00	4.19	0.24	S2_31111676	S2_30710232	206.5	207.5	2.04
	qGLS3_191	3	41	4.89	3.49	-0.22	S3_191702242	S3_180211481	40.5	42.5	3.07
	qGLS3_157	3	89	11.17	8.02	0.34	S3_157562360	S3_150546157	88.5	91.5	3.05
AUDPC	*qAUDC1_283*	1	163	6.06	5.52	6.65	S1_283894617	S1_53456776	158.5	163.5	1.11
	qAUDC1_190	1	372	11.20	10.90	-6.86	S1_190286762	S1_185978658	370.5	372.5	1.06
	qAUDC1_185	1	383	5.51	5.90	5.02	S1_185978658	S1_143231392	382.5	383.5	1.06
	qAUDC9_141	9	146	4.30	3.85	-4.08	S9_141487185	S9_141196498	145.5	146.5	9.06
TLB	*qTLB1_28*	1	268	3.25	6.36	-0.06	S1_28106472	S1_27326414	267.5	269.5	1.02
	qTLB3_2	3	163	6.32	6.32	0.06	S3_2734515	S3_1173815	162.5	163.5	3.01
	qTLB3_225	3	239	4.16	4.05	-0.05	S3_225306785	S3_229691997	230.5	240	3.09
	qTLB6_167	6	311	4.10	5.57	-0.06	S6_167638113	S6_167872283	306.5	313.5	6.08
	qTLB7_146	7	60	3.53	3.82	0.05	S7_146647930	S7_133775178	59.5	61.5	7.03
	qTLB8_170	8	273	4.53	4.34	0.06	S8_170418369	S8_171776990	271.5	275.5	8.08
	qTLB10_87	10	74	3.03	5.19	-0.05	S10_87874180	S10_10422701	73.5	75.5	10.04
	qTLB10_135	10	186	3.48	6.32	0.06	S10_135292723	S10_128274461	185.5	186.5	10.05
	qTLB1_250	1	96	5.36	5.05	-0.14	S1_250341978	S1_253276965	95.5	96.5	1.09
	qTLB2_30	2	204	3.76	11.37	0.20	S2_30341425	S2_27642675	203.5	205.5	2.04
	qTLB3_150	3	96	4.88	4.63	0.13	S3_150546157	S3_146285902	91.5	97.5	3.05
	qTLB4_77	4	94	3.02	4.01	0.13	S4_77362682	S4_37688126	83.5	105.5	4.05
	qTLB8_13	8	271	5.11	7.75	0.23	S8_13252886	S8_170418369	269.5	273.5	8.02
	qTLB1_229	1	70	8.81	9.43	0.17	S1_229375633	S1_232878545	66.5	70.5	1.08
	qTLB2_170	2	62	3.34	3.56	0.10	S2_170427602	S2_164596994	61.5	63.5	2.06
	qTLB3_164	3	185	8.94	12.25	0.20	S3_65853211	S3_12761976	184.5	185.5	3.04
	qTLB3_171	3	192	5.09	7.31	-0.15	S3_65853211	S3_12761976	191.5	192.5	3.04
	qTLB8_141	8	273	3.27	3.38	0.11	S8_170418369	S8_171776990	271.5	275.5	8.08

	qTLB1_186	1	70	5.90	5.31	0.16	S1_229375633	S1_232878545	66.5	71.5	1.08
	qTLB1_28	1	327	4.35	7.30	0.33	S1_286424319	S1_40109570	324.5	328.5	1.11
	qTLB2_167	2	63	5.60	12.33	0.24	S2_164596994	S2_181216395	61.5	63.5	2.07
	qTLB3_88	3	143	5.31	4.84	-0.16	S3_28258001	S3_129315443	142.5	143.5	3.04
	qTLB1_160	1	83	4.90	7.30	0.17	S1_239142914	S1_244605862	82.5	83.5	1.08
	qTLB1_172	1	90	6.19	8.63	-0.19	S1_239142914	S1_244605862	89.5	91.5	1.08
	qTLB3_141	3	98	4.42	5.61	0.15	S3_150546157	S3_146285902	95.5	98.5	3.05
	qTLB6_154	6	0	6.08	7.81	-0.18	S6_138238604	S6_157597489	0	21.5	6.05
AUDPC	*qAUDC1_250*	1	96	4.46	8.65	-4.50	S1_250341978	S1_253276965	95.5	96.5	1.09
	qAUDC1_28	3	143	3.20	6.19	-4.04	S3_28258001	S3_129315443	142.5	144.5	3.04

QTLs were assigned to bins according to the maize reference maps (Maize Data Base 1999).
QTL were named by ‘‘*qNCLB*’’ or “*qGLS*” plus the chromosome bin where it is located.
[a] The maize chromosome where the QTL was located.
[b] The logarithm of odds.
[c] The proportion of phenotypic variance associated with the QTL.
[d] The positive and negative additive effects imply that resistance effects were either from CML511 or CML546 respectively.
[e] Markers flanking the QTL interval.