GENETIC ANALYSIS OF RESISTANCE TO MAIZE LETHAL NECROSIS WITH EMPHASIS ON STRATEGIES FOR IMPROVEMENT OF HOST RESISTANCE

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Submitted in fulfilment of the requirements in respect of the Doctoral Degree in Plant Breeding in the Department of Plant Sciences in the Faculty of Natural and Agricultural Sciences at the University of the Free State

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DELCARATION

I, Daniel Bomet Kwemoi, declare that the thesis that I herewith submit for the Doctoral Degree in Plant Breeding at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education. I further cede copyright of the thesis in favour of the University of the Free State.

Daniel Bomet Kwemoi Date: 30 November 2021

ABSTRACT

Maize lethal necrosis (MLN) is a new disease in Sub-Saharan Africa (SSA) caused by double infection by maize chlorotic mottle virus (MCMV) with any of the many viral agents in the Potyviridae family. It has become one of the key constraints to maize production in the region due to the significant crop losses caused since its emergence in 2011. Sustainable management of MLN is achievable through genetic improvement and replacement of old susceptible varieties with farmer-preferred varieties that combine MLN resistance with tolerance to other prevailing biotic and abiotic stresses. To effectively breed for MLN resistance, it is important to identify sources of resistance, determine the genetic nature of resistance and employ efficient breeding techniques that will result in high genetic gains from selection. This study aimed to dissect the genetic nature of MLN using elite and introduced inbred lines from tropical and temperate maize genetic pools, new biparental populations and potential hybrids. The genetic analysis was conducted in five studies representing the major stages of maize breeding, from pre-breeding to variety development. In the pre-breeding study, the breeding potential for MLN resistance among 18 tropical and temperate inbred and doubled haploid (DH) lines was conducted to identify the best parents for population development. The second study on the genetic potential and usefulness of new pedigree populations was conducted using nine segregating biparental populations derived by crossing susceptible elite and recycled DH lines to two introduced MLN resistant lines from the KS23 pool. The third study, using a section of these populations, selected three sizable populations, which were phenotyped, genotyped and used to validate quantitative trait loci (QTL) linked to MLN resistance in the KS23 genetic background. The fourth study, moving towards product development, entailed determining the potential of new lines and single crosses for use as MLN resistant testers and hybrid parents. In this study, the combining ability for MLN resistance and agronomic traits was estimated among tropical MLN resistant maize lines and prospective single cross testers. Finally, MLN-resistant three-way test cross hybrids (potential new varieties) were evaluated to determine the magnitude of genotype by environment interaction (GEI) and yield stability of hybrids under stress and optimum conditions.

Results indicated overall high genetic variation for MLN resistance among introduced and adapted inbred lines, segregating populations and test cross hybrids. High heritability estimates were also observed among the diverse germplasm categories. With the high heritability estimates observed, significant gains from selection can be achieved for both MLN resistance

and agronomic traits. The pre-breeding assessment identified good inbred lines suitable for formation of new MLN resistant populations. Testcross analysis led to identification of two resistant inbred lines and seven potential single cross testers that could be used for routine MLN resistance breeding. Multi-location experiments on three-way testcross hybrids identified 11 potential hybrids stable under MLN pressure and disease-free conditions. These hybrids could be further tested, released and commercialised. Genetic analysis and QTL mapping using biparental populations led to the validation of one major recessively inherited QTL located on the long arm of chromosome 6, consistently linked to three Kompetitive allele-specific polymerase chain reaction (KASP) markers across three mapping populations. This major candidate QTL needs to be fine-mapped and high-quality markers linked to the QTL selected and used for routine MLN resistance breeding in SSA and globally.

Key words: MLN resistance, pre-breeding, usefulness criterion, combining ability, QTL mapping, linkage analysis, genotype x environment interaction and stability.

DEDICATION

To Emmaculat, Darlene and Durrel - Wife, daughter and son: For your patience, belief and unending support.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACBD	Augmented complete block design
AD	Anthesis date
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
BLUE	Best linear unbiased estimate
BLUP	Best linear unbiased prediction
bp	Base pair
BR	Baker's ratio
ASI	Anthesis-silking interval
BSSS	Iowa B stiff stalk synthetic
BYDV	Barley yellow dwarf virus
CIMMYT	International Wheat and Maize Improvement Centre
CIM	Composite interval mapping
cM	centiMorgan
CP	Coat protein
DH	Doubled haploid
E	Environment
EA	Ear aspect
EH	Ear hight
ELISA	Enzyme-linked immunosorbent assay
EP	Ear position
EPP	Ears per plant
ER	Ear rots
FAW	Fall armyworm
G	Genotype
GCA	General combining ability
GE	Genotype by environment

GEI	Genotype by environment interaction
GEM	Germplasm enhancement of maize
GGE	Genotype and Genotype by Environment Interaction
GLS	Gray leaf spot
GMP	Global Maize Programme
GT	Grain texture
GY	Grain yield
h^2	Narrow-sense heritability
H^2	Broad-sense heritability
HSGCA	Heterotic group's specific and general combining ability
IciMapping	Inclusive composite interval mapping
IITA	International Institute of Tropical Agriculture
IPCA	Interaction principal component analysis
JGMV	Johnsongrass mosaic virus
KASP	Kompetitive allele-specific polymerase chain reaction
LOD	Logarithm of odds
LSD	Least significant difference
MAB	Marker-assisted breeding
MAF	Minor allele frequency
MARS	Marker-assisted recurrent selection
masl	Metres above sea level
miRNA	MicroRNA
MCMV	Maize chlorotic mottle virus
MDMV	Maize dwarf mosaic virus
MIM	Multiple interval mapping
MLM	Mixed linear model
MLN	Maize lethal necrosis
MOI	Grain moisture
MSV	Maize streak virus
MTSI	Multi-trait stability index

MYDV	Maize yellow dwarf virus
NaCRRI	National Crops Resources Research Institute
NARO	National Agricultural Research Organization
NARS	National Agricultural Research Systems
NCBI	National Centre for Biotechnology Information
NE	Number of ears
NIL	Near-isogenic line
NP	Number of plants
NPPO	National plant protection organisations
NSSS	Non-stiff stalk synthetic
PCA	Principal component axis
PH	Plant height
PVE%	Percentage phenotypic variance explained
qPCR	Quantitative real-time polymerase chain reaction
QTL	Quantitative trait loci
rAUDPC	Relative area under disease progress curve
RECORD	Recombination Counting and ORDering
ReML	Restricted maximum likelihood
RFLP	Restriction fragment length polymorphism
R genes	Resistance genes
RIL	Recombinant inbred line
RNAi	RNA interference
RPA	Recombinase polymerase amplification
RT-PCR	Reverse transcription-polymerase chain reaction
SAD	Sum of adjacent criterion
SCA	Specific coming ability
SCMV	Sugarcane mosaic virus
SD	Days to silking
SF-ANOVA	Single factor analysis of variance
SIM	Simple interval mapping

SNP	Single nucleotide polymorphism
SrMV	Sorghum mosaic virus
SSA	Sub-Saharan Africa
SSR	Simple sequence repeat
STMA	Stress tolerant maize for Africa
TBIA	Tissue blot immunoassay
TLB	Turcicum leaf blight
USAID	United States Agency for International Development
WILP	Whole infected leaf powder
WSMV	Wheat streak mosaic virus

CHAPTER 1

General introduction

Maize (*Zea mays* L.) in SSA is a key staple that feeds more than 50% of the region's population (300 million people) and provides an estimated 30% of their calorie requirements (Prasanna et al. 2021). Despite its importance, the annual average on-farm productivity remains low, at <2 ton ha^{-1}, compared to the world average of 5.8 ton ha^{-1} (FAO 2020). This low productivity on-farm in SSA is attributed to persistent, emerging and re-emerging biotic and abiotic stresses, limited access to quality seed and an array of complex socio-economic factors prevailing in farming systems. Addressing biotic and abiotic stress in SSA has been achieved through genetic improvement, an approach that is considered practical and cost-effective. In the last decade, breeding for resistance against common foliar diseases, and tolerance to drought and low soil nitrogen, has resulted in significant genetic gains of up to 141 kg ha^{-1} $year^{-1}$ in eastern and southern Africa (Masuka et al. 2017). Overall, it is estimated that over 53 million farmers have benefited from increased yields due to the adoption of drought-resilient varieties in SSA with a yield of over 25% higher than popular commercial varieties (Cairns and Prasanna 2018; Prasanna et al. 2021).

Despite the success attained in developing stress-resilient varieties in SSA, the emergence of MLN in East Africa in 2011 (Wangai et al. 2012) and the region-wide outbreak of fall armyworm (FAW) in 2015 (FAO 2017), has caused a major setback to breeding gains attained and subsequently a greater challenge to farming communities in the region. Since its discovery in Kenya's Rift Valley region, MLN has continued to cause a major threat to food security due to the rapid spread to non-endemic countries neighbouring Kenya (Mahuku et al. 2015; Fatma et al. 2016). This devastating disease was confirmed by Adams et al. (2014) in Rwanda, the Democratic Republic of Congo (Lukanda et al. 2014), Ethiopia (Mahuku et al. 2015; Mengesha et al. 2019; Terefe and Gudero 2019) and Tanzania (Kiruwa et al. 2020). Unlike many maize diseases, MLN is caused by the double infection and synergistic action by MCMV of the genus *Machlomovirus* and family Tombusviridae with any of the cereal viruses in the Potyviridae family, such as sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), wheat streak mosaic virus (WSMV) or Johnsongrass mosaic virus (JGMV) (Uyemoto et al. 1980; Redinbaugh and Stewart 2018). In East Africa, however, MLN is known to be caused by a combination of MCMV and SCMV (Wangai et al. 2012). Of the two viruses, MCMV has been

found to have a genetic resemblance to other strains in the world, while MCMV is genetically diverse globally and there are strains unique to East Africa (Adams et al. 2013; Wamaitha et al. 2018). Maize infected with individual viruses show disease symptoms with a mild effect on productivity, but a combination of viruses, leading to MLN, can cause up to 100% crop loss depending on the level of resistance of the variety and the prevailing environmental conditions (Wangai et al. 2012; Mahuku et al. 2015). This significant crop loss can greatly impact food, nutritional and income security as well as the general well-being of the large number of households that depend on maize. Several disease management options have been recommended, including routing disease surveillance and forecasting, increased farmer awareness, management of vectors, use of cultural disease and pest control practices, planting disease-free seed and deployment of resistant varieties in affected agro-ecologies (Marenya et al. 2018). Surveillance and management of MLN in endemic and non-endemic areas have been achieved through a concerted effort by countries in SSA to limit further spread within and across boundaries (Prasanna et al. 2020).

Breeding for disease resistance is possible if sources of resistance and effective breeding tools are available and adaptable. Adequate genetic variation, efficient methods and tools for selection and advancement of breeding populations and varieties with support from a vibrant seed system are vital for the release and effective delivery of resistant varieties to farmers. Breeding for MLN resistance in SSA started with screening large tropical maize genetic pools and was supported with the introduction of germplasm with known virus resistance from Asia and the USA (Das et al. 2019; Prasanna et al. 2020). This effort led to the identification of resistant donor lines used to form new breeding populations and first-generation (first cohort) MLN tolerant hybrids. The use of exotic germplasm is necessary to increase the genetic diversity needed to achieve breeding success by introgression of new alleles into adapted backgrounds. Adequate genetic gain, however, depends on the amount of useful genetic diversity generated, selected and advanced in breeding programmes (Hallauer et al. 1988), as well as the number of successful varieties adopted by farmers in MLN endemic areas.

In this study, the usefulness of both elite and exotic germplasm was explored in developing desirable MLN resistant breeding populations, new inbred lines, new hybrids and potential varieties. The genetic nature of resistance was assessed and genomic regions linked to resistance to MLN were validated in tropical maize populations to aid the future implementation of marker-assisted breeding. The five key objectives of the study were to:

i. Determine the genetic potential for resistance to MLN among inbred and DH lines from tropical and temperate genetic backgrounds;
ii. Determine the genetic variation, breeding potential and expected genetic gain from selections among KS23-derived populations;
iii. Validate QTL linked to MLN resistance in the KS23-derived biparental populations;
iv. Estimate the combining ability for MLN resistance and agronomic traits among tropical MLN-resistant maize lines and potential testers;
v. Determine the magnitude of GEI and yield stability of MLN resistant hybrids under stress and optimum conditions.

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CHAPTER 2

Maize lethal necrosis: epidemiology, impact, management and advances in resistance breeding

2.1 Global and regional distribution and impact of maize lethal necrosis

Maize and wheat are the two most important cereals globally (Erenstein et al. 2021). In SSA alone, maize is a key commodity accounting for over 40% of total cereal production. More than 80% of maize grain is consumed by humans, providing an estimated 30% of the total calorie requirements of communities. Regardless of its economic importance, maize productivity remains low in the region despite the increase in area under cultivation over time (Santpoort 2020). The low productivity is mostly attributed to several abiotic and biotic stresses coupled with socio-economic factors (Shiferaw et al. 2011; Prasanna et al. 2021). Biotic stresses such as MLN and associated causal viruses have been among the major biotic constraints to maize production in the Americas since the 1970s. Earlier disease incidences were reported in Peru in 1973 (Castillo and Hebert 1974) and different parts of the USA between 1976 and 1981 (Niblett and Claflin 1978; Uyemoto et al. 1979; Redinbaugh and Stewart 2018) and later in Hawaii in 1990 (Jiang et al. 1992). Globally, the most recent outbreaks of MLN occurred in China in 2010 (Xie et al. 2011), Kenya in 2011 (Wangai et al. 2012), Taiwan in 2014 (Deng et al. 2014) and Ecuador in 2016 (Quito-Avila and Superior 2018) (Figure 2.1). Despite these recent reports, SCMV was already reported in Kenya over four decades ago (Louie 1980).

The first outbreak of MLN in East Africa occurred in the southern Rift Valley region and rapidly spread to the neighbouring countries in less than five years (Wangai et al. 2012; Isabirye and Rwomushana 2016; Redinbaugh and Stewart 2018). In Uganda, the disease was first reported in 2012 in the eastern region neighbouring Kenya (Frank et al. 2016) and more cases were recorded in the eastern and western highland agro-ecological zones (Mudde et al. 2018). Subsequently, regional disease surveillance also reported MLN occurrence in other countries, including Tanzania (Kiruwa et al. 2020), the Democratic Republic of Congo (Lukanda et al. 2014), Ethiopia (Mahuku et al. 2015a, Deressa and Demissie 2017; Fentahun et al. 2017), Rwanda (Adams et al. 2014; Asiimwe et al. 2019) and Tanzania (Kiruwa et al. 2016; 2020). Annual regional disease surveillance and monitoring in SSA indicate that MLN may become a persistent and economically important disease in maize farming systems. However, collective

management of the transboundary disease has limited its spread to countries in southern Africa (Prasanna et al. 2020).

Figure 2.1 Global occurrence and distribution of maize lethal necrosis (MLN).

Red dots indicate countries with MLN**.** (https://www.cabi.org/isc/datasheet/119663#toDistributionMaps)

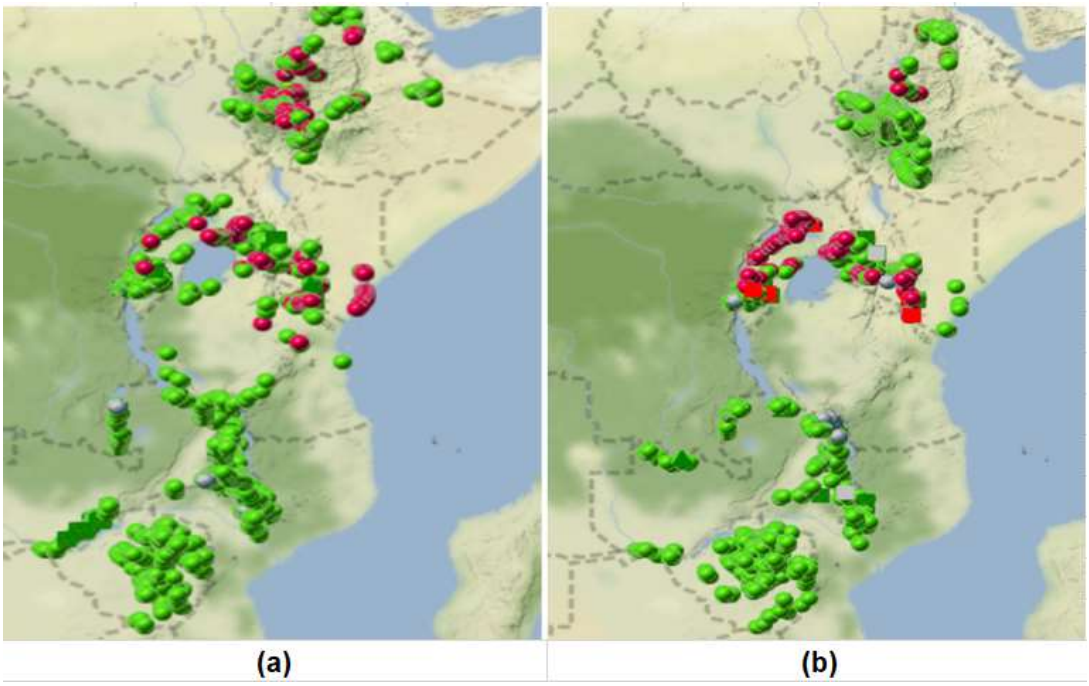

Figure 2.2 Regional maize lethal necrosis surveillance map in eastern and southern Africa in (a) 2019 and (b) 2018.

Green dots represent fields that tested negative for maize chlorotic mottle virus while red dots were fields that tested positive. (https://mln.cimmyt.org/survey-location-map/)

Regional survey data indicated no cases of MLN in southern Africa (Malawi, Zambia and Zimbabwe). There was generally persistent disease in east Africa across years, but with variation between countries (Figure 2.2).

Maize lethal necrosis is known to cause total crop loss in severely infected fields. However, losses ranging from 30 to 100% were initially recorded in East Africa depending on the location and the stage of crop growth. A separate case study in Kenya indicated that lower losses averaging 15% occurred in high altitude areas, while higher losses of up to 59% were seen in the moist transitional zones (De Groote et al. 2016). Overall, the estimated monetary loss in the region was initially estimated at US$ 339 million in 2013 and was projected to rise US$ 418 million by 2018 if management measures were not effectively implemented (Pratt et al. 2017; Redinbaugh and Stewart 2018). This could further impact the food security and livelihood status of over 300 million people who largely depend on maize (Mahuku et al. 2015a; Redinbaugh and Stewart 2018). Additionally, MLN and associated viruses pose a significant threat to other important staple and cash crops in the region, such as sorghum, millet, wheat and sugarcane (Kusia 2015; Braidwood et al. 2018; 2019).

2.2 Symptoms of maize lethal necrosis

The expression of symptoms of MLN depends on the stage of infection and the degree of tolerance in the genetic background of the crop. Overall, bright mosaics develop into tissue chlorosis and necrosis. Subsequently, severe plant stunting and death of terminal leaves occur, which later expand, forming a dead heart (Niblett and Claflin 1978; Mahuku et al. 2015a). Symptoms are observed on plants as early as two weeks after germination up to the ear formation stage. Plants infected late in development remain barren or bear small, poorly filled and malformed ears. Severely damaged cobs are often predisposed to ear rots and many show moulds and mycotoxin contamination (Nelson et al. 2011; Wangai et al. 2012). In general, the severity of symptoms depends on the growth stage at which the plant is infected and, in most cases, the impact is greater if infection occurs early in the cropping cycle (Wangai et al. 2012; Beyene et al. 2017; Jardine 2017). The diverse symptoms of MLN are shown in Figure 2.3

2.3 Epidemiology of maize lethal necrosis and disease management decisions

From a global historical standpoint, MLN is a re-emerging disease in some regions and a new disease spreading to new areas. To effectively manage the disease, it is important to

understand the main and interactive epidemiological factors, agro-ecological and evolutionary factors as well as the prevailing crop management methods (Robert 2001; Jeger 2020). The main epidemiological factors include the presence of causal virus agents and their diversity, host range, vectors and the environment, while the interactive factors that influence the disease spread include virus-plant, virus-vector and vector-plant interactions and the role of ecology and crop management (Jeger 2020). In addition to insect vectors, other mechanisms responsible for the spread of MLN and its causal viruses within and across agro-ecologies include infected seed, infected maize crop debris and contaminated garden tools and soil (Mahuku et al. 2015a; Deressa et al. 2017).

		
Early mosaic and mottling	Shortened internodes and severe chlorotic mottling	Dead hearts and necrosis starting from leaf margins
		
Premature cob drying	Severe chlorosis and leaf necrosis	Poor or no grain filling

Figure 2.3 Maize lethal necrosis symptoms.

2.3.1 Virus genetic diversity, synergism and recombination

Maize lethal necrosis is caused by co-infection and synergistic interaction of MCMV belonging to the genus *Machlomovirus*, family Tombusviridae with any of many viruses from the family Potyviridae, especially SCMV, MDMV, WSMV (Niblett and Claflin 1978; Uyemoto et al. 1979; Redinbaugh and Stewart 2018; Prasanna et al. 2020) and JGMV, which is the most recently confirmed obligatory component of MLN in the region (Stewart et al. 2017). Although these potyviruses have been confirmed in east African farming systems, a combination of MCMV and

SCMV has been identified as the major cause of the current MLN pandemic in the region (Wangai et al. 2012; Redinbaugh and Stewart 2018). Both MCMV and SCMV individually cause disease symptoms, but greater impact is observed when two or more viruses co-infect plants, and could cause complete yield loss when infection occurs early during crop growth (Wangai et al. 2012; Beyene et al. 2017).

Following the emergence of MLN in East Africa in 2011, field surveys, surveillance and virus identification and genetic diversity studies have been conducted on major MLN viruses and analysis of sequence homologies was done to determine the relatedness of virus strains within and between geographic regions. Initial samples collected in Kenya were analysed using tissue blot immunoassay (TBIA) and reverse transcription-polymerase chain reaction (RT-PCR) to confirm the presence of MCMV and SCMV, respectively. Virus amplicon sequences were identical among samples and with the National Centre for Biotechnology Information (NCBI) GenBank sequences for both MCMV (95 - 98%) and SCMV (88 - 96%) (Wangai et al. 2012). In Rwanda, detection of MLN viruses using both enzyme-linked immunosorbent assay (ELISA) and quantitative real-time PCR (qPCR) techniques confirmed the presence of both MCMV and SCMV in samples collected. Deep sequencing of the MCMV coat protein diversity showed a significant relationship among samples collected within the country (> 99% identity) and an equally high identity (> 94%) to curated GenBank sequences from the USA, China, Thailand, Taiwan, Ecuador and Kenya (Xie et al. 2011; Adams et al. 2013; Asiimwe et al. 2019). This similarity suggested limited global diversity of MCMV. On the other hand, higher sequence diversity was observed for SCMV. Sequence homology analysis showed relatedness in SMCV samples from Kenya, Ethiopia, Ecuador and China (Asiimwe et al. 2019). However, there was significant divergence between SCMV sequences from Rwanda and Kenya (Adams et al. 2014). Specifically, single nucleotide polymorphisms (SNPs) and polyprotein sequence alignments distinguished SCMV isolates in Kenya to up to three genetically distinct groups (Wamaitha et al. 2018). A comprehensive genetic diversity study on MLN viruses in Kenya also showed that MCMV had a lower sequence variability while SCMV exhibited higher genetic diversity with possible genetic recombination events (Mwatuni et al. 2020). Similar findings were obtained in comparative studies using samples from China and East Africa (Braidwood et al. 2018; 2019; Mwatuni et al. 2020). The evident virus recombination is a possible reason for the observed genetic diversity of SCMV. This may lead to future threats to crop production as a result of changing virus dynamics arising from virus evolution and emergence of adaptive variants by exchange of genomic segments (King et al. 1982; Martin et al. 2005; Valli et al. 2007; Pérez-

Losada et al. 2015). Because SCMV had been observed earlier in east African cropping systems, it was suspected that the new outbreak of MLN in East Africa was caused by the introduction of MCMV followed by its spread within the region through seed (Asiimwe et al. 2020). However, other factors leading to limited global MCMV diversity and its spread need to be further investigated to inform the development of sustainable disease management options.

Despite the significant role of MCMV and SCMV in the current MLN threat in East Africa, understanding the epidemiological status of other potyviruses is vital for comprehensive MLN management in future. Several factors are known to contribute to the occurrence of epidemics and the emergence of pandemics in cropping systems, including the following: (i) introduction of new pathogens due to limited restriction of movement of plant materials and seed due to free trade and tariff-reduction policies, and increased efficiency of global transportation and transboundary movement systems. Movement of plant materials to new areas favours MLN viruses that are known to be seed-borne or transmitted through plant residues and soil; (ii) transfer of crops from their centres of domestication and use of monocultural practices that encourage the invasion of the introduced crops and build-up of new diseases and (iii) effects of climate change such as global warming that encourages new disease outbreaks (Jones 2021). Such factors could also lead to changes in the dynamics of the current MLN pandemic in SSA. For instance, in the earlier survey by Adams et al. (2014), JGMV and MDMV were not initially detected in samples analysed in Rwanda. However, both viruses were later detected in samples collected from previously surveyed sites in Rwanda, Uganda, Kenya and Tanzania. The JGMV from the three countries had contigs with close matches, signifying a close genetic relationship. In addition, JGMV contributed to MLN symptoms even where SCMV was not detected (Stewart et al. 2017).

2.3.2 Host range, susceptibility and management of maize lethal necrosis and its agents

The presence of the pathogen (MCMV and potyviruses), a conducive environment and a susceptible host or host carriers lead to the presence of the disease. Causal virus agents of MLN are known to thrive in common alternate hosts including up to 14 east African grass-like species such as Johnson grass (*Sorghum halepense* L*. Pers.*), oats (*Avena sativa* L.), millet (*Panicum miliaceum* L.), sugarcane (*Saccharum officinarum* L.), prairie grass (*Bromus catharticus* Vahl), purpletop chloris (*Chloris barbata* Sw.), sour paspalum (*Paspalum conjugatum* Berg.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), Sudan grass (*Sorghum sudanense* (Piper) Stapf), buffalo grass (*Stenotaphrum secundatum* (Walter) Kuntze), eastern

gamagrass (*Tripsacum dactyloides* (L.) L.) and marmalade grass (*Urochloa plantaginea* (Link) R.D. Webster) (Mahuku et al. 2015a). A study conducted by Mudde et al. (2019) showed varying susceptibility to MCMV among tropical weeds, including the African couch grass (*Digitaria abyssinica* ((Hochst. ex A. Rich.) Stapf), Wild African finger millet (*Eleusine africana* Kennedy-O'Byrne) and Itch grass (*Roetboellia cochinchinensis* (Lour.) W.D. Clayton). Grasses found to be susceptible to SCMV were Elephant grass (*Pennisetum purpureum* Schum), Common guinea grass (*Panicum maximum* Jacq) and Itch grass. This implied that common weeds are important reservoirs of MLN viruses in cropping systems. Given the diversity of MLN hosts, weed management and crop rotation are recommended in integrated disease management systems (Mekureyaw 2017; Redinbaugh and Stewart 2018). Crop rotation systems should therefore involve legumes and other non-poaceous crops. In addition to barley, wheat and sugarcane that are known to harbour MLN viruses, a survey confirmed the occurrence of MLN causing viruses in millet, a popular crop in SSA (Kusia 2015).

2.3.3 Vectors and mode of transmission of maize lethal necrosis viral agents

Virus transmission studies on candidate insect vectors for MLN in mainland USA implicated a diversity of beetles and two types of thrips. The major beetle vector species identified were the cereal leaf beetle (*Oulema melanopa* L.), corn flea beetle (*Chaetocnema pulicaria* Melsheimer), flea beetle (*Systena frontalis* (F.)), and southern, western, and northern corn rootworms (*Diabrotica* spp.) that attack plants at larval and adult stages. Studies in Hawaii also identified maize thrips (*Frankliniella williamsi* Hood) and Western flower thrips (*Frankliniella occidentalis* (Pergande)) to be the major vectors of MCMV. Thrips could be the major vectors of MCMV because they feed on a wide range of poaceous and non-poaceous plants and are ubiquitous in cropping agro-ecologies (Nault 1978; Nelson et al. 2011; Redinbaugh and Stewart 2018). Transmission of MCMV by Western flower thrips was also confirmed by Zhao et al. (2014) under screen house conditions. On the other hand, SCMV and other potyviruses are transmitted mainly by diverse aphid species including *Rhopalosiphum maidis* (Fitch), *R. padi* (L.), *Myzus persicae* (Fabricius) and *Schizaphis graminum* (Rondani) (David and Alexander 1984; Noone et al. 1994; Redinbaugh and Stewart 2018). Like MCMV, soil transmission of SCMV is known to occur in sorghum (Bond 1970). Thrips transmit MCMV semi-persistently and the virus remains viable within the insect for up to six days while aphid vector transmission of potyviruses is usually shorter (non-persistent) and the virus remains viable within the stylet for just a few minutes (Cabanas et al. 2013; Gadhave et al. 2020). Understanding modes of vector transmission is vital for vector scouting and implementation of insect pests and vector

management regimes, including the use of effective pesticides (Nelson et al. 2011; Redinbaugh and Stewart 2018). Namikoye et al. (2020) provided recommendations on insect vector scouting techniques and timing of pesticide spray for corn thrips and aphids in maize to maximise returns from pest management. This study recommended an action threshold of six corn thrips and three corn leaf aphids per plant followed by implementation of monthly sprays rather than weekly pesticide application.

2.3.4 Other methods of virus transmission

Earlier studies following systematic sampling and ELISA assays reported low rates (averaging 0.03%) of MCMV transmission through seed (Jensen et al. 1991). Another study by Bockelman et al. (1982) did observe seed transmission of MCMV in maize. However, recent studies in East Africa contradicted the earlier report pointing to very low seed transmission; MCMV incidence of up to 72% was observed in seeds harvested from infected plants. The high stability and survival rate of MCMV could facilitate high transmissibility through the soil, infected plant debris and field equipment or machinery (Mahuku et al. 2015a; Mezzalama et al. 2015). Due to its high stability, MCMV can remain infectious for more than 30 days and can only be deactivated at high temperatures of 80 - 85^{o}C (Uyemoto 1981; Redinbaugh and Stewart 2018). Soil transmission was thought to be promoted by insect larvae in the soil but it was later confirmed that MCMV can survive in crop residues (Uyemoto 1983).

2.3.5 Effect of environment and agro-ecosystems on maize lethal necrosis and disease management decisions

Regional collaborative surveillance for MLN in SSA conducted by CIMMYT and national plant protection organisations indicated the seasonal variation of the disease incidence in farming systems from 2015 to 2019 (Figure 2.4). The sporadic nature of plant viruses is a function of both the environment and the mode of vector transmission, among other factors (Madden et al. 2000; Redinbaugh and Zambrano 2014). Changes in weather conditions, especially seasonal variability in rainfall, drought, carbon-dioxide concentration and high temperatures, affect the responses of both host plants and vectors and subsequently impact disease severity (Jeger 2020).

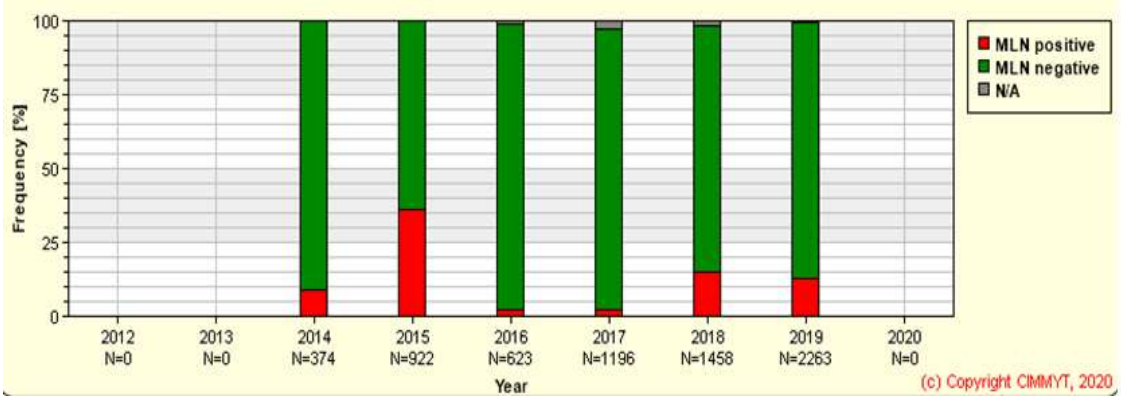

Figure 2.4 Prevalence of maize lethal necrosis of infected farms in Uganda, Ethiopia, Kenya, Tanzania and Rwanda, 2014-2019.

(https://mln.cimmyt.org/survey-frequency-chart/)

2.3.6 Overview of sustainable management options for maize lethal necrosis

Sustainable management of MLN and other crop diseases requires careful consideration of epidemiological components in designing integrated management options (Jeger 2020). Farmer knowledge also plays a key role in the adoption and implementation of integrated disease management strategies (Mudde et al. 2017). Disease surveillance is important in managing spread within cropping systems and across boundaries. A well-coordinated MLN surveillance system in SSA has prevented the spread of the disease from East Africa to southern and western Africa (Mahuku and Kumar 2017; Prasanna et al. 2020). Several approaches to integrated management of MLN have been recommended, including restriction of virus reservoirs such as infected seed, weeds, infected maize, crop rotation, control of vectors and employment of host-plant resistance (Robert 2001; Redinbaugh and Zambrano 2014; Mezzalama et al. 2015). Development and deployment of plant resistance in disease management is an important step in managing MLN and other plant viruses (Beyene et al. 2017; Prasanna et al. 2020). The use of resistant varieties is also considered to be economically viable and environmentally friendly (Redinbaugh et al. 2018). Planting resistant varieties is also sustainable because farmers practice continuous cropping of maize and have limited access to insecticides (Jones et al. 2018). Further, greater effort is often directed towards the development of resistant crops, but less attention is given to understanding the mode of spread of the virus in the field and often there is crop heterogeneity in cropping systems, where both resistant and susceptible varieties are grown (Jeger 2020). Modelling experiments considering seasonal variation, vector transmission and other inoculum sources have recommended the use of mathematical models to guide disease management decisions in cases of MLN, where

epidemiological factors influencing the disease still need to be studied. There is also a need for synchrony in farmer management practices over large areas to increase effectiveness by reducing extraneous sources of the virus (Isabirye and Rwomushana 2016; Hilker et al. 2017; Osunga et al. 2017). So far, first-generation MLN tolerant hybrids have been released and commercialised in Uganda, Kenya and Tanzania to complement other on-going disease prevention strategies such as production and distribution of disease-free seed, cross-border surveillance and management, among other integrated methods used by farmers (Prasanna et al. 2020).

2.4 Genetic resistance to maize lethal necrosis and implications for breeding for durable resistance

2.4.1 Marker-assisted breeding for resistance to maize lethal necrosis

Maize is the main host and most affected by MLN. The impact of the disease is partly dependent on the genetic background of the crop (Beyene et al. 2017). Marker-assisted breeding for virus resistance entails the identification and cloning of genes conferring resistance to both SCMV and MCVM (Redinbaugh et al. 2018). Several serological, metabolic and physiological responses conditioning disease resistance or susceptibility have been suggested (Nicaise 2014; Xia et al. 2018; 2019). The basic indicator of the level of disease resistance is the manifestation of symptoms and disease progression. Artificial screening of 12 maize inbred lines for individual virus resistance indicated differential responses to both MCMV and SCMV, with five lines showing fewer symptoms. Furthermore, detection of viruses in the leaf tissues of all lines suggested that genotypes with few symptoms were only tolerant rather than resistant (Jones et al. 2018). An indicator of virus resistance on molecular level is the measurement of virus titre in the plant tissue as indicated by the proportion of virus RNA sequence reads relative to plant sequence reads (Asiimwe et al. 2019). The MCMV virus titre is generally higher during co-infection of plant tissues with SCMV and other potyviruses (Mbega et al. 2016; Awata et al. 2019a; Leitich et al. 2021). A weak relationship of viral load and expression of symptoms was also observed for cassava brown streak disease, suggesting different mechanisms of disease resistance among genotypes (Kaweesi et al. 2014).

There are several host plant resistance mechanisms and defence pathways against viruses. These resistance mechanisms are deployed depending on the time of infection, location of the plant tissue where the infection first occurs or recognising and targeting viral genomes and/or proteins. In the gene-for-gene model of plant-virus interaction, dominant resistance genes (*R*

genes) are important for pathogen recognition by recognising the viral avirulence gene products and the activation of hypersensitive response leading to programmed cell death around the point of infection, hence preventing further spread of infection. Such *R* genes have been characterised in resistance to *Potato potexvirus X* and *Tobacco mosaic tobamovirus*. Recessively inherited *R* genes are more common in virus resistance systems in plants and thus, important genes of focus in plant breeding. Recessive resistance results from absence of factors in the host that are needed by the virus to complete its virus life cycle (Nicaise 2014). The third form of resistance is RNA interference mediated-resistance (RNAi) or gene silencing. A study by Xia et al. (2019) demonstrated that following coinfection of MCMV and SCMV there was an expression of microRNAs (miRNAs) for both viruses, possibly due to the RNA silencing suppressor protease, HC-Pro. The down-regulation of miR159, miR393 and miR394 was thought to enhance antiviral defence to synergistic infection. Further understanding of gene silencing pathways will be useful in developing targeted disease resistance for MLN causative viruses.

2.4.2 Genetic architecture of resistance to maize lethal necrosis

Native host-plant resistance to viruses exists and the nature of resistance varies with genetic background (Beyene et al. 2017; Jones et al. 2018). Adequate genetic variation for resistance to MCMV and SCMV, and MLN was earlier observed in Hawaiian maize (Nelson et al. 2011). Significant genetic variation, with most inbred lines being moderately tolerant, was seen after screening large CIMMYT genetic pools commonly used in SSA (Beyene et al. 2017; Redinbaugh and Stewart 2018; Das et al. 2019). The polygenic nature of MLN resistance with largely additive and some non-additive gene action was observed in a set of tropical maize lines (Beyene et al. 2017; Nyaga et al. 2020). Another study using a biparental population of tropical maize suggested that additive, dominance and epistatic gene effects contributed to MLN resistance. Epistatic gene effects were observed when there was an interaction between loci as evidenced by QTL with a small percentage of phenotypic variance explained (PVE%) but with a larger additive or dominance effect (Awata et al. 2019b). Although MLN resistance in tropical and temperate maize germplasm is largely quantitative, divergent germplasm backgrounds displayed divergent modes of gene action, such as dominance in inbred line N211, additive in inbred line DR and recessive among inbred lines derived from the KS23 genetic background (Jones et al. 2018; Redinbaugh and Stewart 2018).

The quantitative nature of MLN resistance has further been studied extensively using QTL analysis on diverse global germplasm (details in section 2.5.2). A synthesis of studies on viral and fungal disease resistance in maize showed a tendency of clustering of disease resistance QTL, with each chromosome co-localising resistance QTL for at least two different diseases. Virus resistance QTL were more tightly clustered compared to fungal disease resistance QTL (Wisser et al. 2006a). The major clusters of maize virus resistance genes are found on chromosomes 3, 6 and 10. Resistance to potyviruses is mainly located on chromosome 6, bin 6.01 and linked to simple sequence repeat (SSR) marker umc85 while QTL clusters for diverse viruses are on chromosomes 3 and 10 (Zambrano et al. 2014; Redinbaugh et al. 2018). On the contrary, studies using tropical maize populations showed more diffuse moderate effect QTL for MLN resistance (Gowda et al. 2015; 2018; Awata et al. 2019b; Sitonik et al. 2019). The discovery of a major-effect, recessively inherited disease resistance QTL on chromosome 6 presents the uniqueness of the KS23 genetic background and its usefulness in introgressing MLN resistance into elite lines in SSA (Jones et al. 2018; Redinbaugh and Stewart 2018; Awata et al. 2021). Another peculiar form of inheritance was observed for SCMV in European maize populations that displayed a combination of one dominant (*Scmv1*) and one recessive (*scmv2*) gene controlling disease resistance in inbred line D32, located on chromosomes 6 and 3, respectively (Melchinger et al. 1998).

2.5 Advances in developing resistance to maize lethal necrosis

2.5.1 Identification of potential sources of virus resistance and pre-breeding

In maize and other crops, genetic diversity can be improved by the introduction of new alleles from diverse exotic genetic pools. Frequencies of the new alleles can then be increased through systematic pre-breeding to increase the adaptation of germplasm to the target environment before its utilisation (Hallauer and Carena 2014). Pre-breeding is also applied in developing new breeding populations and identifying heterotic patterns for hybrid maize breeding as suggested by Nass and Paterniani (2000). Success has been reported in germplasm improvement through interpopulation enhancement of temperate and tropical pools (Lu et al. 2009; Mundim et al. 2013; Musundire et al. 2019).

Parental selection is an important step in breeding for any trait or set of traits. Selection of the right parents to be used in crossing is a task faced by plant breeders as it entails maximising genetic variability and the generation of superior recombinant genotypes (Bertan et al. 2014). In response to the emergence of MLN in East Africa in 2011, screening of 25 000 entries from

breeding programmes and gene banks was conducted. Less than 5% of this large pool was found to be tolerant to the disease (Gowda et al. 2015; Redinbaugh and Stewart 2018). Additional screening of 431 tropical elite and low nitrogen tolerant maize lines led to the identification of four inbred lines (CLRCY039, DTPYC9-F46-1-2-1-2-B, CLRCY034 and CLWN270) with tolerance to MLN (Das et al. 2019). Pre-breeding assessment also identified lines with high general combining ability (GCA) for MLN resistance. These lines included three elite lines (CML494, CML550 and CML574), two DH lines (CKDHL120918 and CKDHL0500) and two lines (CKLTI0137 and CKLTI038) developed by improvement of CML494 using off-patent temperate inbred lines from the USA (Beyene et al. 2017). Other resistant DH lines, CKDHL120312 and CKDHL120918 were validated for MLN resistance in artificial screening trials in Kenya prior to use in hybrids and breeding populations (Karanja et al. 2018). To increase the genetic diversity for breeding for MLN resistance in SSA, known multiple virus-resistant lines including OhiV1, OhVRS-1, N11, DR, KS23-6 and KS23-5 were introduced by CIMMYT and national breeding programmes to SSA. The MLN resistant donor lines, KS23-5 and KS23-6 were extracted from the KS23 synthetic developed at Kasetsart University, Thailand. The two lines were accessed and maintained in Wooster, Ohio, USA (Jampatong et al. 2010; Redinbaugh and Jones 2013; Jones et al. 2018). The introduced resistant lines formed a desirable genetic source base to initiate resistance breeding and specifically the improvement of elite SSA lines for MLN resistance through trait introgression (Redinbaugh and Stewart 2018; Prasanna et al. 2020; Awata et al. 2021). Other potential sources of resistance are available globally and could be accessed, including parents and derivatives from European inbred lines D21, D32 and FAP1360A, and USA inbred line Pa405 for SCMV resistance (Kuntze et al. 1997; Melchinger et al. 1998; Xia et al. 1999), Chinese maize line Hunazao4 (Luz and George 2014) and newly introgressed tropical maize lines (Awata et al. 2021).

2.5.2 Progress in resistance quantitative trait loci and marker discovery, validation and deployment

Identification of QTL and markers linked to disease resistance that are applicable across diverse genetic backgrounds and environments can be used in accelerating breeding progress using marker-assisted selection (Bernardo 2008; Semagn et al. 2015; Awata et al. 2019b). Earlier studies mainly mapped regions linked to resistance to individual viral diseases. Of the two viruses, SCMV has been studied more extensively. Using restriction fragment length polymorphism (RFLP) and SSR markers on European biparental and backcross populations, one common dominant locus (*Scmv1*) on chromosome 6 was identified in D21, D32, FAP1360A

and Pa405 backgrounds. Another recessive locus (*scmv2*) in an inbred line D32 background was identified on chromosome 6 (Melchinger et al. 1998; Xia et al. 1999; Dussle et al. 2000). A follow-up project by Tao et al. (2013) combined linkage and association mapping techniques to reduce the map size of the *Scmv1* locus from 18 mbp (mega base pairs) to just 215 kbp while Ding et al. (2012) fine-mapped the *scmv2* locus to 196.5 kbp. This study also predicted two genes, encoding an auxin-binding protein and a Ras homolog (Rho) GTPase-activating protein as candidate genes for the *scmv2* locus. These achievements are desirable steps for the identification of perfect markers for disease resistance improvement and the implementation of map-based gene cloning. A QTL mapping study using Chinese virus-resistant line Hunazao4, on the contrary, identified a wider distribution of SCMV resistance loci in the genome, with variation in their mode of action, depending on plant growth stage. At seedling stage, three QTL were detected on chromosomes 3, 5 and 10, chromosomes 3, 5, 6 and 10 at the stem elongation stage and 1, 3, 5, 6 and 10 at the grain-filling stage. QTL on chromosomes 3 and 10 showed additive gene action while the others were either dominant, partially dominant or over dominant (Luz and George 2014).

Mapping QTL for both MCMV and SCMV has been conducted using tropical and temperate maize populations. Jones et al. (2018) reported diverse disease resistance QTL using maize populations from diverse genetic backgrounds: The temperate line N11 contributed major QTL on chromosomes 3 and 5 while Oh1V11 contributed QTL on chromosomes 1, 2, 3 and 10. A major QTL on chromosome 6 was identified in the Asian KS23 background, common to the two resistant donor lines, KS23-6 and the KS23-5, and accounting for up to 78% of the phenotypic variance. Four major QTL mapping studies using tropical maize diversity panels and DH biparental populations of elite lines revealed a more diffuse distribution of major- and minor-effect resistance loci. Three major QTL for MLN resistance were mapped to chromosomes 3 and 6 (Gowda et al. 2015) and validated (Gowda et al. 2018). Distinct QTL linked to MLN resistance was identified on chromosome 3 (Sitonik et al. 2019), and seven large-effect QTL on chromosomes 3, 6, 8 and 9 (Awata et al. 2019b). These mapping studies in East Africa suggest that MLN resistance QTL in tropical maize germplasm is more widely distributed in the genome than earlier seen in temperate and sub-tropical maize backgrounds.

The diverse distribution of virus resistance QTL among global germplasm further suggests variation of disease resistance genes in different genetic backgrounds. In this case, genome-wide selection may be more effective when breeding using disease-resistant donor lines from

diverse genetic backgrounds, because a more accurate prediction is achieved for the marker-effects involved in selection (Guo et al. 2012). Genome-wide selection has also been found to be cost-effective in maize and wheat, because relatively small training populations can be used to predict large selection sets (Bernardo and Yu 2007; Santantonio et al. 2020).

2.5.3 Achievements in variety development and release

Deployment of resistant varieties is a sustainable and integrated disease management strategy (Jones 2021). However, the release of varieties that combine other desirable adaptive and agronomic traits with farmer-preferred qualities is vital for their adoption. Initially, screening of germplasm in SSA presented a challenge of limited genetic variation for native MLN resistance, with less than 5% of tropical maize germplasm showing tolerance to the new disease and almost all hybrids being susceptible (Prasanna et al. 2015; 2020; Semagn et al. 2015; Marenya et al. 2018). Progress in developing new MLN resistant lines and varieties was made by combining the few adapted tolerant genotypes (such as CML494) with introduced sources of resistance, such as the KS23-derived lines (KS23-5 and K523-6). Effective and rapid screening and identification of new resistant sources was achieved by establishment of the regional screening site at Naivasha, Kenya, with support from the Bill and Melinda Gates Foundation and the Syngenta Foundation for Sustainable Agriculture, and open to both public and private partners globally. The efficiency of the artificial screening site is shown by the capacity to screen a large number of hybrids, populations and inbred lines annually, with high heritability estimates. Over 200 000 genotypes were screened between 2013 and 2020 with broad-sense heritability ranging from 71 - 95%. In addition to artificial screening, rapid generation of new resistant lines has been made using the DH facility operated by CIMMYT. Inter-crossing resistant lines and wide testing of hybrids under artificial inoculation, disease hotspots and optimum conditions led to the release of up to 18 resistant hybrids in East Africa by 2019 (Beyene et al. 2017; Prasanna et al. 2020).

2.6 Useful methods and approaches applicable for enhancing genetic gains in maize lethal necrosis resistance

2.6.1 Considerations for hotspot and optimised artificial screening techniques

Although hotspot screening is less expensive, artificial screening and selection under controlled disease pressure is more reliable. This reliability was observed at the regional MLN screening facility as indicated by high plot heritabilities of up to 0.95. The initial plant samples for extraction of MCMV and SCMV isolates were collected from disease hot spots and maintained separately

on popular susceptible hybrids such as H614 (Prasanna et al. 2020). Appropriate disease detection methods are needed to conduct disease surveys, identify hotspots or identify correct isolates for screening genotypes. Serological techniques (especially ELISA), PCR and next-generation sequencing have been used to detect, identify and quantify plant viruses globally (Awata et al. 2019a). The MLN viruses in East Africa were mainly identified and characterised using ELISA, RT-PCR and deep sequencing of the virus coat protein, combined with sequence homology analysis with already existing sequences in databases (Wangai et al. 2012; Lukanda et al. 2014; Kusia 2015; Mahuku et al. 2015a; b) Reliability of the detection method is important in avoiding false positives and negatives. Mahuku et al. (2015a) and Adams et al. (2014) noted a false negative rate of up to 25% for SCMV using the ELISA technique. This could be due to differences in the source of antisera and differences in coat protein (CP) sequences among SCMV isolates. Despite this error rate, rapid diagnostic kits have been developed to aid large sample analysis during disease surveys. Other more reliable and sensitive methods such as the recombinase polymerase amplification (RPA) assay have been developed for use in virus detection. The new assay, RT-RPA, was found to be ten times more sensitive than the popular RT-PCR method. This technique uses primers designed based on conserved regions of the virus coat protein (Jiao et al. 2019).

An optimised protocol for artificial MLN screening was developed at CIMMYT. This procedure entails systematic inoculum preparation by maintaining the pure isolates separately in screenhouses. Prior to inoculation, the two viruses are mixed at a ratio of 4:1 SCMV:MCMV (Gowda et al. 2015; Sitonik et al. 2019). The main reason for the lower concentration of MCMV is its significant increase of virus titre following co-infection with other viruses (Adams et al. 2013; 2014). A standardised 1 - 9 (1 = totally resistant, 9 = totally susceptible) quantitative scale for scoring disease severity was adopted for inbred lines and hybrids. Time series disease scores can then be used to compute the area under disease progress curve (AUDPC). Forbes et al. (2014) noted that there are errors associated with AUDPC due to differential disease development among genotypes. Using a case of potato blight, two genotypes may have a similar maximum disease score or AUDPC values but one shows early disease initiation with a slow-progressing infection, while the other shows a late disease initiation but with fast-progressing infection. This phenomenon, therefore, implies that AUDPC values do not provide information on the type of resistance among genotypes, nor the potential durability of resistance. The error is resolved by computing the relative area under disease progress curve (rAUDPC) and the susceptibility index.

2.6.2 Parental selection strategies in maize lethal necrosis resistance breeding

2.6.2.1 Diallel analysis and a pre-breeding assessment

Pre-breeding is vital for effective identification and exploitation of genetic resources by selection and introgression of desirable genes from wide genetic backgrounds, while minimising linkage drag (Nass and Paterniani 2000; Sharma et al. 2013). Breeding for MLN resistance was initiated with identification for resistant donors through screening of large germplasm pools adapted to tropical, sub-tropical and temperate regions (Beyene et al. 2017; Das et al. 2019; Prasanna et al. 2020). For effective utilisation of the identified sources of resistance, understanding the genetic nature of resistance as well as determining the breeding value of resistant parents is vital. The breeding values of a parent, measured by the mean value of its progeny, should be attributable to genes involved rather than the genotype (Hallauer et al. 1988). The choice of breeding method using new germplasm depends on trait heritability, gene action, number of genes involved, heterosis and GEI effects (Nass and Paterniani 2000). These factors can be estimated using available biometric methods, including the diallel method, where all possible combinations of crosses are generated using the set of target parents as defined by Hayman (1954). Currently, estimation of parental breeding values is attainable through genomic prediction, especially where the trait is polygenic (Gorjanc et al. 2016).

Diallel mating designs have been adopted for different types of parents, ranging from inbred lines and broad-based populations (Hallauer et al. 2010). To fit the different types of parents, popularly used analysis methods were proposed by Hayman (1954), Griffing (1956), and Gardner and Eberhart (1966). Griffing's approach to diallel analysis has been widely used in maize hybrid breeding. This approach employs four methods that largely depend on the type of material analysed. Method 1 analyses the entire set of parents, F_1's and reciprocals; method 2 involves parents and F_1's only; method 3 analyses F_1's and reciprocals and method 4 analyses F_1's only. Application of Griffing's methods requires proper use of the random model and the fixed model that are often abused (Hallauer et al. 1988; Bernardo 2010). The fixed model analyses parents that are not derived from a particular random mating population, a usual case in hybrid breeding where parents have undergone selection. In contrast, the random model analyses variances due to GCA and specific combining ability (SCA) for parents selected from a single random mating population. In this case, variance due to SCA is associated with dominance variance, while GCA is associated with additive variance, only if variance due to epistasis is negligible (Bernardo 2010).

2.6.2.2 Genetic variance estimation and advancement of pedigree populations

During selection, it is important to apply both genetic variance estimates and mean performance in developing inbred lines and hybrids (Hallauer et al. 1988. Bernardo (2010) highlighted six key uses of genetic variance estimations. They include decisions in designing breeding programmes, allocation of resources for breeding, predicting response to selection, making decisions for marker-assisted breeding, predicting hybrid performance and developing selection indices for multiple traits selection. Genetic variances were first partitioned by Fisher (1918) into additive genetic variance, dominance genetic variance and epistatic variance. The total additive genetic variance is considered as the sum of the additive genetic variances contributed by individual loci, while the dominance genetic variance is the result of within-locus variance after subtracting the additive genetic variance from the total within-locus variance (Acquaah 2012). Epistatic variance refers to the non-additive genetic variance among loci, contrary to dominance genetic variance that is due to non-additive genetic variance within a locus (Hallauer et al. 1988). Estimation of genetic variance can also be conducted in breeding populations, whereby there is an expected decrease in genetic variance within families and an increase among families when continued inbreeding is done, as is the case in maize inbred development (Table 2.1). Mating designs are also used in estimating genetic variance (Bernardo 2010).

Table 2.1 Partitioning of variances under continuous self-pollination

Family	$F^{(a)}$	Between families		Within families		TOTAL	
		σ^2_A	σ^2_D	σ^2_A	σ^2_D	σ^2_A	σ^2_D
HS	0	1/4	0	3/4	1	1	1
HS-$S_1^{(d)}$	0	3/8	0	5/8	1	1	1
HS-$S_2^{(d)}$	0	7/16	0	9/16	1	1	1
FS	0	1/2	1/4	1/2	3/4	1	1
$S_1/F_3^{(e)}$	1/2	1	1/4	1/2	1/2	3/2	3/4
$S_2/F_4^{(e)}$	3/4	3/2	3/16	1/4	1/4	7/4	7/16
$S_3/F_5^{(e)}$	7/8	7/4	7/64	1/8	1/8	15/8	15/64
$S_4/F_6^{(e)}$	15/16	15/8	15/256	1/16	1/16	31/16	31/256
$S_5/F_7^{(e)}$	31/32	31/16	31/1024	1/32	1/32	63/32	63/1024
$S_6/F_8^{(e)}$	63/64	63/32	63/4096	1/64	1/64	127/64	127/4096
$S\infty/F\infty^{(e)}$	1	2	0	0	0	0	2

Modified from Hallauer et al. (1988)

2.6.2.3 Use of testers in parental selection and hybrid development

Testcross selection is a form of progeny test used to determine the combining ability of inbred lines in hybrid breeding or to estimate breeding values of individuals for population

improvement. Combining ability (Sprague and Tatum 1942) of inbred lines is estimated using a broad base heterogenous tester (synthetic) or a narrow genetic base (inbred line or single cross) tester. The ideal tester is therefore one that is genetically diverse from the test population or set of inbred lines, and is able to effectively discriminate among genotypes under selection (Hallauer et al. 1988). In hybrid maize breeding, high yielding varieties are obtained by crossing inbred lines from divergent heterotic groups (Barata and Carena 2006). Maize heterotic groups differ globally. The USA and Canada have the most developed heterotic patterns following inbred line extraction from as early as 1936 and heterotic classifications from the 1960s into the Iowa B stiff stalk synthetic (BSSS) on the seed parent side, and the non-stiff stalk synthetic (NSSS) type on the male side. On the other hand, the heterotic classification in tropical maize pools is curtailed by the complex population structure (Reif et al. 2005). Breeding programmes at CIMMYT and the International Institute of Tropical Agriculture (IITA) have since made progress in heterotic classification and identifying new and effective testers using the heterotic group's specific and general combining ability (HSGCA) method and SNP marker-based methods (Annor et al. 2020; Chisaka et al. 2020). Overall, tropical maize breeding in SSA follows CIMMYT's heterotic groups A and B. However, overlapping heterotic patterns still exist as observed by Semagn et al. (2012), where SNP markers used on a diverse set of 450 inbred lines did not show a clear distinction of heterotic patterns into A and B categories previously assigned on the basis of combining ability with known testers. The constituent inbred lines for popular testers in SSA were all found to be susceptible to MLN (Das et al. 2019). Hence, this study explored the use of some derivative lines identified by Beyene et al. (2017) as candidate testers for selection of new inbred and DH lines for both yield and MLN resistance.

2.6.3 Genotype by environment interaction and stability analysis in maize hybrid selection

Cultivars tend to perform differently across environments, especially when quantitative traits are being evaluated. This phenomenon is often referred to as GEI (Bernardo 2010). Methods have been proposed to analyse GEI based on analysis of variance, linear regression (Scapim et al. 2000; Pixley and Bjarnason 2002) or non-linear analysis, multivariate analysis, biplot (Dolatabad 2010) and/or non-parametric statistics. Newer statistical approaches proposed for the interpretation of GEI are the additive main effects and multiplicative interaction (AMMI) analysis and a modification of the conventional AMMI analysis called genotype and genotype by environment interaction (GGE) biplots (Yan et al. 2007; Badu-Apraku et al. 2011). The GGE analysis groups the genotype effect, which is an additive effect in the AMMI analysis, together

with the genotype by environment (GE) interaction and multiplicative effect, and analyses these effects by principal components (Balestre et al. 2009). The GGE biplot was reported to be superior to AMMI, because it has many visual interpretations that AMMI does not have, especially the visualisation of the “which won where” pattern and any crossover GEI (Yan and Tinker 2006). Yan et al. (2007) in their comparison of the GGE biplot analysis and AMMI analysis concluded that: (i) analysis using both GGE biplot and AMMI combined rather than separate G and GE in mega-environment analysis and genotype evaluation is a better approach; (ii) the GGE biplot is superior to the AMMI graph in mega-environment analysis and genotype evaluation, because it explains more G and GE and has the inner-product property of the biplot; (iii) the discriminating power vs. representativeness view of the GGE biplot is effective in evaluating test environments, which is not possible in AMMI analysis and (iv) model diagnosis for each dataset is useful, but accuracy gained from model diagnosis should not be overstated. Stability analysis is one of the ways of exploiting GEI. In many cases breeders ignore GEI by using across-site means while others reduce the effect of GEI by partitioning environments into clusters. Another option is to exploit GEI conducting stability analysis (Bernardo 2010). According to Lin et al. (1986), three considerations determine the choice of method used in stability analysis: (i) a cultivar is considered stable if its among-environment variance is small; (ii) stability assumes the response to the environments to be parallel to the mean response of all genotypes in the trial and (iii) a genotype is stable if there is a small residual mean square from regression on the environmental index. For this reason, a breeder needs to choose an appropriate method before recommending varieties to farmers. One disadvantage of popular parametric and non-parametric methods is that selection of desirable genotypes is mainly based on a single trait. A new method, multi-trait stability index (MTSI), proposed by Olivoto et al. (2019) is applicable for identifying superior genotypes with high mean performance across traits by taking into consideration the correlation structure of the traits. This method could be useful in selection of new MLN resistant hybrids across both MLN infested and disease-free environments.

2.7 Perspectives for durable multiple disease resistance in sub-Saharan Africa

Maize streak viruses and common fungal diseases, especially Turcicum leaf blight, gray leaf spot (GLS), common rust and rots are the key biotic stress conditions in SSA (Vivek et al. 2010; Asea et al. 2012; Tembo et al. 2016; Nkurunziza et al. 2019). Progress in breeding for multiple resistance to these prevalent diseases has been made using both conventional and modern techniques. Molecular markers targeting *msv1*, the most important locus for maize streak virus

(MSV) resistance, have been successfully deployed to screen populations for resistance to MSV in CIMMYT's marker-assisted breeding approaches (Semagn et al. 2015). However, the emergence of MLN presented new challenges to breeders in the region. The quantitative nature of maize resistance have been extensively studied and used to develop methods for developing multiple disease resistance, but resistance to new diseases such as MLN is controlled by rare alleles that may suffer possible negative genetic linkage drag or pleiotropic effects during direct resistance introgression (Yang et al. 2017). Pyramiding MLN resistance into already existing multiple resistant sources is a sustainable and practical approach. This approach limits resistance breakdown resulting from virus mutations (Nicaise 2014). The clustering tendency of MLN resistance genes presents an opportunity for deploying markers linked to multiple viruses and to apply simpler pedigree breeding and marker-assisted selection (Wisser et al. 2006b).

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CHAPTER 3

Diallel analysis for dual resistance to maize lethal necrosis and Turcicum leaf blight among tropical and temperate maize germplasm

Abstract

Breeding for combined disease resistance is an economically sustainable option for disease management. However, breeders face the challenge of a narrow genetic base for resistance to new diseases such as MLN. Introduced germplasm is characterised by poor adaptability, linkage drag and incompatibility of alleles in introduced disease resistant donor lines. In this study, genetic analysis was done for combined resistance to MLN and Turcicum leaf blight (TLB) among tropically adapted African and Asian lines, and temperate virus resistant lines. A 19-parent diallel analysis was conducted with the following key objectives: (i) to determine the level of useful genetic variation and heritability for MLN and TLB resistance among tropical and temperate inbred and DH lines; (ii) to estimate the combining ability and identify donor lines with dual MLN and TLB resistance, and (iii) to identify potential MLN resistant single cross hybrid combinations for developing new disease resistant segregating populations. Analysis of variance (ANOVA) showed significant variation for both MLN resistance ($P \leq 0.001$) and TLB ($P \leq 0.05$). General combining ability was found to be predominant over SCA. Highly significant GCA variance among male and female parents was observed for MLN resistance ($P \leq 0.001$) and only significant among female parents ($P \leq 0.05$) for TLB resistance. Non-significant reciprocal effects, and the interaction of GCA and SCA with the environment, were observed for both diseases. Significant correlation of GCA with mean performance of parents for both TLB and MLN resistance was seen. However, the correlation of GCA effects with line means between the two diseases was negative and non-significant, suggesting the possibility of combining resistance with the need to independently screen for the two diseases. Nine tropical lines, CML494, CML574, CLRCY034, CML543, DTPY-F46, CLYN261, CML550, CKDHL120312 and CKDHL120918, had significant negative GCA effects ($P \leq 0.05$) desirable for MLN resistance, the best combiners being CKDHL120312, CKDHL120918 and DTPY-F46. Among introduced MLN resistant donors, temperate inbred line Oh101-1-B-2 was among the top four best combiners while Asian lines KS523-5 and KS523-6 both had significant positive combining ability effects for MLN resistance despite their high *per se* resistance. Inbred line KS523-5, however, combined well for TLB resistance, unlike KS523-6. MLN resistant lines, CKDHL120918, CKDHL120312, CML543 and CML574 had desirable combining ability for both

TLB and MLN resistance while CML494, CML550 and KS523-6 combined for susceptibility to TLB. The three elite tropical lines (NML97, CML312 and CML444), on the contrary, had high GCA effects for TLB resistance but were susceptible to MLN. A total of 21 single crosses with significant SCA effects for MLN resistance were identified. These crosses can be used for the production of new breeding populations for combined disease resistance. In the short term, adapted lines with dual resistance to MLN and TLB can be used for the production of new disease resistant hybrids while non-adapted lines are best suited for introgression of resistance into susceptible elite lines.

Key words: Combining ability, exotic and elite germplasm, heritability

3.1 Introduction

The occurrence of MLN in East Africa since 2011 (Wangai et al. 2012) continues to pose a threat to crop productivity and food security. If it continues to spread to other countries in SSA, the disease threatens maize production on more than 26 million hectares cultivated by mainly small-holder farmers in the region (Mahuku et al. 2015). Initially, a combination of maize MCMV and SCMV were identified as the key causes of the MLN epidemic in eastern Africa. However, other viruses such as JGMV, MDMV, maize yellow dwarf virus (MYDV), sorghum mosaic virus (SrMV) and barley yellow dwarf virus (BYDV) have, through field surveys conducted in the region, been associated with MLN (Stewart et al. 2017; Kiruwa et al. 2020). Documented losses due to MLN are known to range from 30 - 100%, depending on the stage of infection during the plant growth cycle (Mahuku et al. 2015). To initiate breeding for MLN resistance, pre-breeding was conducted, which included screening a large number of adapted tropical maize inbred lines, hybrids and open-pollinated varieties to identify MLN resistant donor lines. This effort yielded limited success since only 5% of over 25 000 entries were found to be tolerant (Gowda et al. 2015; Redinbaugh and Stewart 2018). Additional screening of 431 tropical elite and low nitrogen tolerant maize lines led to the identification of four inbred lines with tolerance to MLN (Das et al. 2019). Known virus resistant lines from the USA and Asia (Redinbaugh et al. 2004; Jones et al. 2018) were introduced to enrich the genetic base for MLN resistance and to introduce unique alleles to African germplasm. Through artificial screening, the introduced virus resistant lines from the Asian KS23 population were identified as key sources of resistance to MLN in eastern Africa (Redinbaugh and Stewart 2018). These lines have been used in mapping disease resistance QTL (Jones et al. 2018) and for trait introgression to improve elite lines for MLN resistance (Prasanna et al. 2020).

Maize is a widely adapted crop species in terms of altitude and latitude, temperature and light intensity (Bouchet et al. 2013; Andorf et al. 2019). This wide adaptation offers an opportunity for improvement of existing maize germplasm using lines from exotic gene pools, making it possible to attain significant genetic gains from trait introgression (Hallauer and Carena 2014). This genetic gain from introgression is attained from utilising existing wide genetic variability and the introduction of novel genes from exotic germplasm (Wen and Guo 2012). The major setback to direct utilisation of exotic germplasm is the limited adaptability, susceptibility to endemic diseases and genetic drag due to non-complementarity of alleles for genes controlling target traits (Menkir et al. 2015). Economically important fungal diseases such as GLS and TLB are prevalent in mid-altitude tropical environments with warmer temperatures such as eastern Africa (Vivek et al. 2010). An appropriate strategy is therefore to incorporate MLN resistance alleles from exotic sources into adapted elite backgrounds that have undergone selection for resistance to common diseases, and desirable agronomic and farmer-preferred traits. Another setback in the utilisation of exotic germplasm is the divergence in heterotic patterns and the imposed negative genetic linkage drag or pleiotropic effects if used directly in breeding (Yang et al. 2017). This constraint often makes introduced germplasm less useful for direct formation of new varieties, hence the need for pre-breeding assessment to guide trait introgression efforts (Hallauer and Carena 2009). Temperate germplasm has been successfully introgressed into tropical maize lines without significant distortion of heterotic patterns (Musundire et al. 2019). On the other hand, tropical pools have been utilised to improve temperate maize for yield and disease resistance through the germplasm enhancement of maize (GEM) project (Balint-Kurti et al. 2006). Effective utilisation of tropical x temperate GEM lines was achieved by aligning BSSS and NSS derived lines with tropical heterotic groups A and B respectively. This alignment has been made possible by using information generated from phenotypic, molecular diversity and association studies, and hybrid prediction (Wen and Guo 2012). This strategy can be employed to avoid overlapping heterotic patterns when using introduced MLN resistant donors crossed with tropical elite lines.

Determining the potential of introduced inbred lines to improve adapted elite tropical maize lines is vital for developing new MLN resistant populations, inbred lines and hybrids, while retaining existing resistance and agronomic traits. The present challenge is to combine MLN resistance with resistance to TLB and other common diseases considered as must-have-traits in most breeding programmes in SSA. The major setback with pyramiding disease resistance is the ability to recover F_2 plants or segregating families carrying all resistance loci in a homozygous

state. Increasing the chance of selecting multiple disease resistant lines from a population therefore requires screening of large numbers of F_2 individuals (Pratt et al. 2004). Due to interaction among loci controlling disease resistance, the correlation of a line's performance with its combining ability influences successful pyramiding of diseases into one genetic background (Vivek et al. 2010).

The aim of this study was to use the diallel design to determine the breeding potential of a collection of tropical and temperate inbred and DH lines for combined resistance to MLN and TLB. The study was conducted using adapted tolerant and susceptible tropical inbred lines, Asian-sourced lines and multiple virus resistant lines from the USA to: (i) determine the level of useful genetic variation and heritability for MLN and TLB resistance among tropical and temperate inbred and DH lines, (ii) estimate the combining ability for resistance to MLN and TLB among tropical and temperate lines, and (iii) identify potential disease resistant parental lines and single cross hybrid combinations for developing new populations.

3.2 Materials and methods

3.2.1 Genetic material and mating design

A diverse collection of 19 inbred lines used in this study included six MLN tolerant tropical lowland lines from CIMMYT Mexico, three popular CIMMYT tester lines (one in heterotic group A and two in heterotic group B), two new MLN tolerant lines identified at CIMMYT Kenya, three elite lines from NARO Uganda, three Asian lines (two MLN resistant donors and one susceptible line), and two multiple virus resistant lines from Ohio, USA (Table 3.1) (Zambrano et al. 2014; Beyene et al. 2017; Jones et al. 2018; Das et al. 2019). The purpose of this diverse collection was to capture adequate genetic variation from different gene pools for combining resistance to MLN, a new disease, with TLB (a prevalent foliar disease in East Africa and other tropical regions) and key agronomic traits. The inbred lines were crossed in a diallel design using paired rows, at NaCRRI, Uganda in the first season (March - August) of 2017 (2017A). Both forward and reciprocal crosses were generated and evaluated for combining ability and to estimate reciprocal effects associated with disease resistance.

Table 3.1 Inbred and doubled haploid lines used in diallel analysis

Entry	Line name	Source	MLN resistance	TLB resistance
1	CML494	CIMMYT, Tropical lowland	Tolerant	Tolerant
2	CML574	CIMMYT, Tropical lowland	Tolerant	Tolerant
3	CLRCY034	CIMMYT, Tropical lowland	Tolerant	Tolerant
4	CML543	CIMMYT, Kenya tester B	Tolerant	Resistant
5	DTPY-F46	CIMMYT, Tropical lowland	Tolerant	Susceptible
6	CLYN261	CIMMYT, Tropical lowland	Tolerant	Tolerant
7	CML550	CIMMYT, Tropical lowland	Tolerant	Resistant
8	KS523-5	Kasetsart University, Thailand	Resistant	Tolerant
9	KS523-6	Kasetsart University, Thailand	Resistant	Susceptible
10	CKDHL120312	CIMMYT, Kenya	Tolerant	Resistant
11	CKDHL120918	CIMMYT, Kenya	Tolerant	Resistant
12	Oh101-1-B-2	Ohio, USA	Tolerant	Tolerant
13	Oh1VIxOh28RI-70245	Ohio, USA	Tolerant	Susceptible
14	CML312	CIMMYT, tester A	Susceptible	Resistant
15	CML444	CIMMYT, tester B	Susceptible	Resistant
16	NML85	NARO, Uganda	Susceptible	Susceptible
17	NML88	NARO, Uganda	Susceptible	Resistant
18	NML97	NARO, Uganda	Susceptible	Resistant
19	KS523-4	Kasetsart University, Thailand	Susceptible	Tolerant

MLN, Maize lethal necrosis; TLB, Turcicum leaf blight; NARO, National Agricultural Research Organization; CIMMYT, International wheat and maize improvement centre

3.2.2 Field trial design

Using CIMMYT's Fieldbook software (Bänziger and Vivek 2007), a total of 344 entries, consisting of all crosses (F_1's and reciprocal crosses), 19 parents (17 inbred and two DH lines), and two checks were used to constitute an augmented complete block design (ACBD) of six blocks, 59 entries/block and the two checks randomised within each block. All trials were planted in single rows of 5 m in length, 0.25 m between plants and 0.75 m between rows.

3.2.3 Artificial screening for resistance to maize lethal necrosis

Artificial MLN screening site

Artificial inoculation of plots with the two MLN viruses (MCMV and SCMV) was conducted at the regional MLN screening facility in Naivasha, Kenya, during the second season (September 2017 - January 2018) (2017B) and the first season (March – August) of 2018 (2018A). The Naivasha

screening facility is located along the Nakuru-Nairobi highway near Naivasha Township ($36^0$23'43''E and $0^0$41'22''S). At an altitude of 1,884 metres above sea level (masl), the station receives an average of 120 and 131 mm of rainfall over two seasons, with the first season in mid-March and the second starting in mid-September. The small amounts of rainfall received combined with high temperatures ranging from 23 - 28^{o}C qualifies the site as a dry high-altitude zone.

An optimised virus inoculation protocol described by Gowda et al. (2015) was used. Pure isolates for MCMV and SCMV, respectively, were propagated and maintained in separate screen houses using a susceptible genotype. The purity of isolates was ascertained using ELISA. To prepare inoculum, random leaf samples from the SCMV and MCMV production screen houses were collected and ground separately before combining at an optimised SCMV:MCMV ratio of 4:1. Inoculation was done twice, at the 5th and 6th week after planting, using a motorised backpack mist blower at a pressure of 10 kg cm^{-2}. The second inoculation was done to ensure minimal escapes in the test plots.

Assessment of MLN severity and disease progress curve

Visual scoring for MLN symptoms was done following CIMMYT's standardised scoring system using a quantitative scale of 1 - 9 (http://mln.cimmyt.org/mln-scoring/). Scoring for disease symptoms started two weeks after the second inoculation and was repeated every fortnight. Using this scale, different manifestations of disease symptoms were recorded as follows: 1 = no visible symptoms, 2 = fine chlorotic specks, but vigorous plants, 3 = mild chlorotic streaks on emerging leaves, 4 = moderate chlorotic streaks on emerging leaves, 5 = chlorotic streaks and mottling throughout the plant, 6 = intense chlorotic mottling throughout the plant, necrosis of leaf margin, 7 = excessive chlorotic mottling, mosaic and leaf necrosis and dead heart symptoms, 8 = excessive chlorotic mottling, leaf necrosis, dead heart and premature death of plants, and 9 = complete plant necrosis and dead plant (Figure 3.1).

The four consecutive MLN scores were used to calculate the average score, as well as the AUDPC. The susceptibility score was determined following the mid-point procedure described by Shaner and Finney (1977), adopted by Forbes et al. (2014). Relative AUDPC and susceptibility scores were calculated to compensate for problems associated with the sensitivity of AUDPC to variation in disease development. A common case could be an early-onset and slow-progressing infection or late-starting, fast-progress of symptoms.

		
1 = No visible symptoms	2 = Fine chlorotic specks, vigorous plants	3 = Mild chlorotic streaks on emerging leaves
		
4 = Moderate chlorotic streaks on emerging leaves	5 = Chlorotic streaks and mottling throughout plant	6 = Intense chlorotic mottling throughout plant, necrosis of leaf margin
		
7= Excessive chlorotic mottling	8 = Excessive chlorotic mottling, leaf necrosis, dead heart and premature death	9 = Complete plant necrosis, and dead plant

Figure 3.1 Quantitative scores for maize lethal necrosis symptoms in experimental plots.

Source: http://mln.cimmyt.org/mln-scoring

The rAUDPC, therefore, was calculated by dividing the AUDPC by the “maximum potential” AUDPC. The AUDPC was calculated using the midpoint formula:

$$\text{AUDPC} = \sum_{n=1}^{n-1} \left(\frac{yi + yi + 1}{2} \right) + (ti + 1 - ti)$$

Where t is the time of each reading, y is the score of symptoms at each reading and n is the number of readings. The variable t was considered as days after inoculation.

To help classify genotypes into various classes of resistance/tolerance, a susceptibility score for MLN symptoms was calculated from AUDPC or rAUDPC, by adjusting the severity scores of the test entries using a standard susceptible entry in the trial. The susceptibility value was calculated as follows:

$$Sx = \frac{Dy}{Dx} Sy$$

Where, Sy and Dy represent, respectively, the assigned susceptibility scale value and observed disease measure (AUDPC or rAUDPC) for the standard genotype, and Sx and Dx represent, respectively, the calculated susceptibility scale value and observed disease measurement for the genotype in question.

3.2.4 Hotspot screening for resistance to Turcicum leaf blight

Diallel crosses and parents were evaluated at three MLN-free, TLB hotspot sites: Namulonge, Bulindi and Bulegeni, in 2017A. In these hotspot sites, disease inoculum was boosted and uniform plot infection was ensured using the whole infected leaf powder (WILP) procedure (Dutta et al. 2010). Scoring for TLB was done twice during the period of crop growth. The first disease scores were taken after pollen shed when there were observable differences between plots for the severity of disease symptoms. The second score was done 14 days from the date of the first score and the two scores were averaged for every plot.

3.2.5 Data management and statistical analysis

Data on response to MLN was collected over two screening seasons and analysed using the ACBD in R-software, Version 3.0" developed by CIMMYT (Rodríguez et al. 2017). Analysis of variance for combining ability effects and variance components were estimated using R-software, Version 3 (© 2009-2018 RStudio, Inc.) following the model below. To satisfy Griffing (1956) method 1 (model I), effects due to parents were considered fixed, thus, interpretation of results was limited to the germplasm used in the study.

Linear model: $Y_{ijk} = \mu + g_i + g_j + S_{ij} + e_{ijk}$

Where Y_{ijk} is the observed value for the ij^{th} cross grown in the k^{th} environment, μ is the overall mean of crosses, g_i is GCA effect for the i^{th} parent, g_j = GCA effect for the j^{th} parent, S_{ij} is the SCA (interaction due to crossing the i^{th} and j^{th} parents) and e_{ijk} is the error term.

The relative importance of GCA and SCA effects was determined using Baker's ratio (BR) calculated as $2\delta^2_{GCA}/ (2\delta^2_{GCA} + \delta^2_{SCA})$; where $2\delta^2_{GCA}$ and δ^2_{SCA} are variances due to GCA and SCA effects, respectively (Baker 1978). Pearson's correlation of mean parent performance with corresponding GCA effects was conducted for both MLN and TLB and across MLN and TLB scores.

3.3 Results

3.3.1 Genetic variation and heritability among single cross hybrids and their parents

Analysis of variance showed that there was a highly significant variation ($P \leq 0.001$) for MLN severity scores at both early and late growth stages of disease progression. This result was further confirmed by the highly significant variation among crosses and parents for computed AUDPC and MLN susceptibility values. On the other hand, genetic variation for TLB severity was significant ($P \leq 0.05$). Unlike individual MLN scores, AUDPC and disease susceptibility, the environmental variance was highly significant when the four MLN severity scores were averaged (MLN_AV) ($P \leq 0.001$). Likewise, highly significant environmental variance was observed for TLB severity (Table 3.2).

Table 3.2 Analysis of variance for the diallel for resistance to maize lethal necrosis and Turcicum leaf blight

Source	Variances							
	MLN1	**MLN2**	**MLN3**	**MLN4**	**MLN_AV**	**MLN_AUDPC**	**MLN_SC**	**TLB**
GCA-female	28.70***	86.44***	97.72***	167.80***	8.24***	15741***	102.04***	2.11*
GCA-male	24.25***	79.65***	95.04***	153.21***	65.72***	146340***	97.85***	1.81
Reciprocal	0.32	0.00	0.44	0.38	0.34	314	0.24	1.81
Environment (E)	0.01	0.00	9.35*	0.92	286.88***	1225	0.78	78.20***
SCA	4.53***	0.13	0.92	0.00	0.62	1103	0.74	0.62
GCA-female x E	0.18	1.96	0.81	2.29	0.48	2090	1.27	0.10
GCA-male x E	0.76	1.06	2.27	3.54	0.20	3002	1.93	0.03
Residuals	0.32	1.08	1.66	2.68	0.85	1894	1.25	0.48
δ^2_G	0.04	0.19	0.32	0.59	0.14	390.49	0.26	0.03
δ^2_m x E	0.01	0.01	0.01	0.03	0.00	33.25	0.02	0.03
δ^2_f x E	0.01	0.01	0.01	0.00	0.00	15.31	0.01	0.02
δ^2_f	0.07	0.44	0.71	1.11	0.38	902.14	0.60	0.04
δ^2_m	0.08	0.40	0.58	1.00	0.34	782.65	0.51	0.08
δ^2_e	0.21	0.35	0.44	0.62	0.25	397.90	0.26	0.29
Heritability (h^2)	**0.42**	**0.71**	**0.75**	**0.77**	**0.74**	**0.81**	**0.81**	**0.29**
GCA:SCA ratio	**0.78**	**0.81**	**0.80**	**0.78**	**0.83**	**0.81**	**0.81**	**0.78**

Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN; MLN_SC, calculated MLN susceptibility score; TLB, Turcicum leaf blight; GCA, general combining ability; SCA, specific combining ability; *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; δ^2_G, δ^2_f, δ^2_m, δ^2_m x E, δ^2_f x E, δ^2_e, variance components due to genotype, female parents, male parents, the interaction of male parent with the environment, interaction of female parent with environment and error variance, respectively.

3.3.2 Combining ability, reciprocal effects and heritability estimates for maize lethal necrosis and Turcicum leaf blight

The total genetic variance was further partitioned into variance due to GCA, SCA, reciprocal effects and interaction of GCA with the environment (Table 3.2). Highly significant GCA variance for MLN was observed for both male and female parents (P ≤ 0.001), whereas GCA variance for TLB for female parents was significant (P ≤ 0.05). Variance due to SCA was only significant during the early development of MLN symptoms. Reciprocal effects were not significant for either disease. Variance due to the environment was significant (P ≤ 0.05) for MLN development after 42 days (MLN3) stage and highly significant (P ≤ 0.001) for average MLN severity scores while the interaction of GCA with the environment was non-significant for all disease parameters. Heritability based on variance components was high for all MLN measurements, except the initial score. Computation of AUDPC and susceptibility scores for MLN improved heritability from approximately 0.75 to 0.81. The GCA:SCA ratio (Baker's ratio) for MLN resistance was high. According to Baker (1978), the closer the ratio is to unity, the higher the

predictability of hybrid performance based on GCA. Results from this study, hence, imply that for MLN resistance, variance due to GCA is more important than variance due to SCA in determining the performance of progeny from inbred parents (Table 3.2).

Estimation of GCA effects for parents (Table 3.3) confirmed that the nine MLN tolerant tropical inbred and DH lines (CML494, CML574, CLRCY034, CML543, DTPY-F46, CLYN261, CML550, CKDHL120312 and CKDHL120918) showed significant negative GCA effects but the GCA effects varied between stages of MLN disease development and between genotypes ($0.001 \geq P \leq 0.05$). Out of the nine tropical maize lines, the best combiners for MLN resistance were second-generation MLN resistant lines (CKDHL120312 and CKDHL120918) and yellow inbred line DTPY-F46 with consistently high GCA effects ($P \leq 0.001$) at early and late stages of disease development and AUDPC. Next to these three high-GCA lines, temperate inbred lines Oh101-1-B-2 and tropical line CML550 had significant GCA effects for most MLN resistance parameters. On the contrary, both susceptible tropical tester CIMMYT lines (CML312 and CML444) and NARO-Uganda elite lines (NML85, NML88 and NML97) had highly significant positive GCA effects ($P \leq 0.001$) for MLN severity and AUDPC. Albeit the high *per se* MLN resistance, Asian lines KS523-5 and KS523-6 did not have good combining ability for MLN resistance, as shown by the significant positive GCA effects. Asian susceptible line, KS523-4 and temperate line Oh1VIxOh28RI both showed non-significant GCA effects for MLN scores and AUDPC.

Separate analysis of combining ability for TLB resistance showed varying magnitudes of resistance and GCA effects among the diverse collection of MLN resistant and MLN susceptible lines (Table 3.3). Tropical lines with significant negative GCA effects for MLN resistance, but with significant positive GCA effects for TLB were CML494 ($P \leq 0.05$), CML550 ($P \leq 0.001$) and DTPY-F46 ($P \leq 0.001$). Second-generation MLN resistant lines, CKDHL120312 and CKDHL120918, showed highly significant GCA effects for MLN resistance ($P \leq 0.001$) but non-significant effects for TLB resistance. The two Asian lines both combined for MLN susceptibility but varied in combining ability effects for TLB. Of these lines, KS523-6 had highly significant positive GCA effects while KS523-5 had a significant negative GCA effect for TLB resistance ($P \leq 0.05$). The two USA temperate lines also had contrasting combining ability for MLN and TLB. Inbred line Oh101-1-B-2 showed a highly significant negative effect for MLN resistance but with a non-significant GCA effect for TLB resistance, while Oh1VIxOh28RI-70245 had a non-significant effect on MLN resistance, but had highly significant positive GCA effects for TLB resistance.

Table 3.3 General combining ability effects for maize lethal necrosis and Turcicum leaf blight among diverse tropical and temperate inbred and doubled haploid lines

Parent	Source	Name	GCA effects							
			MLN1	MLN2	MLN3	MLN4	MLN_AV	MLN_AUDPC	MLN_SC	TLB
1	CIMMYT, Tropical lowland	CML494	-0.14*	-0.35*	-0.26	-0.57*	-0.21	-13.69*	-0.35*	0.14*
2	CIMMYT, Tropical lowland	CML574	-0.23**	-0.17	-0.13	-0.12	-0.08	-6.80	-0.17	-0.18**
3	CIMMYT, Tropical lowland	CLRCY034	-0.22**	-0.20	0.12	0.21	0.02	-1.33	-0.03	-0.09
4	CIMMYT, Kenya tester B	CML543	-0.18**	-0.14	-0.02	0.15	-0.04	-2.60	-0.06	-0.20**
5	CIMMYT, Tropical lowland	DTPY-F46	-0.30***	-0.96***	-1.13***	-1.62***	-0.86	-43.19***	-1.11***	0.24***
6	CIMMYT, Tropical lowland	CLYN261	-0.17*	-0.21	-0.39*	-0.50*	-0.18	-13.25*	-0.33*	-0.18**
7	CIMMYT, Tropical lowland	CML550	0.00	-0.31*	-0.45*	-1.06***	-0.39	-18.29**	-0.47**	0.36***
8	Thailand	KS523-5	0.00	0.41*	0.38*	0.94***	0.30	17.64*	0.45*	-0.16*
9	Thailand	KS523-6	0.18**	0.45**	0.66**	0.83**	0.45	22.91**	0.59**	0.61***
10	CIMMYT, Kenya	CKDHL120312	-0.27***	-0.92***	-1.48***	-1.62***	-1.08	-47.16***	-1.21***	0.05
11	CIMMYT, Kenya	CKDHL120918	-0.28***	-0.86***	-0.87***	-1.06***	-0.71	-34.00***	-0.87***	0.10
12	USA	Oh101-1-B-2	-0.10	-0.58***	-0.60**	-0.45*	-0.51	-20.65**	-0.53**	-0.08
13	USA	Oh1VIxOh28RI-70245	0.02	0.01	-0.28	-0.35	-0.14	-6.15	-0.16	0.39***
14	CIMMYT, tester A	CML312	0.08	0.45**	0.57**	0.44	0.40	17.98*	0.46*	-0.34***
15	CIMMYT, tester B	CML444	0.20**	0.50**	0.59**	0.40	0.46	19.65**	0.51**	-0.30***
16	NARO, Uganda	NML85	0.57***	0.99***	0.92***	0.80**	0.68	36.92***	0.95***	0.07
17	NARO, Uganda	NML88	0.47***	1.05***	0.99***	1.29***	0.81	41.40***	1.05***	-0.09
18	NARO, Uganda	NML97	0.43***	0.84***	1.26***	2.05***	1.03	47.25***	1.21***	-0.27***
19	Thailand	KS523-4	-0.03	0.00	0.14	0.25	0.04	3.34	0.08	-0.07
	GCA:SCA ratio		**0.78**	**0.81**	**0.80**	**0.78**	**0.83**	**0.81**	**0.81**	**0.78**

Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN; MLN_SC, calculated MLN susceptibility score; *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; TLB, Turcicum leaf blight severity score; GSC, general combining ability; SCA, specific combining ability

Tropical lines that showed desirable combining ability for MLN resistance, as well as TLB, were CML574, CLYN261 and CML543. Overall, MLN susceptible testers, CML312 and CML444, and elite line NML97 were the best combiners for TLB resistance ($P \leq 0.001$).

Combining ability analysis also led to the selection of 21 crosses (6%) with significant SCA effects for MLN resistance. These crosses were generated from mainly MLN resistant lines. Parents 11 (CKDHL120918), 1 (CML494) and 12 (Oh101-1-B-2) contributed to at least four MLN resistant combinations with significant negative SCA values. The rest of the MLN resistant lines and, uniquely, susceptible Asian line KS523-4, had one to three resistant cross combinations (Table 3.4).

3.3.3 Correlation of combining ability effects with mean performance for disease resistance

Pairwise correlation of mean performance of individual lines with corresponding GCA effects for both TLB and MLN severity and AUDPC were conducted to determine the relationship in inheritance patterns of the two diseases. There was a significant positive correlation ($0.001 \geq P \leq 0.05$) of MLN mean scores and AUDPC and significant correlation ($P \leq 0.01$) of mean severity with line GCA effects for TLB resistance. Contrary to this, analysis for both diseases indicated a consistent negative but non-significant correlation of line *per se* mean for TLB and corresponding GCA effects for MLN resistance and vice versa (Table 3.5).

3.4 Discussion

This study was conducted to determine the breeding potential of tropically adapted and introduced germplasm for developing MLN resistant breeding populations and possibly hybrids targeting eastern Africa that recently experienced an outbreak of MLN, a new disease endemic in the Americas. The introduction of exotic germplasm from the USA and tropical Asia (Thailand) aimed at complementing the limited genetic base for MLN resistance among adapted African tropical gene pools. This effort to widen the genetic base for MLN resistance was prompted by the limited genetic base observed in an initial extensive screening of germplasm from gene pools, advanced breeding lines and commercial parental lines, hybrids and open-pollinated varieties when the disease was first reported in East Africa (Beyene et al. 2017; Das et al. 2019).

Table 3.4 Specific combining ability effects for response to maize lethal necrosis for selected diallel hybrids

Cross†	MLN1	MLN2	MLN3	MLN4	MLN_AV	MLN_AUDPC	MLN_SC
10 X 19	-0.96**	-2.80***	-3.03***	-4.21***	-2.64***	-117.74***	-3.01***
1 X 11	-0.59	-1.75***	-1.54*	-1.78*	-1.58**	-62.57**	-1.61**
10 X15	-0.68*	-0.7	-1.90**	-2.85***	-1.95**	-61.04**	-1.57**
7 X 13	-0.27	-1.29*	-2.11***	-2.54***	-1.56*	-67.38***	-1.74***
1 X 5	-0.28	-1.43**	-1.66**	-1.78*	-1.03	-57.64**	-1.51**
8 X 12	-0.27	-1.08*	-1.77**	-1.81*	-1.43*	-54.48**	-1.40**
2 X 11	-0.51	-1.05*	-1.21*	-1.95**	-0.94	-48.87*	-1.28*
6 X 12	-0.2	-0.96	-1.57**	-1.90*	-1.09	-50.17*	-1.29*
11 X 14	-0.42	-0.51	-1.30*	-2.04**	-0.8	-42.57*	-1.10*
5 X 9	-0.37	-1.34**	-0.99	-1.18	-0.74	-43.54*	-1.12*
6 X 13	-0.16	-0.64	-1.92**	-2.22**	-1.00	-52.54**	-1.34**
5 X 8	-0.23	-1.37**	-0.97	-1.35	-0.96	-43.71*	-1.13*
1 X 10	-0.27	-0.64	-1.35*	-1.75*	-0.92	-42.11*	-1.08*
3 X 7	-0.25	-0.87	-1.59**	-1.54*	-0.71	-47.03*	-1.21*
11 X 19	-0.22	-0.9	-0.76	-1.94**	-1.65**	-38.16	-0.98
9 X 19	-0.70*	-1.24*	-0.64	-0.94	-0.79	-37.77	-0.98
4 X 12	-0.27	-0.58	-1.17*	-1.47*	-1.08	-36.74	-0.94
2 X 6	-0.68*	-0.78	-0.47	-1.11	-1.57**	-29.75	-0.78
1 X 13	-0.72*	-0.83	0.14	-1.26	-1.55*	-23.22	-0.58
11 X 12	-0.03*	-0.73	-1.31*	-1.74*	-0.12	-41.29*	-1.09*
8 X 9	0.03	-0.52	-0.78	-1.55*	-1.39*	-28.71	-0.73

Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN; MLN_SC, calculated MLN susceptibility score; *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; † numbers 1-19 for crosses correspond to parents in Table 3.1

Table 3.5 Summary of correlation coefficients of combining ability effects with per se parental means for maize lethal necrosis and Turcicum leaf blight

***Per se* mean**	**GCA effect**							
	*g*MLN1	*g*MLN2	*g*MLN3	*g*MLN4	*g*MLN_AUDPC	*g*MLN_AV	*g*MLN_SC	*g*TLB
*μ*MLN1	0.59**	0.45	0.32	0.21	0.36	0.33	0.36	-0.14
*μ*MLN2	0.71***	0.61**	0.49*	0.46*	0.55*	0.52*	0.55*	-0.24
*μ*MLN3	0.70***	0.65**	0.60**	0.54*	0.62**	0.61**	0.62**	-0.39
*μ*MLN4	0.43	0.37	0.32	0.27	0.34	0.34	0.34	-0.32
*μ*MLN_AUDPC	0.70***	0.61**	0.52*	0.47*	0.56*	0.54*	0.56*	-0.32
*μ*MLN_AV	0.68**	0.59**	0.49*	0.43	0.53*	0.52*	0.53*	-0.31
*μ*TLB	-0.05	-0.15	-0.20	-0.29	-0.20	-0.21	-0.20	0.60**

GCA, general combining ability; *g*MLN1, *g*MLN2, *g*MLN3, *g*MLN4, *g*MLN_AUDPC, *g*MLN_AV, *g*MLN_SC and *g*TLB, GCA effects for respective maize lethal necrosis (MLN) and Turcicum leaf blight (TLB) severity scores, MLN area under disease progress curve (AUDPC), mean of MLN scores (AV) and calculated MLN susceptibility score (SC); *μ*MLN1, *μ*MLN2, *μ*MLN3, *μ*MLN4, *μ*MLN_AUDPC, *μ*MLN_AV, *μ*TLB, individual (*per se*) line means for respective MLN and TLB severity scores, MLN area under disease progress curve, mean of MLN scores (AV) and calculated MLN susceptibility score (SC); *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05

In this study, a diverse collection of 19 lines was included, with 13 having varying levels of MLN resistance and five susceptible tropical and testers lines, and one susceptible line from the KS23 pool. These lines were evaluated for the level of existing genetic variation, combining ability across the representative gene pools, estimates of heritability and mode of gene action for disease resistance. Understanding these breeding parameters could be used to design effective ways to utilise the available and acquired MLN resistant donor lines. This initial genetic study could also guide the choice of efficient breeding methods to alleviate breeding challenges associated with using exotic germplasm, such as limited adaptability, susceptibility to endemic diseases, and linkage drag associated with lack of complementarity in favourable loci in divergent genetic backgrounds.

Results from the evaluation of diallel crosses and parents confirmed the existence of adequate genetic variation for resistance to MLN and TLB. Significant genetic variation is the premise for selection of desirable progenies and success in genetic improvement for combined MLN and TLB resistance in east African breeding populations. The adequate genetic variation coupled with high heritability suggest that adequate genetic gains can be expected from selection for MLN and TLB resistance. However, selection for the two diseases needs to be conducted in

separate screening trials due to the lack of a positive correlation between MLN and TLB resistance, an indication that indirect selection for resistance to one disease is not possible during direct selection for another. Similar observations were made by Vivek et al. (2010) when diverse tropical lines were evaluated for six African diseases, including TLB. The development of the CIMMYT regional screening facility and existence of disease hotspots and screening protocols in East Africa offers an opportunity for rapid disease resistance breeding.

Combining ability and estimation of its effects have been used widely in maize breeding, especially in hybrid breeding programmes, to identify the best parental combinations and maximising variance in breeding populations (Fasahat 2016). Results of this study indicated that variance due to GCA was higher than for SCA. This suggests the predominance of additive gene action governing resistance to MLN and TLB in breeding populations derived from both tropical and temperate sources. Significant variation in the magnitude of GCA effects was observed for MLN and TLB resistance, making it possible to select parents with duel disease resistance. Disease resistant lines with significant negative GCA effects are desirable for the production of resistant breeding populations and hybrids. Using tropical maize germplasm, Beyene et al. (2017) observed highly significant GCA-based prediction accuracy for MLN resistance and other key traits among new hybrids. These results were confirmed by Nyaga et al. (2020), where the correlation of GCA effects was high with hybrid performance.

In the current study involving two foliar diseases, the negative non-significant correlation of GCA effects for TLB and MLN implies that with effective disease screening, it will be possible to combine alleles for resistance to the two diseases in new inbred elite lines. Successful combining of MLN resistance into tropical lines is achievable since elite lines and testers such as CML312, CML444, NML97, CML543 and NML88 evaluated in this study are good combiners for TLB resistance. On the other hand, elite lines CML494 and CML550 need to be improved for TLB resistance. Adapted lines CKDHL120918, CKDHL120312, CML543 and CML574 could be used directly in forming new hybrids with combined MLN and TLB resistance, forwarding breeding to form improved disease resistant parental lines. These dual disease resistant lines, except yellow line CML574, are good potential hybrid parents because they are white-grain types, preferred by consumers in eastern Africa. Temperate inbred line, Oh101-1-B-2 with good GCA for MLN and TLB could be used in developing disease resistant backcross populations using tropical maize lines. Asian sourced lines were found to have the highest *per se* resistance to MLN but with undesirable significant positive GCA effects. This implies that these lines

cannot be used in producing resistant single cross hybrids in combination with African elite lines. This could also suggest that Asian lines KS523-5 and KS523-6 have unique recessive MLN resistance alleles or QTL that need to be introgressed into at least two elite maize lines followed by crossing the resultant lines carrying the complementary recessive alleles to form a resistant hybrid product. Generally, the 21 cross combinations with high SCA for MLN resistance are candidates for pedigree, backcross and recurrent selection populations targeting MLN resistance using both temperate and tropical lines. The heterotic orientation of donor and elite lines needs to be validated to minimise overlap in heterotic groups of new resistant inbred lines derived from these founder lines and to maximise genetic variance in hybrids developed from newly introgressed lines.

3.5 Conclusions

This study ascertained the usefulness of inbred virus resistant lines sourced from tropical and temperate germplasm pools. Collections from the USA, Africa, Mexico and Asia form a representative genetic base for developing MLN resistant breeding populations from which new disease resistant lines could be extracted. The observed genetic variation and high heritability present an opportunity for rapid genetic progress from using the available lines to develop lines with dual resistance to MLN and TLB using recurrent and backcross selection methods. The prevalence of additive gene action over non-additive gene action suggests that rapid progress can be expected from recurrent selection. Adapted lines with high combining ability for resistance to both MLN and TLB could be used in forming new varieties in the short term. Less adapted disease resistant lines could be used in medium to long-term trait introgression, considering selection for desirable agronomic traits and resistance to other economically important diseases such as MSV and GLS.

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CHAPTER 4

Genetic potential for resistance to maize lethal necrosis among KS23-derived maize pedigree populations

Abstract

To achieve adequate genetic gains for MLN resistance, there is need to deploy efficient, cost-effective breeding techniques and making selections in breeding populations derived from carefully selected parents. In this study, nine pedigree populations were evaluated with the following objectives: (i) to determine the magnitude of genetic variation for MLN resistance among KS23-derived biparental populations, (ii) to determine the usefulness and expected gain from selection under artificial MLN pressure among KS23-derived derived biparental populations and, (iii) to select and advance segregating lines with desirable MLN resistance. Analysis of variance showed highly significant genetic variation for MLN resistance among full-sib families in each biparental population. With the exception of two populations, MLNR-P14 and MLNR-P17, the biparental populations had high heritability estimates for MLN severity (0.6 - 0.77). Based on lower usefulness values, populations MLNR-P16, MLNR-P18 and MLNR-P21 could be prioritised for advancement under limited resources. Overall, KS23-6 was a better MLN resistant donor as shown by higher negative usefulness values, and higher number of lines selected (22%) compared with KS23-5 (11%). Selected lines require further testing for agronomic performance and resistance to common foliar diseases.

Key words: genetic variation, expected gain, analysis of variance, heritability

4.1 Introduction

Since the first report of MLN in SSA in 2011 (Wangai et al. 2012), resistant breeding populations, parental lines and hybrids have been developed using both conventional and modern breeding methods. Albeit the increasing use of modern tools such as DH technology, marker-assisted breeding and genomic selection, pedigree selection is still used by breeders in the region, alongside other commonly used conventional methods such as mass and recurrent selection. Pedigree selection entails deriving new inbred lines by subsequent self-pollination with strict record keeping of F_2 narrow base populations; improved open-pollinated varieties such as composites and synthetics; backcross populations and germplasm mixtures. Similar to inbred progeny selection, major advantages of pedigree selection are the enhanced variability

among progenies, exposure and elimination of undesirable recessive traits in the process of self-pollination and selection, and the high degree of traceability of pedigrees through record keeping. The major disadvantage of the method is the prolonged interval between selection cycles and possible linkage and inbreeding effects when selected lines are recombined (Hallauer et al. 1988a). The prolonged cycle of selection is often reduced by off-season nurseries. The DH technology is gaining ground in SSA because of reducing selection cycle time. Inducer-based DH technology can reduce the generations of inbred lines from six to nine seasons to only two seasons, with enhanced selection efficiency. Increased use of DH technology was facilitated by the investment in DH facilities at CIMMYT to serve both the National Agricultural Research System (NARS) and private sector and international research centres, to generate new maize lines resistant to MLN and other traits (Chaikam et al. 2019).

Stress-tolerant parental lines and hybrids have commonly been developed and released in SSA using both pedigree and DH selection methods (Beyene et al. 2015; 2017). In this study, selected pedigree populations deliberately developed for selection under MLN infestation, were evaluated for their breeding potential. Ideally, desirable segregating populations should have large genetic variation combined with a favourable mean to enable attainment of maximum potential selection gain (Bernardo 2020). Usually, the limited genetic diversity of elite maize germplasm raises concerns about the potential to breed for new constraints such as MLN. To address this concern, breeders adopted the usefulness criterion for determining the potential of breeding populations and their ability to generate superior lines (Schnell 1983; Melchinger 1987; Hallauer et al. 1988a). The "usefulness" of a cross (in this case, a biparental population) is defined as the trait mean of a defined upper fraction of its progeny, which is defined as the expected population mean plus the expected selection gain as a function of the selection intensity, square-root of the trait heritability, and the genetic standard deviation of the population (Schnell and Utz 1975; Lehermeier et al. 2017).

The usefulness criterion therefore, is a more informative method that combines the mean performance, genetic variance and heritability to identify populations with high breeding potential for prioritisation under limited resources. Thus, this principle was used in this study, with three specific objectives: (i) to determine the magnitude of genetic variation for MLN resistance among KS23-derived biparental populations; (ii) to determine the usefulness and breeding potential of KS23-derived derived biparental populations selected under artificial MLN pressure; and (iii) to select and advance partial inbred lines with desirable MLN resistance.

4.2 Materials and methods

4.2.1 Biparental populations

A total of five DH lines initially developed through forward breeding of CIMMYT elite lines for improved drought tolerance and combining ability for yield, were crossed with two introduced tropical yellow inbred lines, KS23-5 and KS23-6, with the highest *per se* MLN resistance under artificial inoculation. Nine pedigree starts used in this study were selected and advanced to the F_3 generation in season 2015B (Table 4.1). Each biparental population used in this study had a large number (>100) of randomly sampled $F_{2:3}$ families to obtain adequate variation for genetic analysis. Across populations, 1 301 $F_{2:3}$ half-sib families were screened under artificial MLN infestation for two seasons, 2016B and 2017A. From each population lines with a score < 4.0 on a 1-9 MLN severity scale were selected from advancement.

Table 4.1 Biparental populations evaluated for maize lethal necrosis resistance

Population	Pedigree	Families evaluated	Families selected	% selected
MLNR-P13	(CML543/KS523-5)F3	104	25	24
MLNR-P14	((KU1403 x 1368)-7-2-1-1-B-B/CML444)-B-8-7-3-2-4-1-2-B-B-B/KS523-5)F3	121	5	4
MLNR-P15	(CML543/CML444//CML543)DH5-B-B-B/KS523-5)F3	172	24	14
MLNR-P16	((CML543/CML444//CML543)DH6-B-B-B/KS523-5)F3	158	21	13
MLNR-P17	(([LZ956441/LZ966205]-B-3-4-4-B-5-B*7/LaPostaSeqC7-F71-1-2-1-1-BBB)-1-7-1-1-BB-B/KS523-5)F3	168	19	11
MLNR-P18	(CKML543/KS23-6)F3	146	14	10
MLNR-P19	(((KU1403 x 1368)-7-2-1-1-B-B/CML444)-B-8-7-3-2-4-1-2-B-B-B/KS23-6)F3	156	48	31
MLNR-P20	((CML543/CML444//CML543)DH5-B-B-B/KS23-6)F3	138	32	23
MLNR-P21	((CML543/CML444//CML543)DH6-B-B-B/KS23-6)F3	138	32	23

4.2.2 Field experimentation

Field experiments were conducted for two seasons, 2016B (September 2016 - January 2017) and 2017A (March - August 2017), under artificial inoculation with MLN at the regional screening facility in Naivasha (detailed site characteristics described in Chapter 3). Full-sib families (inbred lines per population) were planted in an alpha lattice design with two replications. All populations were planted in one block under similar management practices and time of inoculation with MLN viruses, following the optimised MLN screening protocol, earlier

elaborated on in section 3.2.3. Because the test materials were inbred, scoring for disease symptoms started two weeks from the second inoculation and was repeated weekly for four weeks instead of a fortnight as for hybrids. Average disease scores, AUDPC and susceptibility scores were computed.

4.2.3 Statistical analysis

Analysis of variance (ANOVA) within every single population and computation of family means within each population were conducted using the restricted maximum likelihood (REML) method in the R programme embedded in CIMMYT Fieldbook Software (Bänziger and Vivek 2007). Further estimates of heritability and best linear unbiased prediction (BLUP) of each family across testing seasons were done using the REML method in the R programme embedded in META-R software (Alvarado et al. 2016). In this case, seasons and replications were treated as random effects while entries were fixed. Estimated BLUPs were then pooled and used to analyse variances of full-sib families (within populations).

Prediction of genetic gain and calculation of the usefulness criterion:

The usefulness value (expected genetic gain) for each pedigree population was computed using the usefulness value calculated based on F_3 families as described by Hallauer et al. (1988a):

$$\text{Usefulness criterion } U = (\ddot{X} + \Delta G)$$

Where (ΔG) is the expected genetic gain from selection calculated as Heritability (H^2) x Selection differential (S). The selection differential (S) = Mean of selected families ($\ddot{X}_S$) - Mean of the overall population ($\ddot{X}$).

4.3 Results

Analysis of variance among F_3 families in each pedigree population was first conducted. Results showed highly significant genotypic variation for both early and late MLN disease development and disease progress curve ($P \leq 0.001$) among families of each population, except for MLNR-P14 (Tables 4.2 and 4.3). Genotype x season variance was also highly significant among families, except in populations MLNR-P14 and MLNR-P19. Populations MLNR-P14 and MLNR-P17 had moderate heritability estimates for MLN scores, while the rest of the populations recorded high heritability estimates that ranged from 0.59 - 0.77. Despite single-season

evaluation due to limited seed, high heritabilities (> 0.70) were attained consistently for both MLNR-P15 and MLNR-P21.

Overall, populations MLNR-P15, MLNR-P19, and MLNR-P20 had lower family mean ($\ddot{x}$) scores for MLN severity, while population MLNR-P14 had with very high, undesirable mean scores. For advancement, lines with final MLN scores of < 2.0 (on a scale of 1–9) and a few moderately MLN tolerant lines with mean scores (MLN_AV) of < 4.0, were selected based on the least significant difference (LSD). Population MLNR-P18 had the largest expected gain due to selection with reduced disease severity of up to 1.55 units lower than the previous generation. Populations derived from sister DH lines (MLNR-P15 and MLNR-P16; and MLNR-20 and MLNR-21) were comparable in performance and also had high usefulness values and expected gains from selection.

Comparison of usefulness values for disease severity scores, MLN_AUDPC MLN_SC across populations indicated that populations MLNR-P21, MLNR-P20, MLNR-P18, MLNR-P19, MLNR-P16 had overall lower *U* values, hence were considered better selection populations. MLNR-P14 was the worst performing population with exceptionally high *U* values and most of the families were rejected. Results further indicated differences in usefulness values for two populations derived from crossing susceptible parents to different donors. Overall, KS23-6 was a better donor as shown by a higher number of lines selected (22% for KS23-6 and 11% for KS23-5). On average, lower usefulness values were observed for MLN scores in populations involving KS23-6 (average *U* = 3.5) compared to populations derived from KS23-5 (average *U* = 4.2). The usefulness values for disease progress (AUDPC) were lower for KS23-6 populations, ranging from 58.79 - 83.53 (average = 71.1) compared with populations derived from KS23-5 that ranged from 63.24 - 121.81 (average = 81.1)

Table 4.2 Genetic variability and usefulness values of pedigree populations based on the KS23-5 donor line

Trait	δ^2_g	δ^2_{gxs}†	δ^2_e	H^2	$\ddot{X}$	$\ddot{X}_s$	S	ΔG	*U*
MLNR-P13: (CM543/KS23-5)F3									
MLN2	0.63***	0.48***	0.54	0.62	4.41	3.43	-1.22	-0.76	3.65
MLN4	1.07***	0.48***	0.92	0.69	5.17	4.05	-1.32	-0.92	4.26
MLN_AV	0.67***	0.43***	0.47	0.67	4.45	3.42	-1.24	-0.83	3.62
MLN_AUDPC	617.11***	855.27***	581.47	0.52	113.05	79.81	-53.25	-27.61	85.43
MLN_SC	0.83***	0.63***	0.59	0.64	4.05	2.07	-1.38	-0.89	3.16
MLNR-P14: ((KU1403 x 1368)-7-2-1-1-B-B/CML444)-B-8-7-3-2-4-1-2-B-B-B/KS23-5)F3									
MLN2	0.29**	0.00	0.93	0.56	6.56	4.51	-0.92	-0.51	6.04
MLN4	0.19	0.21*	0.71	0.40	7.34	4.42	-1.70	-0.67	6.66
MLN_AV	0.18**	0.05	0.47	0.55	6.47	3.98	-1.27	-0.70	5.77
MLN_AUDPC	95.43**	12.87	245.55	0.58	137.86	84.71	-27.46	-16.05	121.81
MLN_SC	0.24**	0.03	0.63	0.58	6.78	4.36	-1.41	-0.82	5.96
MLNR-P15: ((CM543/CML444//CM543)DH5-B-B-B/KS23-5)F3									
MLN2	0.43***	-----	0.50	0.63	3.87	2.53	-1.29	-0.82	3.05
MLN4	0.97***	-----	0.72	0.73	4.73	2.90	-1.83	-1.34	3.39
MLN_AV	0.56***	-----	0.38	0.75	4.08	2.61	-1.46	-1.08	2.99
MLN_AUDPC	243.77***	-----	180.61	0.73	85.26	54.76	-30.19	-22.03	63.24
MLN_SC	0.76***	-----	0.56	0.73	4.77	3.08	-1.67	-1.22	3.55
MLNR-P16: ((CM543/CML444//CM543)DH6-B-B-B/KS23-5)F3									
MLN2	0.56***	0.34***	0.85	0.59	4.27	2.89	-1.53	-0.91	3.36
MLN4	1.32***	0.26*	1.14	0.76	5.23	3.17	-2.11	-1.60	3.63
MLN_AV	0.75***	0.20**	0.69	0.73	4.42	2.87	-1.66	-1.22	3.20
MLN_AUDPC	329.01***	94.69**	321.00	0.72	93.93	61.44	-35.08	-25.28	68.65
MLN_SC	0.85***	0.26***	0.83	0.72	4.78	3.12	-1.79	-1.29	3.49
MLNR-P17: (([LZ956441/LZ966205]-B-3-4-4-B-5-B*7/LaPostaSeqC7-F71-1-2-1-1-BBB)-1-7-1-1-BB-B/KS23-5)F3									
MLN2	0.23**	0.35***	0.48	0.43	4.36	3.26	-1.19	-0.51	3.85
MLN4	0.48***	0.70***	0.73	0.48	5.26	3.73	-1.65	-0.79	4.47
MLN_AV	0.31***	0.1***	0.39	0.51	4.50	3.27	-1.32	-0.67	3.83
MLN_AUDPC	131.52***	179.23***	177.32	0.50	94.96	69.68	-27.29	-13.52	81.44
MLN_SC	0.34***	0.46***	0.45	0.50	4.79	3.50	-1.40	-0.69	4.09

Maize lethal necrosis (MLN) severity ratings 2 and 4, subsequent MLN severity scores at 28 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN; MLN_SC, calculated MLN susceptibility score; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; δ^2_g, genotype variance; δ^2_{gxs}, genotype x season variance; δ^2_e, residual variance; H^2, heritability; $\ddot{X}$, mean of families; $\ddot{X}_s$, mean of selected families; S, selection differential ($\ddot{X}$ - $\ddot{X}_s$); ΔG, expected genetic gain; *U*, usefulness criterion ($\ddot{X}$ + ΔG); † missing δ^2_{gxs} values for MLNR-P15 due to single season evaluation

Table 4.3 Genetic variability and usefulness values of pedigree populations based on the KS23-6 donor line

Trait	δ^2_g	δ^2_{gxs}	δ^2_e	H^2	$\ddot{X}$	$\ddot{X}_s$	S	ΔG	*U*
MLNR-P18: (CM543/KS23-6)F3									
MLN2	1.15***	0.47***	1.09	0.69	4.99	2.92	-2.21	-1.53	3.46
MLN4	1.29***	0.48**	1.41	0.69	5.55	3.29	-2.29	-1.57	3.98
MLN_AV	0.99***	0.39***	0.86	0.71	4.83	2.89	-2.00	-1.41	3.42
MLN_AUDPC	458.43***	177.21***	410.91	0.71	103.17	61.68	-43.23	-30.50	72.68
MLN_SC	1.17***	0.42***	1.04	0.71	5.19	3.10	-2.18	-1.55	3.64
MLNR-P19: (((KU1403 x 1368)-7-2-1-1-B-B/CML444)-B-8-7-3-2-4-1-2-B-B-B/KS23-6)F3									
MLN2	0.56***	0.34	0.76	0.61	3.96	2.63	-1.28	-0.78	3.19
MLN4	0.67***	0.27	1.00	0.63	4.65	3.01	-1.29	-0.82	3.83
MLN_AV	0.48***	0.26	0.58	0.64	3.98	2.61	-1.13	-0.72	3.26
MLN_AUDPC	217.53***	122.51	274.07	0.63	84.91	55.09	-25.02	-15.67	69.24
MLN_SC	0.55***	0.31	0.69	0.63	4.28	2.83	-1.29	-0.81	3.47
MLNR-P20: ((CM543/CML444//CM543)DH5-B-B-B/KS23-6)F3									
MLN2	0.70***	0.213***	0.58	0.74	4.23	2.88	-1.37	-1.01	3.22
MLN4	1.20***	0.30**	1.02	0.75	4.93	3.21	-1.74	-1.30	3.63
MLN_AV	0.72***	0.222***	0.54	0.74	4.26	2.89	-1.38	-1.02	3.23
MLN_AUDPC	380.34***	137.72***	316.68	0.72	108.65	70.10	-34.89	-25.12	83.53
MLN_SC	0.72***	0.30***	0.65	0.70	4.67	2.99	-1.54	-1.07	3.60
MLNR-P21: ((CM543/CML444//CM543)DH6-B-B-B/KS23-6)F3									
MLN2	0.77***	----	0.65	0.70	3.76	2.88	-1.37	-0.96	2.80
MLN4	1.41***	----	0.90	0.76	5.09	3.21	-1.74	-1.32	3.77
MLN_AV	0.75***	----	0.47	0.76	4.02	2.89	-1.38	-1.05	2.97
MLN_AUDPC	354.72***	----	213.47	0.77	85.62	70.10	-34.89	-26.82	58.79
MLN_SC	0.98***	----	0.59	0.77	4.49	2.99	-1.54	-1.18	3.31

Maize lethal necrosis (MLN) severity ratings 2 and 4, subsequent MLN severity scores at 28 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN; MLN_SC, calculated MLN susceptibility score; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001, δ^2_g, genotype variance; δ^2_{gxs}, genotype x season variance; δ^2_e, residual variance; H^2, heritability; $\ddot{X}$, mean of families; $\ddot{X}_s$, mean of selected families; S, selection differential ($\ddot{X}$ - $\ddot{X}_s$); ΔG, expected genetic gain; *U*, usefulness criterion ($\ddot{X}$ + ΔG); † missing δ^2_{gxs} values for MLNR-P20 due to single season evaluation

4.4 Discussion

Initially, the major hindrances to breeding for MLN resistance in SSA was the limited genetic variation for resistance and lack of suitable resistant donor lines. This problem was resolved by rapid germplasm screening and the introduction of exotic resistant sources such as the KS23-derived inbred lines (Redinbaugh and Stewart 2018; Das et al. 2019; Prasanna et al. 2020). To guide effective breeding, genetic analysis in this chapter was conducted using nine pedigree populations derived from elite inbred lines crossed with the two donor lines, KS23-5 and KS23-

6, to determine their genetic potential and usefulness in the extraction of new inbred lines. In addition to the choice of breeding germplasm, the genetic nature of disease resistance also dictates the choice of selection technique to be used. Earlier studies pointed to the quantitative nature of MLN resistance with an array of major- and minor-effect QTL distributed in tropical maize germplasm (Gowda et al. 2015; 2018; Beyene et al. 2017). However, a study by Jones et al. (2018) noted differences in the genetic nature of resistance in different genetic backgrounds. Specifically, the KS23 genetic background was found to exhibit a major recessive QTL on chromosome 6 when crossed with temperate USA inbred lines. A similar pattern was observed in Chapter 3 whereby the combining ability effects for MLN resistance among the two KS23 lines were significantly positive (unfavourable), despite their significantly low *per se* mean disease severity values.

The major challenge in selecting recessive loci is assosiated with the ability to accelerate the recurrent genome recovery or the maintenance of the desirable recessive alleles in backcrossing programmes. Subsequently, at the end of the selection cycle, recreation of resistant hybrids using the new recombinant resistant inbred lines (RILs) or near-isogenic lines (NILs) requires the two parental lines to contain the fixed recessive locus. This implies that the performance of this hybrid could also be affected by parental line purity and segregation within the recessive resistance locus. To efficiently select recessive loci, modern tools that reduce cycle time and cost need to be applied (Hospital 2005; Xu et al. 2017). A recent comparative study found that the use of DH lines with KS23 donor parents led to higher expected genetic gain than marker-assisted backcrossing (Awata et al. 2021). An earlier study by Jumbo et al. (2011) found a slight but significant difference among conventional methods, with the modified single seed decent method being better than DH and pedigree methods in resultant yield gains among lines derived from GEM. These two studies imply that the choice of breeding methods depends on the germplasm used and the focus traits. Despite the increased adoption of modern breeding methods, pedigree selection is still a popular method in SSA maize breeding programmes. This is due to major advantages such as ease of pedigree tracking and the opportunity for adequate recombination of loci to occur between selfing and advancement. With adequate recombination for a highly heritable trait such as MLN resistance, adequate genetic gain is achieved by advancing to the next generation only those segregating lines or plants with desired characters (Haullauer et al. 1988b).

In pedigree breeding under MLN pressure, high selection gains are attained by advancing superior populations with favourable mean values, high genetic variance and subsequently desirable usefulness values determined by the magnitude of heritability and the selection intensity (Bernardo 2020). Only when these high performing populations have been selected, then genetic gain in hybrids can be enhanced by selecting only those families or individuals with high means and intercrossed as suggested by simulation models used by Bernardo (2003). The current study showed that most populations evaluated were suitable for selecting new resistant MLN lines using the pedigree method. The observed high heritabilities among populations imply that selection of MLN resistant inbred lines could be effective in early generations. However, in a maize hybrid system, the usefulness method based on *per se* performance of segregating families or partial inbred lines does not provide adequate information about the suitability of the inbred lines for developing hybrids that can be commercialised. Early generation testcross evaluation of the partial inbred lines is therefore required to complement line *per se* selection.

The nine breeding populations showed varying breeding potential as indicated by the expected genetic gain and usefulness values. This could make it easier to prioritise populations that are more promising when breeding resources are limited. Due to the recessive nature of the KS23 QTL, populations used in this study, except MLNR-P19, had less than 25% selected partial inbred lines with desirable MLN resistance, even after considering some families with moderate resistance (score < 4 on a 1-9 scale).

The significant genotype x season variance suggested differential genotype performance in different seasons under artificial screening conditions. This implies that selection among populations could require replicated screening of populations over multiple seasons. This procedure is time-consuming and resource constraining, especially when the number of populations is large. It is even more costly in the case of phenotypic selection under artificial MLN pressure, which necessitates screening part of the seed from segregating families while the other part is planted in a non-MLN nursery for advancement, to avoid the spread of the disease through seed. In such a situation, optimisation and application of marker-assisted breeding, DH induction and genomic selection are needed. These methods require the choice of the best resistant donors and elite lines to generate new populations, and decisions on the number of populations and individuals to evaluate per population. Lines generated through DH induction do not need advancement through generations, and can be evaluated directly in replicated trials across seasons. Combined with DH induction, genomic selection will enable

cost effectiveness in selection of large populations, hence, increasing the probability of identifying new desirable lines. Other modifications such as S_1 family selection followed by DH induction have been suggested for increasing genetic gains (Wegenast et al. 2008).

The usefulness criterion has long been applied in selecting the best breeding populations under limited resources. However, the major problem in large maize breeding programmes that do selections among many biparental or DH populations, is the risk of lowering the selection differential. The size of the selection differential depends on the total number of individuals evaluated and selected. Selecting more individuals versus selecting only a few individuals when the total number of individuals in a population (population size) is large, will have little negative impact on the selection differential. Contrary to this, selecting more individuals versus selecting only a few will greatly reduce the selection differential if the population size is small. It is therefore not a problem if large numbers of individuals can be evaluated, but it is with constrained resources. Other experimental considerations such as the number of progenies, replications and environments should be part of the decision process. For instance, with higher plot heritability, a higher selection intensity is needed to warrant increase in replications (Da Silva Filho 2013).

Despite the challenges in optimising breeding, Beyene et al. (2015) demonstrated the efficiency of genomic selection over pedigree breeding in improvement of yield and stress-tolerance in tropical maize. The application of genomic selection can decrease cycle time and costs in hybrid breeding, particularly by rapidly establishing heterotic pools, reducing testcrossing and limiting the erosion of genetic variance (Labroo et al. 2021). However, most breeding programmes do not implement genomic selection due to limited capacity for consistent genotyping and bioinformatics, hence the need to build capacity among breeding programs (Shamshad and Sharma 2018; Gedil and Menkir 2019). Technically, use of genomic selection requires optimised breeding schemes and training population design (Bassi et al. 2016).

Breeding programmes will therefore need to optimise both genetic gains and breeding cost, especially in incorporating new traits such as MLN resistance, to effectively widen the genetic base, while ensuring consistency and without disrupting variety release (Allier et al. 2019). This optimisation is centred on balancing the number of populations with the budget spent for each population (Riedelsheimer and Melchinger 2013). Alternatively, while applying genomic selection, increased gains using DH and pedigree populations can be achieved by incorporating

whole-genome information in computation of usefulness values (genomic usefulness criterion) rather than selection based on mean genomic estimated breeding values (Lehermeier et al. 2017).

4.5 Conclusions

A total of 160 (12%) MLN tolerant partial inbred lines were selected out of 1 301 families evaluated in this study, based on the mean performance of populations derived from the two resistant donor parents, KS23-5 and KS23-6. Despite high heritability estimates, usefulness values showed that populations from KS23-6 could yield more lines that are resistant. Greater success in breeding MLN resistant varieties could therefore be attained by using the most effective donor parent and increasing the diversity of susceptible elite lines to be introgressed. Based on results of this study, three populations, MLNR-P16, MLNR-P18 and MLNRP-21, could be prioritised for advancement under limited resources. Further simulation studies need to be conducted to determine the optimum number of populations and individuals required to maximise gains for MLN resistance. In addition, to aid product development, testcross evaluation will be needed to determine the potential for yield and combining ability of new MLN resistant lines for other desirable agronomic, adaptive and farmer-preferred traits.

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CHAPTER 5

Mapping quantitative trait loci linked to maize lethal necrosis resistance in tropical maize populations

Abstract

The recent MLN infections, caused by co-infection of maize with MCMV and a second virus usually from the family Potyviridae, are causing significant losses for farmers in SSA. Preliminary genetic studies suggested a polygenic nature of resistance to the disease. Screening large collections of maize inbred lines revealed KS23-5 and KS23-6 as two of the most promising donors of MLN resistance alleles. The two MLN resistant donor lines were developed at the University of Hawaii, USA, based on a source population constituted using germplasm from Kasetsart University, Thailand. A previous study using KS23 crossed to temperate lines from USA identified a major recessive MLN resistance QTL on chromosome 6. The KS23-derived sister lines have, therefore, been used as donor parents in the region. The current study was conducted to determine the presence and pattern of distribution of this major QTL from the KS23-background, in tropical maize populations. The specific objectives were to (i) map the distribution of MLN resistance QTL in tropical x KS23 biparental populations and (ii) identify candidate markers linked to major MLN resistance QTL in these populations. Three tropical lines were crossed to 2 donor lines KS23-5 and KS23-6 and 3 resultant biparental populations were chosen for QTL mapping based on artificial screening results. Analysis of variance revealed significant genotypic variance and moderately high heritability for MLN disease severity and AUDPC. Quantitative trait loci analysis revealed one major effect QTL on chromosome 6 within a confidence interval of 85 - 157 Mbp in all three populations. Nine SNP markers were closely linked to major QTL across early and late disease development stages. Two of these markers overlapped in two populations. Three markers were linked to major QTL in one population while the other two populations each had two markers linked to major disease resistance QTL. The candidate QTL and the closely linked markers could be useful for MAB and backcrossing to efficiently improve MLN resistance in elite tropical maize genetic backgrounds.

Key words: Biparental populations, linkage mapping, KS23 donors, quantitative trait loci

5.1 Introduction

The outbreak of MLN in eastern Africa in 2011 (Wangai et al. 2012) has caused an economically important biotic stress affecting the maize industry in SSA. Breeding for resistance commenced with the search for resistant inbred lines from locally adapted genetic pools as well as introductions from Asia and USA. Current efforts are being undertaken to develop adapted and productive inbred lines and hybrids for SSA farmers. Genetic studies of MLN resistance using tropical maize pointed to a polygenic nature, which is largely additive in nature (Beyene et al. 2017). Breeding for MLN resistance is further complicated by the existence of a few major effect QTL with an array of minor effect QTL in different genetic backgrounds (Gowda et al. 2015; 2018; Awata et al. 2019; Prasanna et al. 2020). Due to the quantitative nature of resistance, different genotypes deploy different resistance mechanisms against MLN viruses (Jones et al. 2018). This phenomenon has also been observed for several important adaptive and agronomic traits that are conditioned by the collective and interactive effect of many gene clusters (QTL) positioned in diverse chromosomal regions (Mulualem and Bekeko 2016). These desired genomic regions and linked markers need to be mapped effectively and validated for use in MAB.

Several QTL mapping approaches have been suggested, each having strengths and weaknesses. For instance, the single marker method, also known as single factor analysis of variance (SF-ANOVA) is less complex in application and has been used with isozymes (Edwards et al. 1987). This method, however, becomes less accurate as the distance between markers and QTL increases, it cannot determine whether a marker co-segregates with one or more QTL and the effects of QTL are likely to be underestimated due to complexities brought about by variation in recombination frequencies. Another QTL mapping method, simple interval mapping (SIM) (Lander and Botstein 1989), is able to compensate for recombination between markers and QTL, thus increasing the power of QTL detection. The disadvantage of SIM is the inability to account for genetic variance caused by multiple QTL segregation in a mapping population. The third QTL mapping method, composite interval mapping (CIM) (Jansen and Stam 1994; Zeng 1994), has enhanced QTL mapping power by combining interval mapping for a single QTL with multiple marker-QTL regression analysis. An advancement of interval mapping referred to as multiple interval mapping (MIM) (Kao et al. 1999) allows for identifying the position of a QTL between marker intervals and can decipher interactions between QTL. The QTL mapping approaches have therefore evolved with the need to improve the power and accuracy of QTL identification (Kearsey and Farquhar 1998; Mulualem and Bekeko 2016).

Successful deployment of mapped QTL and linked markers requires validation in the target germplasm pool. The QTL validation process involves confirming if QTL truly exist in the target germplasm and confirming the effectiveness of linked markers, detected in the initial mapping population, in selecting desirable genotypes in a separate population or set of populations from different germplasm pools (Collard et al. 2005). Using a combination of linkage and association mapping approaches, several small to medium effect, and few large effect QTL controlling MLN resistance, have been identified in genetic mapping studies using diverse tropical maize populations (Gowda et al. 2015; 2018; Awata et al. 2019; Sitonik et al. 2019).

A separate study by Jones et al. (2018) mapped disease resistance QTL for MLN and individual MLN viruses using populations derived from inbred lines from USA and Asia (Kaeppler et al. 1998; Brewbaker 2009; Jampatong et al. 2010). This study indicated differences in response to disease, mechanisms of MLN resistance and distribution of disease resistance QTL in diverse genetic backgrounds. Unlike other genetic sources that have a combination of major and minor effect QTL, the two Asian lines (KS23-5 and KS23-6) were found to have a major QTL on chromosome 6, accounting for phenotypic variance of up to 78%. These two lines are popular MLN resistant donor parents in east African breeding programmes. Another major finding of this study suggested that the major QTL in the KS23 background is recessive in nature as shown by the negative skewness in the phenotypic distribution towards the susceptible parent among populations containing KS23 as parent. The recessive nature was confirmed by the pattern of disease development whereby, individuals that were heterozygous for markers linked to the major resistance QTL were comparable to the susceptible parent.

In this study, the occurrence and pattern of previously mapped MLN resistance QTL from the KS23 background are investigated in new populations derived from crosses of African elite lines with the KS23 MLN resistant donors. The specific objectives were to (i) map the distribution of MLN resistance QTL in tropical x KS23 biparental populations and (ii) identify candidate markers linked to major MLN resistance QTL in tropical x KS23 biparental populations.

5.2 Materials and methods

5.2.1 Mapping populations

The three biparental populations used for linkage mapping were developed by crossing two Asian MLN resistant donor lines (KS23-5 and KS23-6) to three CIMMYT lines. The CIMMYT parental lines consisted of one elite line, CML543, and two DH lines extracted from a cross of

recombining CML543 and CML444. The MLN resistant donor lines, KS23-5 and KS23-6, were extracted from the KS23 synthetic line developed at Kasetsart, Thailand. The two lines were maintained in Wooster, Ohio, USA (Jones et al. 2007; Jampatong et al. 2010) and were accessed by CIMMYT-Kenya and NaCRRI for MLN resistance breeding (Table 5.1). Each F_1 cross was self-pollinated and respective F_2 seed harvested and bulked. During the first cropping season of 2016 (March - August) (2016A), the bulked F_2 seed of each population was planted and at least 200 unselected F_2 plants self-pollinated. Following the single seed decent method, each self-pollinated cob was harvested and shelled individually (ear-to-packet) to form corresponding F_3 families. Only families with adequate seed for evaluation in at least two seasons' replicated trials were selected and sent to the regional MLN screening facility, while remnant seed was planted in the field to sample leaf tissue for DNA extraction.

Table 5.1 Quantitative trait loci mapping populations and respective families

Population	Pedigree	F_3 families
MLNR-P20	((CM543/CML444//CM543)DH5-B-B-B/KS23-6)F_3	138
MLNR-P16	((CM543/CML444//CM543)DH6-B-B-B/KS23-5)F_3	158
MLNR-P13	(CM543/KS23-5)F_3	104
Total		390

5.2.2 Phenotyping and phenotypic data analysis

Artificial screening for reaction to MLN was conducted at the regional screening facility in Naivasha, Kenya (https:/mln.cimmyt.org) during the second cropping season of 2016 and repeated during the first cropping season of 2017. Families within each biparental population were planted in an alpha-lattice design with two replications. Each experimental unit was a single row plot measuring 4 m in length, 0.75 m between rows and 0.2 m within rows. Each of the three populations was planted as a separate trial and all trials were planted adjacent to each other in one artificial screening block. All trials were subjected to uniform standard agronomic practices (fertiliser levels, irrigation and weed management). Standardised inoculation procedures and phenotypic data collection were conducted on the mapping populations as described in section 3.2.3. Inoculation of lines was done at the V4-6 stage and repeated one week later. Inoculated plots were rated for MLN severity using a scale of 1 to 9, as described in section 3.2.3. Disease severity data were recorded three weeks after the second inoculation followed by 7-day intervals thereafter. Four ratings were taken for each of the F_3 validation populations and used to compute AUDPC.

To estimate the level of genetic variation in each mapping population, replicated families were subjected to ANOVA for each of the four consecutive MLN scores (MLN1, MLN2, MLN3 and MLN4) and AUDPC. Analysis of variance was conducted using ReML in the R-programme embedded in CIMMYT's fieldbook software (Bänziger and Vivek 2007). Estimates of broad sense heritability and best linear unbiased estimate (BLUE) and BLUPs for each family across testing seasons were computed using mixed linear model (MLM) embedded in META-R software (Alvarado et al. 2016). Seasons, replications and interaction of genotype with season were considered as random effects while genotypes were considered fixed effects.

5.2.3 Genotyping, linkage analysis and quantitative trait loci mapping

Each of the 390 F_3 families from the three biparental populations (Table 3.1) was planted in four-metre rows of 16 plants per row. Fourteen days after emergence, leaf punches from the top, youngest leaves of seedlings were obtained from the first five plants in each family row and bulked into a single well in a 96-well leaf tissue plate. The plated samples were preserved with silica packs and shipped to LCC Genomics Ltd in the UK for DNA extraction and purification using the throughput extraction oKtopure™platform that employs the sbeadex™ magnetic bead based extraction technique (https://www.biosearchtech.com).

The three families were genotyped using 447 low-density polymorphic SNP markers spread across the maize genome, using the KASP genotyping system of LGC Genomics Ltd. The KASP set was selected from the ready-to-use set of > 1 200 markers in the KASP™ SNP genotyping library for maize developed for CIMMYT's Global Maize Programme and the Generation Challenge Programme (Jones et al. 2009; Semagn et al. 2014; https://www.biosearchtech.com). The genotypic data was filtered at a minor allele frequency (MAF) of > 0.05 and less than 10% missing data per sample. The number of SNPs was further reduced by selecting homozygous and polymorphic markers between the two parents of each population. Filtering resulted in 361 SNPs (Appendix 1) being used in construction of linkage maps in each of the three biparental mapping populations using inclusive composite interval mapping, IciMapping, version 4.1 software (http://www.isbreeding.net; Meng et al. 2015). The mapping procedure described by Meng et al. (2015) was used as follows: first, redundant SNPs were removed using the BIN function and the remaining high-quality markers were used to construct genetic linkage maps using the MAP function. The MAP function uses stepwise regression to select the most significant markers and a likelihood ratio test to calculate the logarithm of odds (LOD) scores for each marker at the critical threshold of > 3.0 LOD and a

maximum distance of 30 centiMorgans (cM) between two loci. The three major steps involved in construction of a linkage map were: identification of linkage groups, ordering SNP markers using the RECORD (REcombination Counting and ORDering) algorithm and rippling was done using the SAD (sum of adjacent criterion) to confirm the marker order. Recombination frequencies between two linked loci were converted to map distances in cM using the Kosambi mapping function (Kosambi 1944). Phenotypic data was computed into BLUPs for each of the four MLN scores and AUDPC was used in QTL analysis. The PVE explained by each QTL and across all QTL for each trait was also estimated. Both additive and dominance effects of each QTL were estimated and defined based on the positive or negative direction of the additive effects of each QTL. Observed genotype frequencies were tested for deviations from Mendelian segregation distortion and devaition from expcted ratios and the allele frequency of 0.5 using a χ^2. Selected high quality markers were used to contruct the linkage maps for each of the three mapping populations.

After construction of the linkage map in the IciMapping sofware, QTL anlysis was conducted using the BIP fucntion. Using a customised MS Excel file, general information on the population type (in this case $F_{3,}$), mapping function, marker space type (position), marker space units (cM), number of linkage groups (chromosomes = 10), size of mapping populations (number of families), and the parameters (traits MLN1, MLN2, MLN2, MLN4 and MLN_AUDPC) was entered. Number of markers per chromosome, linkage map, genotypic data and phenotypic data were also imported into the BIP window prior to running the function. Using the stepwise regression method, the probability was set at 0.01 and the scanning step was 1 cM. A threshold LOD score of > 3.0 was set by using more than 1 000 permutations and a P value of ≤ 0.05 was used to determine the significance of a QTL.

5.3 Results

5.3.1 Genetic variation and heritability

Across the two seasons of artificial MLN screening, highly significant genotypic variation ($P \leq 0.001$) was observed among families within each population for all disease scores and AUDPC, except for initial MLN score in MLNR-P13 ($P \leq 0.05$). At maximum disease progress (MLN4), the severity across of the three populations ranged from 3.05 (minimum) to 7.46 (maximum) and mean disease severity ranged from 4.88 to 5.13. AUDPC ranged from 80.98 (minimum) to 187.05 (maximum). Broad-sense heritability estimates were moderate to high for all MLN scores

as well as the MLN disease progress curve, ranging from 0.48 to 0.76. Generally, lower heritability was observed in the initial MLN scores (MLN1 and MLN2) (Table 5.2).

5.3.2 Linkage analysis and identification of disease resistance quantitative trait loci

Linkage analysis and map construction were conducted independently for each biparental population to determine the consistency of QTL detection between populations at different stages of disease development. Across mapping populations, linkage analysis results disclosed major QTL on chromosome 6 consistent across MLN severity scores and AUDPC (Figure 5.1). In two mapping populations MLNR-P16 and MLNR-P20, the major QTL on chromosome 6 were consistent across early and late stages of disease development and were flanked by at least one of the overlapping SNP markers, PZA00223_4, PZA02673_1, S6_157568432, S6_125593444 and S6_156386857. The major effect QTL was mapped to 82 - 87 cM on chromosome 6 and explaining up to 47.6% of the phenotypic variation. However, for MLNR-P13, the major QTL on chromosome 6 was larger, spanning 87 - 93 cM and linked to another additional maker (Tables 5.3 - 5.5).

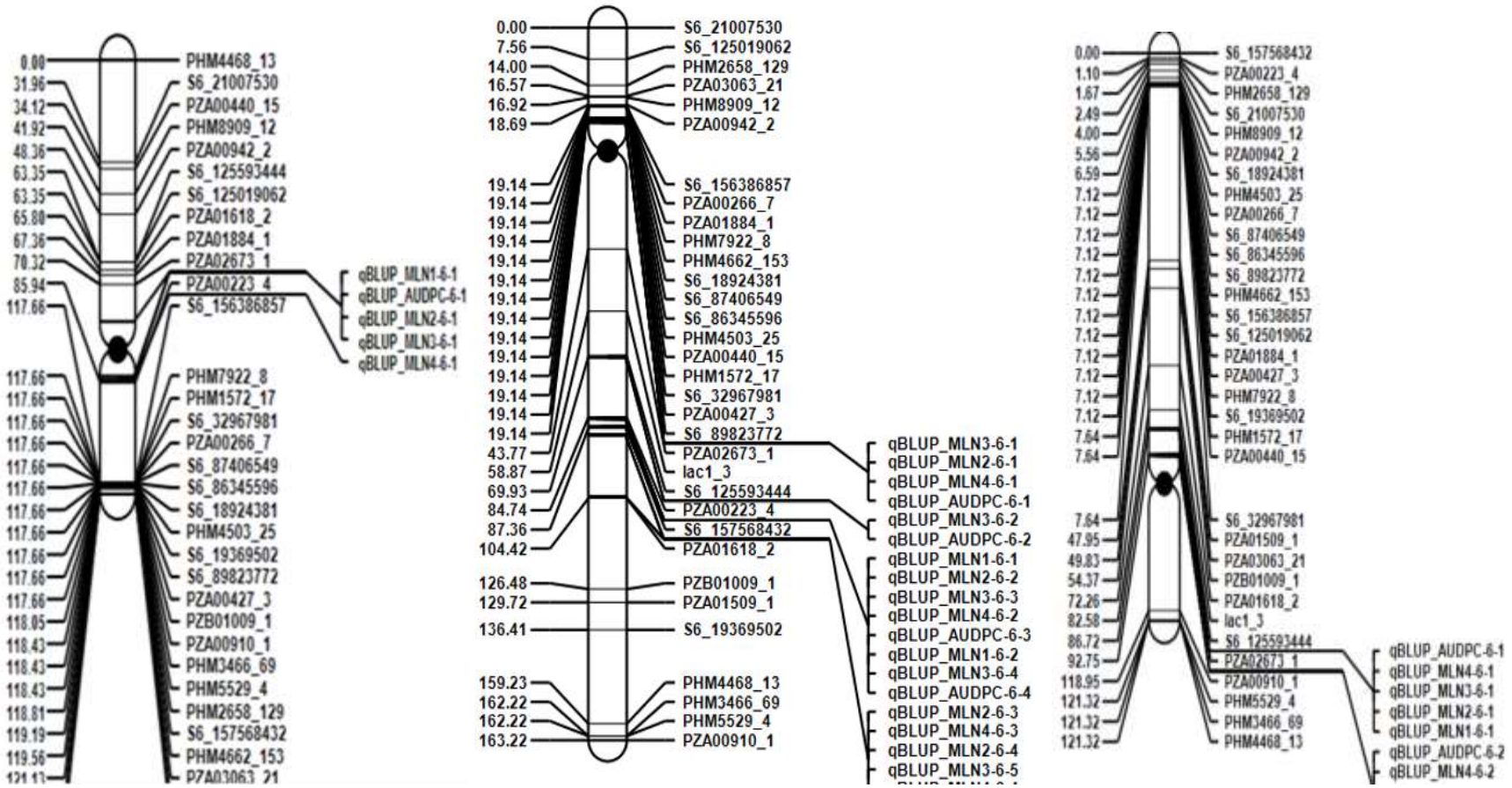
In MLNR-P20, the major QTL was flanked by SNP markers PZA02673_1 and PZA00223_4 in both early to late stages of disease development and S6_156386857 at late stages of disease development. In the same population, another moderately high effect QTL (qMLN2-7-1) was identified on chromosome 7, at 112 cM between markers PHM9162_135 and PZA00424_1. This QTL explained up to 14.4% of phenotypic variation during early disease development and AUDPC. Five small effect QTL were identified and distributed among chromosomes 1, 8, 9 and 10, explaining between 2.6 and 8.9% of the phenotypic variance. Overall, 13 SNP markers were associated with both major and minor effect QTL controlling MLN resistance in MLNR-P20 (Table 5.3).

In MLNR-P16, the major QTL on chromosome 6, consistent across MLN severity scores and AUDPC, was flanked by two of the following three markers: PZA00223_4, S6_157568432 and PZA01618_2. Another four minor effect QTL were identified on chromosomes 4, 5, 6, 9 and 10, explaining between 3.7 and 4.9% of the phenotypic variance and linked to 10 markers (Table 5.4).

Table 5.2 Variation and heritability for maize lethal necrosis resistance traits in three biparental mapping populations

Trait	MLN resistance parameters							
	Mean	Min	Max	Range	δ^2_g	δ^2_{gxs}	δ^2_e	H^2
MLNR-P20: ((CM543/CML444//CM543)DH5-B-B-B/KS23-6)F_3								
MLN1	3.41	2.81	4.07	1.26	0.30***	0.22***	0.39	0.59
MLN2	4.30	3.03	6.20	3.17	0.70***	0.21***	0.58	0.74
MLN3	4.67	3.28	6.33	3.05	0.72***	0.31***	0.78	0.67
MLN4	4.88	3.25	6.69	3.44	1.20***	0.30**	1.02	0.75
MLN_AUDPC	131.17	90.53	181.67	91.14	380.34***	137.72***	316.68	0.72
MLNR-P16: ((CM543/CML444//CM543)DH6-B-B-B/KS523-5)F_3								
MLN1	3.23	2.44	4.14	1.70	0.30***	0.22***	0.51	0.56
MLN2	4.18	2.88	5.79	2.91	0.56***	0.34***	0.85	0.59
MLN3	4.80	2.83	6.61	3.78	0.90***	0.24**	1.01	0.71
MLN4	5.13	3.05	7.46	4.41	1.32***	0.26*	1.14	0.76
MLN_AUDPC	131.60	80.98	178.99	98.01	329.01***	94.69**	321.00	0.72
MLNR-P13: (CM543/KS523-5)F_3								
MLN1	3.34	2.60	4.36	1.76	0.27*	0.3***	0.43	0.48
MLN2	4.32	3.25	6.28	3.03	0.63***	0.48***	0.54	0.62
MLN3	4.74	3.39	6.46	3.07	0.71***	0.53***	0.67	0.62
MLN4	5.08	3.37	6.79	3.42	1.07***	0.48***	0.92	0.69
MLN_AUDPC	132.54	95.04	187.05	92.01	617.11***	855.27***	581.47	0.52

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$, δ^2_g, genotype variance; δ^2_{gxs}, genotype x season variance; δ^2_e, residual variance; H^2, broad-sense heritability; MLN1, 2, 3 and 4, first, second, third and fourth MLN severity scores; MLN_AUDPC, area under MLN disease progress curve



(a) MLNR-P20 **(b) MLNR-P16** **(c) MLNR-P13**

Figure 5.1 Major-effect quantitative trait loci (QTL) linked to maize lethal necrosis resistance on chromosome 6 in three mapping populations.

Values one the left side of the chromosome represent marker position (cM) and to the right are single nucleotide polymorphism (SNP) markers. Identified QTL are indicated to the right of the SNP markers as qBLUP followed by the measured trait

Table 5.3 Quantitative trait loci linked to maize lethal necrosis resistance across disease progress stages in population MLNR-P20

Trait	QTL name†	Chr	Position (cM)	Flanking markers		LOD	PVE (%)	Add	Dom	QTL confidence interval	
				Left marker	Right marker					Left marker	Right marker
MLN1	qMLN1-6-1	6	82	PZA02673_1	PZA00223_4	15.76	40.45	0.20	0.12	78.5	85.5
MLN1	qMLN1-9-1	9	0	PHM4604_18	PZB00221_3	3.35	6.67	0.18	-0.05	0.0	0.50
MLN2	qMLN2-6-1	6	85	PZA02673_1	PZA00223_4	28.29	45.57	0.65	0.22	81.5	87.5
MLN2	qMLN2-8-1	8	68	PHM5805_19	PZA00498_5	3.47	3.79	-0.10	-0.25	64.5	69.5
MLN2	qMLN2-1-1	1	78	PZA03194_1	PZA01019_1	3.24	3.42	0.19	0.05	75.5	78.5
MLN2	qMLN2-7-1	7	112	PHM9162_135	PZA00424_1	3.13	14.39	-0.05	0.68	75.5	124.5
MLN3	qMLN3-6-1	6	85	PZA02673_1	PZA00223_4	29.08	32.00	0.62	0.25	81.5	87.5
MLN3	qMLN3-10-1	10	20	S10_97796845	PHM15331_16	3.99	8.94	0.03	0.77	15.5	27.5
MLN3	qMLN3-9-1	9	12	S9_37149685	PZA00708_3	3.83	8.80	-0.08	0.90	6.5	19.5
MLN3	qMLN3-1-1	1	82	PZA01019_1	PHM5622_21	3.75	2.99	0.21	-0.05	79.5	89.5
MLN3	qMLN3-7-1	7	11	PHM7898_10	PHM9162_135	3.64	9.17	0.21	0.66	6.5	11.5
MLN3	qMLN3-7-2	7	109	PHM9162_135	PZA00424_1	3.48	9.24	-0.06	0.66	94.5	127.5
MLN4	qMLN4-6-1	6	86	PZA00223_4	S6_156386857	31.03	47.59	0.72	0.38	82.5	87.5
MLN4	qMLN4-9-2	9	72	PZA00708_3	PZA00832_1	3.18	2.88	-0.08	-0.28	61.5	72.5
AUDPC	qAUDPC-6-1	6	84	PZA02673_1	PZA00223_4	29.48	35.27	19.08	7.10	81.5	87.5
AUDPC	qAUDPC-7-1	7	116	PHM9162_135	PZA00424_1	3.37	9.82	-1.41	18.58	75.5	137
AUDPC	qAUDPC-1-1	1	77	PZA03194_1	PZA01019_1	3.34	2.59	5.52	0.23	75.5	78.5
AUDPC	qAUDPC-8-1	8	68	PHM5805_19	PZA00498_5	3.28	2.66	-2.61	-7.25	64.5	69.5

QTL, quantitative trait loci; Chr, chromosome; Position, cM distance from the distal end of the chromosome; cM, centiMorgan; LOD, logarithm of odds; PVE, phenotypic variation explained; Add, additive effect of QTL; Dom, dominance effect of QTL; † QTL naming was done with letter "q" indicating QTL, followed by abbreviation of trait name, the chromosome and marker position, respectively; Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; AUDPC, area under disease progress curve for MLN

Table 5.4 Quantitative trait loci linked to maize lethal necrosis resistance across disease progress stages in population MLNR-P16

Trait	QTL name†	Chr	Position (cM)	Flanking markers		LOD	PVE (%)	Add	Dom	QTL confidence interval	
				Left marker	Right marker					Left marker	Right marker
MLN1	qMLN1-6-1	6	85	PZA00223_4	S6_157568432	17.41	23.95	0.40	0.16	84.5	85.5
MLN1	qMLN1-6-2	6	87	PZA00223_4	S6_157568432	18.07	27.53	-0.46	0.19	86.5	87.5
MLN2	qMLN2-6-2	6	85	PZA00223_4	S6_157568432	16.85	13.85	0.57	0.19	84.5	85.5
MLN2	qMLN2-6-3	6	89	S6_157568432	PZA01618_2	24.54	18.85	-0.69	-0.09	87.5	91.5
MLN2	qMLN2-6-4	6	104	S6_157568432	PZA01618_2	5.49	4.92	0.25	0.42	102.5	104.5
MLN3	qMLN3-6-2	6	70	S6_125593444	PZA00223_4	5.14	3.08	-0.30	-0.04	69.5	71.5
MLN3	qMLN3-6-3	6	85	PZA00223_4	S6_157568432	23.11	22.89	0.86	0.20	84.5	85.5
MLN3	qMLN3-6-4	6	87	PZA00223_4	S6_157568432	25.85	26.06	-0.97	0.26	86.5	87.5
MLN4	qMLN4-4-1	4	4	S4_17742333	S4_9850443	4.14	3.73	0.82	-0.58	3.5	4.5
MLN4	qMLN4-5-1	5	118	PHM563_9	S5_202816906	3.75	4.63	0.81	-1.50	112.5	121.5
MLN4	qMLN4-6-1	6	23	S6_89823772	PZA02673_1	4.27	5.54	0.79	-1.82	20.5	23.5
MLN4	qMLN4-6-2	6	85	PZA00223_4	S6_157568432	22.35	12.27	1.013	0.23	84.5	85.5
MLN4	qMLN4-6-3	6	89	S6_157568432	PZA01618_2	30.44	15.68	-1.19	-0.08	87.5	90.5
MLN4	qMLN4-6-4	6	104	S6_157568432	PZA01618_2	5.58	3.81	0.39	0.71	102.5	104.5
MLN4	qMLN4-9-1	9	93	PHM229_15	PZA01715_2	4.26	3.93	0.02	1.63	89.5	95.5
MLN4	qMLN4-10-1	10	10	PZA03713_1	PZA01001_2	3.70	4.69	-0.76	-1.67	9.5	10.5
AUDPC	qAUDPC-6-3	6	85	PZA00223_4	S6_157568432	22.02	21.44	22.99	6.43	84.5	85.5
AUDPC	qAUDPC-6-4	6	87	PZA00223_4	S6_157568432	24.81	25.07	-26.50	7.90	86.5	87.5

QTL, quantitative trait loci; Chr, chromosome; Position, cM distance from the distal end of the chromosome; cM, centiMorgan; LOD, logarithm of odds; Add, additive effect of QTL; Dom, dominance effect of QTL; PVE, phenotypic variation explained; † QTL naming was done with letter "q" indicating QTL, followed by abbreviation of trait name, the chromosome and marker position, respectively; Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; AUDPC, area under disease progress curve for MLN

Table 5.5 Quantitative trait loci linked to maize lethal necrosis resistance across disease progress stages in population MLNR-P13

Trait	QTL name†	Chr	Position (cM)	Flanking markers		LOD	PVE (%)	Add	Dom	QTL confidence interval	
				Left marker	Left marker					Left marker	Right marker
MLN1	qMLN1-6-1	6	87	S6_125593444	PZA02673_1	4.20	6.95	-0.21	0.10	86.5	87.5
MLN1	qMLN1-6-2	6	93	PZA02673_1	PZA00910_1	7.24	10.54	0.28	0.17	92.5	98.5
MLN2	qMLN2-4-1	4	6	PZA02457_1	S4_19430220	3.21	8.18	-0.24	1.27	5.5	6.5
MLN2	qMLN2-6-1	6	87	S6_125593444	PZA02673_1	3.78	4.26	-0.29	0.22	86.5	87.5
MLN2	qMLN2-6-2	6	93	PZA02673_1	PZA00910_1	6.53	6.40	0.42	0.27	91.5	96.5
MLN2	qMLN2-7-1	7	79	PZA02872_1	PZA00424_1	3.87	8.47	0.35	1.39	69.5	83.5
MLN3	qMLN3-6-1	6	87	S6_125593444	PZA02673_1	4.34	6.24	-0.35	0.25	86.5	87.5
MLN3	qMLN3-6-2	6	93	PZA02673_1	PZA00910_1	8.70	10.16	0.51	0.34	92.5	96.5
MLN3	qMLN3-7-1	7	75	PZA02872_1	PZA00424_1	3.98	12.13	0.39	1.38	63.5	81.5
MLN4	aMLN4-6-1	6	87	S6_125593444	PZA02673_1	3.17	10.27	-0.37	0.29	86.5	87.5
MLN4	qMLN4-6-2	6	93	PZA02673_1	PZA00910_1	11.78	29.65	0.77	0.33	92.5	96.5
AUDPC	qAUDPC-6-1	6	87	S6_125593444	PZA02673_1	4.51	9.72	-10.65	6.95	86.5	87.5
AUDPC	qAUDPC-6-2	6	93	PZA02673_1	PZA00910_1	8.41	15.22	15.20	9.40	92.5	96.5
AUDPC	qAUDPC-7-1	7	77	PZA02872_1	PZA00424_1	3.73	16.74	12.16	41.12	65.5	82.5

QTL, quantitative trait loci; Chr, chromosome; Position, cM distance from the distal end of the chromosome; cM, centiMorgan; LOD, logarithm of odds; Add, additive effect of QTL; Dom, Dominance effect of QTL; PVE, phenotypic variation explained; † QTL naming was done with letter "q" indicating QTL, followed by abbreviation of trait name, the chromosome and marker position, respectively Maize lethal necrosis (MLN) severity ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; AUDPC, area under disease progress curve for MLN

In MLNR-P13 the major QTL on chromosome 6 was flanked by two of the following three markers: S6_125593444, PZA02673_1 and PZA00910_1. Another average effect QTL was mapped to chromosome 7 between, 75 - 79 cM and explaining up to 16.7% of the phenotypic variance. This QTL, unlike in MLNR-P20 was significant in both early and advanced stages of disease development. Only one minor effect QTL was mapped to chromosome 4 in this population (Table 5.5). Overall, in addition to the one major QTL on chromosome 6 consistent across populations, 13 minor-effect MLN resistance QTL were identified on chromosomes 1 (77 - 82 cM), 4 (4 - 6 cM), 5 (118 cM), 6 (23 cM), 7 (11 cM), 7 (75 - 79 cM), 7 (109 - 116), 8 (68 cM), 9 (12 cM), 9 (72 cM), 9 (93 cM), 10 (10 cM) and 10 (20 cM) within the three mapping populations (Tables 5.3 - 5.5).

5.4 Discussion

Maize lethal necrosis and other major foliar diseases continue to threaten food security and cause impact losses in major maize growing areas in SSA (De Groote et al. 2015; 2016; Redinbaugh and Stewart 2018; Prasanna et al. 2020). Genetic improvement for resistance to MLN in eastern Africa commenced with identification of resistant sources among adapted germplasm (Beyene et al. 2017; Das et al. 2019) as well as introductions of exotic sources. The introduced resistance sources were selected from successfully identified sources of resistance to multiple viruses, including constituent MLN viruses (Kaeppler et al. 1998; Redinbaugh et al. 2004; 2018; Brewbaker 2009; Redinbaugh and Jones 2013; Jones et al. 2018; Redinbaugh and Stewart 2018). The introduced virus resistant inbred lines were studied for both adaptability and breeding potential and crossed to adapted tropical lines to form new breeding populations for QTL mapping and other genetic studies.

Efforts towards MAB for MLN were incorporated in on-going efforts to identify QTL and markers linked to tolerance and resistance to diverse prevailing stresses such as drought, nitrogen use efficiency and MSV in SSA (Semagn et al. 2015). Specifically, genetic analysis and QTL mapping studies for MLN resistance using tropical maize association panels and biparental populations in SSA revealed the quantitative nature of disease resistance as well as the diverse distribution of major and minor effect QTL in the tropical maize genome (Gowda et al. 2015; 2018; Nyaga et al. 2019; Awata et al. 2019; Sitonik et al. 2019). Based on findings of Jones et al. (2018) using temperate germplasm, new mapping populations were developed to study the stability of the major recessive QTL for the KS23 genetic background in tropical populations.

Two donor lines KS23-5 and KS23-6 were used to form several populations from crosses of these lines to elite CIMMYT and NARO lines.

In the current study linkage mapping and QTL analysis were used in three mapping populations developed from the KS23-5 and KS23-6 donor lines. A major QTL was confirmed on the long arm of chromosome 6 in all three populations. The existence of one major QTL on chromosome 6 consistent across mapping populations, average effect QTL on chromosome 7 (MLNR-P20 and MLNR-P13) and an array of minor effect QTL distributed on different chromosomes in the three populations and at different stages of disease development further confirms the quantitative nature of MLN resistance. Major QTL on chromosome 6 was consistent between 82 - 87 cM for MLNR-P20 and MLNR-P16 but larger (87 - 93 cM) in MLNR-P13. This suggests that recombination for this major QTL varies with genetic backgrounds. Another possible reason for this variation is that the first two populations were formed from crosses involving sister DH lines.

Different mechanisms of resistance controlled by different genes have been suggested, including restricting virus growth or moderating pathogen effects. These methods of resistance are known to vary among genetic backgrounds, and genes controlling resistance need to be further studied. For instance, MSV resistance was not related to MCMV resistance when different inbred lines were studied (Jones et al. 2018). It was therefore necessary to infect maize plants with both viruses in order to measure their synergistic effect causing MLN. It was however earlier observed that virus resistance genes in maize tend to cluster on specific genomic regions, especially on chromosomes 3 and 6 (Redinbaugh et al. 2004; 2018; Zambrano et al. 2014). Contrary to the clustering tendency, major and minor MLN resistance QTL in tropical maize were found to be more disperse in distribution. The major QTL from the KS523 background on the other hand is unique and more consistent across populations and disease development stages.

Overall, this study confirmed that MLN resistance is quantitative with few distinct major QTL that could be the major focus for MAB. In addition to identifying the position, the average effect of the QTL on traits, is vital (Bernardo 2020). This was indicated by either the positive or the negative sign of the dominance effect and the proportion of phenotypic variation explained. On the other hand, based on the sign of the additive effect, the source of the favourable alleles for MLN resistance was determined, with negative values pointing to the source of the favourable allele being the resistant donor line. In this current study, most favourable alleles appeared to be

mainly from the KS23 background. However, CML543 appeared to contribute to reduced disease severity and AUDPC. The elite line, CML543 was among the tolerant lines identified during the initial screening of tropical pools (Das et al. 2019). CML543 also showed a significant contribution to MLN QTL in the putative QTL mapping studies at CIMMYT (Gowda et al. 2018), making it a good candidate for introgression of MLN resistance into adapted tropical backgrounds using MAB.

5.5 Conclusions

This study confirmed the presence and transfer of major QTL for MLN resistance from the non-native KS23 background to the African maize background. The major QTL and linked markers on chromosomes 6 and the average effect QTL on chromosome 7 could be deployed in MAB. There is also a need to enrich breeding populations by combining both major and minor QTL to develop durable resistance to MLN in tropical maize populations. This durable resistance could be attained through MARS and genomic selection. Although the sister lines KS23-5 and KS23-6 were found to be resistant to MLN, they may not be used directly in hybrid products due to limited adaptability, and the recessive nature of the resistance QTL. However, markers linked to major QTL need to be added to those from earlier studies to constitute a customised set of markers and low-cost SNP chips for effective MLN introgression in tropical germplasm. In eastern Africa where DH technology is becoming increasingly important, selected markers linked to major effect QTL will be useful for F_2 population enrichment prior to DH induction, hence, increasing efficiency in breeding for MLN resistance.

5.6 References

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CHAPTER 6

Combining ability analysis for grain yield, agronomic traits and maize lethal necrosis resistance in tropical maize

Abstract

Maize lethal necrosis is a viral disease of maize that occurred recently in eastern Africa, causing significant yield losses. Cost-effective management of MLN requires rapid development and deployment of resistant hybrids. The present study aimed at assessing GCA and SCA effects of parents for grain yield, agronomic traits and resistance to MLN, and explore their use in hybrid development. Combining ability analysis using the line by tester design was conducted in maize inbred and DH lines by growing 104 three-way hybrids developed by crossing 13 lines with eight single cross testers. The 104 three-way hybrids along with commercial checks were evaluated under artificial MLN inoculation, optimum conditions and random abiotic and biotic stresses. Genetic variation among testcross hybrids was highly significant for grain yield, MLN resistance, agronomic traits and foliar diseases. High broad-sense heritability estimates (0.66 - 0.95) were observed for both agronomic and MLN resistance traits, whereas moderate values (0.22 - 0.45) were obtained for other foliar diseases under natural infestation. Under artificial MLN inoculation, both line and tester GCA variances were highly significant for grain yield, anthesis date (AD), MLN severity scores, MLN AUDPC and susceptibility index. However, non-significant SCA variance was observed for all traits except for anthesis-silking interval (ASI). Analysis across all environments revealed that GCA variance for both lines and testers were significant for grain yield and foliar diseases. Baker's ratio revealed that GCA was predominant for all traits, suggesting that additive gene action was more prevalent than non-additive gene effects. Two lines, CKDHL140910 and CKDHL120312, contributed favourable GCA effects to MLN resistance while CKSBL10194 and CKSBL10205 contributed unfavourably to MLN resistance. Tester 2 had an undesirable positive GCA effect for MLN resistance. Four identified resistant hybrids with high SCA for grain yield under stress and optimum conditions can be recommended for further testing for commercial release. Generally, adequate genetic variation for disease resistance and agronomic traits makes the selection of new MLN resistant parents and hybrids possible. Importantly, high GCA lines and available single cross testers can be used for future breeding to develop new MLN resistant commercial lines and hybrids.

Key words: Elite and DH lines, single cross testers, Line by tester analysis, gene action

6.1 Introduction

Ever since the emergence of MLN in eastern Africa in 2011 (Wangai et al. 2012), disease management measures have been recommended and implemented by cross-functional teams across eastern and southern Africa (Prasanna et al. 2020). Integrating stable, high-yielding, disease resistant varieties into cropping systems is considered an economical and environmentally friendly approach to manage MLN in SSA (Beyene et al. 2017; Prasanna et al. 2020). New MLN resistant varieties should combine desirable agronomic qualities with resistance to prevalent diseases such as TLB, GLS, MSV and ear rots (ER) that often lead to low on-farm yields and low grain quality for SSA farmers who grow susceptible varieties (Vivek et al. 2010; Kibe et al. 2020; Tembo et al. 2020). Other important constraints limiting potential yields routinely addressed through multiple stress-tolerant variety development in SSA include unpredictable drought, low soil nitrogen, heat stress, floods and salinity (Ertiro et al. 2017; Ligate et al. 2017; Makumbi et al. 2018; Prasanna et al. 2020).

Breeding efforts to address MLN were spearheaded by CIMMYT in 2012. The major pre-breeding activity was to identify resistant/tolerant lines from gene banks and elite collections that constitute parents of commercial hybrids in the region (Prasanna 2014; Redinbaugh and Stewart 2018; Das et al. 2019; Nyaga et al. 2020; Prasanna et al. 2020). Resistant lines identified through extensive screening were further used to develop a new generation of MLN resistant lines through pedigree selection, DH technology, marker-assisted recurrent selection (MARS) and temperate introgression using off-patent inbred lines, especially from the USA (Beyene et al. 2017). Newly developed MLN resistant inbred and DH lines constitute a desirable genetic base for the development of new disease resistant hybrids. The resistant hybrids once released, will form an integral part of sustainable disease management in affected farming systems in SSA. Some of the high performing parental lines could be used in forward breeding and rapid recycling to generate new versions of inbred lines (Bernardo 2010) that will further increase rates of genetic gains under MLN pressure and other economically important abiotic and abiotic stresses.

Developing high yielding MLN resistant hybrids requires identification of parental lines with both high *per se* mean performance and adequate heterosis when intercrossed. The major disadvantage of *per se* performance is that individual line performance is not adequately correlated with hybrid performance, especially in cross-pollinated crops such as maize, where hybrid vigour is desired. This limited relationship of the parent line performance with hybrid

performance is associated with the degree of dominance at contributing loci (Bernardo 2010). Whereas individual selection is effective in identifying lines with high means for target traits, it is not possible to predict line performance in hybrid combinations and their respective combining abilities, unless crosses are made and progenies evaluated (Bertan et al. 2014). Other modern techniques such as genomic prediction can be effectively used to select superior parental lines without evaluating their progenies (Crossa et al. 2017). The testcross method is still popular in selecting hybrid parents in many breeding programmes (Fasahat 2016; Beckett et al. 2019). This approach is used in hybrid maize breeding to estimate the combining ability and heterotic inclination of new inbred and DH lines and to maximise their use in new hybrid combinations (Aslam et al. 2017; Meena et al. 2017; Tolera et al. 2017; Fan et al. 2018).

Progress in identifying new yield testers for SSA breeding programmes has been made (Annor et al. 2020; Chisaka et al. 2020), however, the new and commonly used testers in the region's maize breeding programmes are susceptible to MLN, except for a few moderately resistant lines such as CML543 and CML494 (Prasanna 2014; Beyene et al. 2017; Das et al. 2019). These tester lines have weak combining ability for MLN resistance as was observed in a genetic study by Beyene et al. (2017). This current study was therefore conducted to identify new MLN resistant lines and single cross testers for use as hybrid parents or utilisation as MLN resistant testers in current and future breeding pipelines. The objectives of this study were (i) to estimate combining ability (GCA and SCA) among MLN resistant lines and single cross testers and (ii) to identify parent lines, testers and three-way cross hybrids that combine MLN resistance with desirable adaptive and agronomic traits.

6.2 Materials and methods

6.2.1 Genetic material and mating design

A total of 104 three-way testcross hybrids were generated by crossing 13 MLN tolerant lines with eight candidate MLN resistant single cross testers. The MLN tolerant lines used in this study were selected based on a previous study (Beyene et al. 2017) and their characteristics are provided in Table 6.1. Four of the tester parents were extracted using MARS or DH development from populations whose parentage included the MLN resistant elite line, CML494. In the line x tester mating design, single cross testers were used as female parents while the MLN tolerant lines were used as male parents.

Table 6.1 Lines and testers crossed and evaluated under MLN and non-MLN conditions

Line	Line code	Genetic background	Heterotic group
1	CKLTI0043	Temperate introgression of CML539	A
2	CKLTI0026		
3	CKSBL10194	Drought, insect and MLN tolerant	Unknown
4	CKSBL10205		
5	CKDHL120341	Forward breeding using CML202	B
6	CKDHL120358		
7	CKDHL141105	Forward breeding using CML312	A
8	CKDHL142425	Forward breeding using CML536	A
9	CKDHL140910		
10	CKDHL142806		
11	CKDHL140475	Forward breeding using CML538	A
12	CKDHL143607		
13	CKDHL120312	Forward breeding using CML78	A
	Testers		
1	CKLTI0227/CKLMARSI0022	Tropical marker-assisted recurrent selection (MARS) line x temperate introgression of CML494	B
2	CKLTI0227/CKLMARSI0029		
3	CKLTI0227/CKDHL120918		
4	CKLTI0137/CKLMARSI0022		
5	CKLMARSI0037/CKLTI0139		
6	CKDHL120918/CKLTI0138		
7	CKDHL120918/CKLMARSI0022	Tropical MARS line x forward breeding line	B
8	CKDHL120918/CKLMARSI0029		

6.2.2 Trial sites

Testcross hybrids were evaluated under artificial MLN inoculation for two seasons at the MLN screening facility located in Naivasha in Kenya (site details provided in section 3.2.3). The testcross hybrids were further evaluated at two TLB and GLS hotspots, Namulonge and Bulindi, in Uganda. Bulindi experienced optimum conditions while Namulonge, and two more sites, Serere and Tororo, were considered random abiotic stress sites as described by Makumbi et al. (2018). All experiments at Naivasha were conducted under supplementary irrigation, while other sites in Uganda were entirely rain fed. Tororo was selected because of its position in an MLN-endemic region that frequently experiences multiple constraints such as unreliable rainfall, low

soil fertility and common pests and diseases (Kagoda et al. 2016). The experimental sites are further described in Table 6.2.

Table 6.2 Description of trial sites

Site	Latitude	Longitude	Altitude (masl)	Testing season	Site attribute
Naivasha	00^0 41'S	36^0 23'E	1 884	2018 A & B	Artificial MLN screening
Bulindi	01^0 48'N	31^0 47'E	1 020	2017A	Optimum, TLB and GLS hotspot
Namulonge	00^0 32'N	32^0 37'E	1 150	2017B	Random abiotic stress, TLB and GLS hotspot
Serere	01^0 51'N	33^0 45'E	1 080	2017A	Random abiotic stress
Tororo	00^0 36'N	34^0 09'E	1 169	2017A	Random abiotic stress

MLN, maize lethal necrosis; TLB, Turcicum leaf blight; GLS, Gray leaf spot; testing season A (March - August), testing season B (September - January of following year); masl, metres above sea level

6.2.3 Field trials and data collection

Field experimental design and trial management

A total of 104 testcross hybrids were evaluated alongside six commercial checks, making a total of 110 experimental entries. An alpha lattice design (Williams 1976) with five plots per block x 22 blocks with two replications was used across the test sites. Each experimental plot consisted of two rows spaced 0.75 m apart and 0.25 m between plants, translating to a plant population density of 53 333 plants ha^{-1}. Standard agronomic, cultural and pest management practices were applied according to the recommendation for each site.

Screening for maize lethal necrosis resistance

Testcross hybrids were artificially screened for MLN resistance, following an optimised protocol detailed in section 3.2.3 (Gowda et al. 2015; MLN Information Portal 2020). The severity of MLN symptoms was recorded two weeks after the final inoculation, following a quantitative scale of 1-9 (1 = no visible symptoms, 9 = complete plant necrosis) (http://mln.cimmyt.org/mln-scoring/; section 3.2.3). The AUDPC and susceptibility scores for MLN were computed using four consecutive scores recorded every 14 days from the first score. The susceptibility score was determined following the mid-point procedure by Shaner and Finney (1977), adopted by Forbes et al. (2014). The rAUDPC and susceptibility scores were calculated to compensate for shortfalls associated with the sensitivity of AUDPC to variation in disease development. A common case could be an early-onset and slowly progressing infection or late-onset and fast-progress of symptoms. The rAUDPC was calculated as described in section 3.2.3.

An MLN susceptibility score was calculated classify genotypes into various classes of resistance as described in section 3.2.3.

Assessment of reaction to natural infestation with common foliar diseases

Symptoms of major diseases, TLB, GLS and MSV were scored twice during the crop growth period. The first disease scores were taken after pollen shed when there were observable differences between plots for disease symptom severity. A quantitative scale of 1 - 5 (1 = no disease symptoms, 5 = severe symptoms) (CIMMYT 1999; Setyawan et al. 2017) was used to record the plot severity for each of the three diseases. The second score was done 14 days from the date of the first score and the two scores were averaged for every plot.

Assessment of key agronomic traits

Key agronomic traits were recorded at each of the test sites. Days to anthesis (AD) were counted as the number of days from planting to when 50% of plants in a plot shed pollen, while ASI was calculated as the difference between days to silking (SD) and AD. Plant and ear heights were measured after completion of pollen shed in all plots. Plant height (PH) in cm was calculated as the average height of five randomly selected plants measured from the base of the plant to the first tassel branch. Ear height (EH) was taken as the average height on the same plant, measured from the base of the plant to the node bearing the uppermost ear. Ear position (EP) was calculated as the ratio of EH : PH. At harvest, grain yield (GY) was estimated from total plot weight adjusted to 12.5% moisture content and expressed in ton ha^{-1}. Ear aspect (EA) was scored using a scale of 1 - 5 for all ears in a plot, where 1 = very good, assigned to big uniform, appealing cobs and 5 = very poor, assigned to cobs with inferior characteristics such as small non-uniform cobs with gaps and/or uneven kernels. Grain texture (GT) was scored using a scale of 1 - 5, where 1 = entirely shiny-flint kernels and 5 = entirely dent kernel type. Ears per plant (EPP) was calculated as the ratio of the number of ears (NE) harvested to the number of plants (NP) at harvest (CIMMYT 1999).

6.2.4 Data analysis

Analysis of variance and estimation of combining ability and heritability were done using Analysis of Genetic Designs with R for Windows (AGD_R, Version 5.0) (Rodríguez al. 2018). The ReML method was used to estimate variances. Comparison of combining ability effects for lines and testers was conducted for yield and agronomic traits for different management

conditions. Combining ability for MLN resistance was computed based on individual disease severity, mean of severity scores, AUDPC and adjusted susceptibility scores.

Line x tester analysis (Kempthorne 1957) was conducted, where parents and testcross hybrids were considered fixed factors, whereas environments were considered random, according to the linear model below:

$$Y_{ijk} = \mu + l_k + mi + f_j + s_{ij} + ml_{ik} + fl_{jk} + sl_{ijk} + e_{ijk}$$

Where Y_{ijk} = observed value per experimental unit, μ = grand mean, l_k = effect of the k^{th} location, m_i = GCA effect of the i^{th} line, f_j = GCA effect of j^{th} tester, s_{ij} = SCA effect of the i^{th} line and the j^{th} tester, ml_{ik} = interaction effect of i^{th} line GCA by the k^{th} location, f_{jk} = interaction effect of j^{th} tester GCA by the k^{th} location, sl_{ijk} = interaction effect of the i^{th} line and the j^{th} tester SCA by the k^{th} location and e_{ijk} is the experimental error.

As proposed by Baker (1978), the relative importance of GCA and SCA was calculated as a ratio of respective variance components as $2\sigma^2_{GCA}/(2\sigma^2_{GCA} + \sigma^2_{SCA})$. Broad-sense ($H^2$) and narrow-sense ($h^2$) heritability was calculated as follows:

$$H^2 = \frac{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCA}}{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCA} + \sigma^2_{I\times SCA} + \sigma^2_{e}} \quad ; \quad h^2 = \frac{\sigma^2_{GCAf} + \sigma^2_{GCAm}}{\sigma^2_{GCAf} + \sigma^2_{GCAm} + \sigma^2_{SCA} + \sigma^2_{I\times SCA} + \sigma^2_{e}}$$

Where H^2 = Broad-sense heritability, $\sigma^2_{GCAm} = \sigma^2_{GCAf}$ = GCA variance for female parents (Testers) GCA variance due for male parents (Lines), σ^2_{SCA} = SCA variance, σ^2_{lxSCA} = SCA x location interaction variance, σ^2_e =error variance.

6.3 Results

6.3.1 Genetic variation, variance components and heritability estimates

Analysis of variance across environments showed that the main effect due to environment was highly significant ($P \leq 0.001$) among testcrosses for GY, AD and ASI under artificial MLN inoculation, random abiotic stress, optimum management and across all environments (Tables 6.3 and 6.4). A highly significant environmental effect was observed for key agronomic traits such as grain moisture content, EP, EPP, GT, HC and EA. There was also a highly significant environmental effect on major foliar diseases, TLB, GLS and MSV (Table 6.5). There was

significant genetic variation (P ≤ 0.001) among testcross hybrids for GY and other key agronomic traits under stress and non-stress conditions (Tables 6.3, 6.4 and 6.5). Weaker significant variation (P ≤ 0.05) was observed or TLB, GLS and MSV under natural infestation (Table 6.5). Overall, the interaction effect of lines with the environment across traits was stronger compared with testers. Under MLN artificial inoculation, there was highly significant variation among testcross hybrids for disease severity scores, AUDPC and the susceptibility score, except in the initial score (during early disease development). Broad sense heritability estimates were high (0.61 - 0.95) for all traits except early MLN score (MLN1), GLS, TLB and MSV with moderate values (0.22 to 0.46) (Tables 6.3, 6.4 and 6.5).

6.3.2 Estimation of combining ability

Line x tester analysis indicated that GCA effects of lines were highly significant (P ≤ 0.001) for all agronomic and disease response traits except for MSV under optimum and stress conditions (Tables 6.3, 6.4 and 6.5). Under MLN, GCA variances for both lines and testers were highly significant for GY (P ≤ 0.001), AD, average MLN score, MLN AUDPC and susceptibility score (P ≤ 0.01). Contrary to this, the SCA effect was only significant (P ≤ 0.01) for ASI under the same conditions. Line GCA interaction with the environment was highly significant (P ≤ 0.001) for GY and MLN disease parameters except MLN2, while tester GCA interaction with the environment was significant (P ≤ 0.05) for GY, ASI and the final MLN score. Under MLN, increased heritability for MLN resistance was attained at later stages of disease development and was more pronounced when AUDPC and susceptibility scores were computed (Table 6.3). Analysis of combining ability across all test environments showed that GCA effects due to both lines and testers were significant for EH, HC, GT and response to foliar diseases TLB, GLS and MSV. However, the tester GCA effect was non-significant for grain moisture (MOI), EPP and EA (Table 6.5).

For all traits measured under MLN pressure, MLN-free abiotic stress and optimum conditions, GCA:SCA ratios (Baker's ratios) were low for ASI and MSV, but relatively higher and closer to unity for all other traits. These results indicated that GCA is more prominent than SCA in the expression of these target traits, except for ASI and resistance to MSV where both GCA and SCA contributed to genetic variance among hybrids.

Table 6.3 Line x tester mean squares, variance components and trait heritability under artificial MLN conditions

		Mean squares									
Source	**Df**	**GY**	**AD**	**ASI**	**MLN1**	**MLN2**	**MLN3**	**MLN4**	**MLN_AV**	**MLN_AUDPC**	**MLN_SC**
Environment (E)	1	363.44***	33776.60***	6.42***	166.81***	293.57***	396.24***	199.78***	230.50***	454811.46***	290.55***
Testcrosses	103	3.41***	20.29***	0.58**	0.24*	0.411**	0.71***	1.08***	0.39***	706.08***	0.42***
GCA-Line	12	17.97***	118.79***	1.11***	0.65***	1.83***	3.55***	6.64***	2.32***	4106.41***	2.46***
GCA-Tester	7	4.32***	18.02**	0.38	0.30	0.38	0.66*	0.79*	0.37**	659.86**	0.39**
SCA-Line x Tester	84	1.25	6.43	0.52**	0.17	0.21	0.31	0.31	0.11	224.16	0.13
E x Testcross	71	1.30	6.68	0.55**	0.23	0.25	0.36	0.51*	0.16	289.30	0.17
E x Lines	12	3.68***	10.00	0.34	0.61***	0.38	1.02***	1.48***	0.48***	893.49***	0.53***
E x Tester	7	2.90*	3.41	1.15**	0.26	0.14	0.44	0.90*	0.10	181.93	0.11
E x Line x Tester	52	0.53	6.48	0.52*	0.17	0.24	0.26	0.35	0.11	211.93	0.13
Residuals	62	1.03	6.10	0.28	0.17	0.24	0.26	0.34	0.12	229.81	0.13
		Variance components									
δ^2_G		0.58	3.86	0.05	0.00	0.04	0.09	0.15	0.06	107.82	0.06
δ^2_m		0.44	4.24	0.04	0.00	0.05	0.08	0.17	0.06	102.02	0.06
δ^2_f		0.01	0.25	0.00	0.00	0.00	0.00	0.00	0.00	8.15	0.00
δ^2_{mf}		0.10	0.07	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00
δ^2_A		2.34	5.42	0.18	0.01	0.17	0.36	0.59	0.24	431.29	0.26
δ^2_D		0.42	0.26	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.00
δ^2_E		0.40	1.83	0.14	0.05	0.06	0.09	0.12	0.04	69.74	0.04
H^2		0.87	0.90	0.69	0.22	0.74	0.82	0.83	0.86	0.86	0.86
h^2		0.47	0.70	0.17	0.04	0.45	0.45	0.58	0.63	0.61	0.61
GCA:SCA		0.81	0.99	0.52	1.00	1.00	0.88	1.00	1.00	1.00	1.00

Df, degrees of freedom; GY, grain yield (t ha^{-1}); AD, days to anthesis; ASI, anthesis-silking interval; Maize lethal necrosis (MLN) ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN symptoms; MLN_SC, calculated MLN susceptibility score; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05; Variance components: δ^2_G, total genotypic variance; δ^2_m, line GCA variance δ^2_f, tester GCA variance; δ^2_{mf}, SCA (male x female) variance; δ^2_A additive variance; δ^2_D dominance variance; δ^2_E environmental variance; H^2, broad-sense heritability; h^2, narrow-sense heritability; GCA, general combining ability; SCA, specific combining ability

Table 6.4 Line x tester mean squares, variance components and heritability for grain yield and maturity under combined stress and non-stress environments

	Across MLN-free optimum and random abiotic stress conditions								Across all environments			
	Random abiotic stress				Random abiotic stress and optimum conditions				Optimum, random abiotic stress and MLN conditions			
Source	**Df**	**GY**	**AD**	**ASI**	**Df**	**GY**	**AD**	**ASI**	**Df**	**GY**	**AD**	**ASI**
Environment (E)	2	27.11***	160.25***	36.75***	3	111.98***	97.14***	25.68***	5	134.86***	13362.13***	32.91***
Testcrosses	103	0.74**	15.19***	3.29***	103	1.22***	13.55***	2.48***	103	2.61***	24.97***	2.29***
GCA-Line	12	2.94***	104.28***	12.66***	12	4.22***	93.44***	9.33***	12	12.65***	181.51***	8.41***
GCA-Tester	7	1.09*	13.70***	3.00	7	2.06**	11.35***	2.31	7	4.01***	11.87**	2.26
SCA-Line x Tester	84	0.40	2.60	1.98	84	0.73	2.33	1.52	84	1.06*	3.71	1.43
E x Testcross	206	0.51	2.79	1.98	309	0.74	3.12	1.80*	483	1.05***	4.77*	1.40*
E x Lines	24	0.85**	5.08*	3.738***	36	1.31***	8.53***	3.84***	60	3.19***	13.21***	2.81***
E x Tester	14	0.50	1.77	1.76	21	0.48	2.91	1.52	35	1.16*	4.45	1.23
E x Line x Tester	168	0.47	2.55	1.74	252	0.69	2.37	1.53	388	0.71	3.59	1.20
Residuals	180	0.44	3.19	1.69	240	0.62	3.06	1.40	302	0.73	3.90	1.16
						Variance components						
δ^2_G		0.05	2.37	0.24		0.07	1.50	0.09		0.15	1.99	0.08
δ^2_m		0.05	2.38	0.19		0.05	1.54	0.09		0.12	2.17	0.06
δ^2_f		0.01	0.16	0.01		0.01	0.10	0.01		0.02	0.06	0.01
δ^2_{mf}		0.00	0.00	0.04		0.01	0.00	0.00		0.03	0.00	0.02
δ^2_A		0.18	9.49	0.94		0.28	6.00	0.37		0.62	7.97	0.33
δ^2_D		0.00	0.00	0.16		0.03	0.00	0.00		0.14	0.00	0.06
δ^2_E		0.09	0.47	0.33		0.10	0.36	0.23		0.09	0.40	0.12
H^2		0.67	0.95	0.77		0.76	0.94	0.61		0.89	0.95	0.76
h^2		0.41	0.84	0.35		0.39	0.82	0.30		0.51	0.85	0.32
GCA:SCA		1.00	1.00	0.84		0.91	1.00	1.00		0.79	1.00	0.81

Df, degrees of freedom; GY, grain yield (t ha^{-1}); AD, days to anthesis; ASI, anthesis-silking interval; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05; Variance components: δ^2_G, total genotypic variance; δ^2_m, line GCA variance δ^2_f, tester GCA variance; δ^2_{mf}, SCA (male x female) variance; δ^2_A additive variance; δ^2_D dominance variance; δ^2_E environmental variance; H^2, broad-sense heritability; h^2, narrow-sense heritability; GCA, general combining ability; SCA, specific combining ability

Table 6.5 Line x tester mean squares, variance components and heritability for important agronomic traits and common foliar diseases across test environments

		Mean squares									
Source	**Df**	**MOI**	**EH**	**EP**	**EPP**	**HC**	**GT**	**EA**	**TLB**	**GLS**	**MSV**
Environment (E)	5	915.74**	19051.03***	0.559***	7.22***	744.30***	26.25***	21.99***	20.24***	15.92***	53.30***
Testcrosses	103	5.79***	246.21***	0.003***	0.07***	3.72***	1.19***	0.37***	0.371*	0.26**	0.378*
GCA-Line	12	16.24***	1118.56***	0.015***	0.24***	19.90***	3.57***	1.71***	0.88***	0.60***	0.60*
GCA-Tester	7	6.89	679.08***	0.006***	0.03	7.05***	7.57***	0.26	1.077***	0.42*	0.66*
SCA-Line x Tester	84	4.20	85.39*	0.002*	0.05	1.14	0.32	0.19	0.24	0.19	0.32
E x Testcross	483	4.26*	84.14***	0.002*	0.06**	1.88***	0.49***	0.31***	0.34*	0.32***	0.39**
E x Lines	60	5.43**	183.93***	0.003***	0.17***	6.83***	2.06***	0.96***	1.23***	1.28***	0.67***
E x Tester	35	4.97	147.03***	0.003***	0.05	3.87***	0.97**	0.27	0.53**	0.43**	0.38
E x Line x Tester	388	4.02	64.59	0.00	0.04	1.01	0.23	0.22	0.19	0.17	0.35*
Residuals	302	3.50	58.13	0.00	0.04	1.06	0.24	0.22	0.26	0.16	0.27
		Variance components									
δ^2_G		0.15	18.47	0.00	0.00	0.24	0.12	0.01	0.00	4.39	4.87
δ^2_m		0.12	12.68	0.00	0.00	0.21	0.03	0.01	0.00	0.01	0.00
δ^2_f		0.01	4.66	0.00	0.00	0.03	0.09	0.00	0.01	0.00	0.00
δ^2_{mf}		0.02	2.37	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.01
δ^2_A		0.61	73.87	0.00	0.00	0.97	0.50	0.03	0.02	0.01	0.01
δ^2_D		0.10	9.48	0.00	0.00	0.02	0.05	0.00	0.00	0.02	0.03
δ^2_E		0.36	7.53	0.00	0.00	0.16	0.04	0.03	0.03	0.04	0.04
H^2		0.66	0.92	0.87	0.55	0.86	0.93	0.49	0.39	0.45	0.46
h^2		0.25	0.64	0.49	0.17	0.59	0.68	0.24	0.19	0.15	0.14
GCA:SCA		0.84	0.88	0.82	0.79	0.98	0.91	1.00	0.96	0.68	0.54

Df, degrees of freedom; MOI, grain moisture; EH, ear height; EP, ear position; EPP, ears per plant; HC, husk cover; GT, grain texture; EA, ear aspect; TLB, Turcicum leaf blight; GLS, gray leaf spot; MSV, maize streak virus; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05; Variance components: δ^2_G, total genotypic variance; δ^2_m, line GCA variance δ^2_f, tester GCA variance; δ^2_{mf}, SCA (male x female) variance; δ^2_A additive variance; δ^2_D dominance variance; δ^2_E environmental variance; H^2, broad-sense heritability; h^2, narrow-sense heritability; GCA, general combining ability; SCA, specific combining ability

6.3.4 General and specific combining ability effects under different management conditions

Estimation of GCA effects of lines under MLN artificial inoculation identified two lines, CKDHL140910 (line 9) and CKDHL120312 (line 13), with significant positive GCA effects while line 11 (CKDHL140475) had significant negative GCA effect ($P \leq 0.05$) for GY. For MLN resistance, line 9 had significant negative GCA effects ($P \leq 0.05$) while line 13 had highly significant negative ($P \leq 0.001$) GCA as shown by the effect for MLN severity and AUDPC. Line 13 (CKDHL120312) showed highly significant GCA effects for reduced AD while line 11 (CKDHL140475) and 12 (CKDHL143607) had significant CGA effects for earliness but also for reduced GY (Table 6.6).

With non-significant negative GCA effects for grain yield, the two insect-resistant lines, 3 (CKSBL10194) and 4 (CKSBL10205) showed significant positive GCA effects for MLN resistance at early disease development (MLN2). These two lines therefore showed combinability for MLN susceptibility due to the significant positive GCA effects at early disease development (Table 6.6). Lines 9 (CKDHL140910) and 13 (CKDHL120312) were consistently high general combiners for yield under optimum and random abiotic stress conditions. In addition to MN resistance line 9 (CKDHL140910) combined well for TLB and GLS resistance. These two lines showed desirable GCA effects for reduced ASI (Line 9) and earliness (Line 13) as shown in Table 6.7. GCA effects for testers were not significant for most traits except grain texture, whereby tester 4 (CKLTI0137/CKLMARSI0022) and 5 (CKLMARSI0037/CKLTI0139) generally combined well for dent grain texture while tester 7 (CKDHL120918/CKLMARSI0022) and 8 (CKDHL120918/CKLMARSI0029) combined well for flint grain texture. Only tester 2 (CKLTI0227/CKLMARSI0029) showed significant GCA effects for increased overall susceptibility to MLN and AUDPC (Table 6.8). Lines and testers showed variable GCA effects for individual resistance to GLS and TLB under natural disease pressure. However, lines 4, 6, 7, 9 and 10 and, testers 1, 7 and 8 combined well for resistance to both diseases, indicated by their negative GCA effects (Figure 6.1).

Table 6.6 General combining ability effects of lines under MLN conditions in 2017A (March – August) and 2017B (September – January 2018)

Line[+]	GY	AD	ASI	MLN1	MLN2	MLN3	MLN4	MLN_AV	MLN_AUDPC	MLN_SC
1	0.11	1.39	0.13	0.05	0.05	0.12	0.11	0.08	3.56	0.08
2	-0.07	1.13	0.09	-0.13	-0.07	0.19	0.24	0.07	3.03	0.08
3	-0.45	0.29	-0.19	0.16	0.23*	0.24	0.32	0.25	10.14	0.24
4	-0.44	0.89	0.09	0.18	0.23*	0.06	0.01	0.10	5.09	0.12
5	-0.33	0.98	-0.15	0.21	0.16	0.15	0.11	0.15	6.36	0.16
6	-0.31	0.04	0.09	0.02	0.13	0.00	0.13	0.07	2.93	0.07
7	0.65	1.43	0.30*	-0.05	0.04	0.19	0.19	0.10	4.49	0.11
8	-0.15	0.25	-0.11	-0.01	0.06	0.03	0.32	0.10	3.45	0.08
9	0.94*	0.36	-0.03	-0.11	-0.18	-0.23	-0.64*	-0.29*	-11.23*	-0.27*
10	0.33	1.86*	0.09	-0.01	-0.08	-0.19	-0.31	-0.15	-6.12	-0.15
11	-0.96*	-1.55*	-0.04	0.11	0.00	0.17	0.39	0.17	6.00	0.15
12	-0.09	-1.55*	-0.09	-0.13	-0.10	-0.18	-0.15	-0.14	-5.86	-0.14
13	0.78*	-5.52***	-0.18	-0.29	-0.47***	-0.56***	-0.73***	-0.51***	-21.86**	-0.53***

GY, grain yield (t ha^{-1}); AD, days to anthesis; ASI, anthesis-silking interval; Maize lethal necrosis (MLN) ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN symptoms; MLN_SC, calculated MLN susceptibility score; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05; [+]Line, refer to Table 6.1 for line names.

Table 6.7 General combing ability effects of lines under stress and non-stress conditions

Line	Across random abiotic stress environments			Across non-MLN, random abiotic stress and optimum environments			Across all environments (MLN, random stress and optimum)		
	GY	AD	ASI	GY	AD	ASI	GY	AD	ASI
1	0.11	0.88*	0.35	0.16	0.87	0.30	0.12	0.96*	0.16
2	0.21	-0.13	0.34	0.28	-0.07	0.27	0.14	0.37	0.15
3	0.23	-1.16*	0.07	0.05	-0.93	0.07	-0.12	-0.47	-0.02
4	-0.24	0.02	0.03	-0.12	0.05	-0.04	-0.20	0.41	0.00
5	-0.33	0.72	0.41	-0.29	0.42	0.53	-0.26	0.61	0.18
6	0.03	-0.36	0.20	0.07	-0.23	0.28	-0.08	-0.10	0.16
7	-0.39	1.78***	0.55*	-0.17	1.50	0.61	0.12	1.38***	0.35*
8	-0.19	0.70	-0.35	-0.36	0.50	-0.40	-0.22	0.36	-0.20
9	0.54*	0.58	-0.67*	0.61*	0.55	-0.80*	0.64***	0.41	-0.34*
10	-0.15	2.74***	-0.26	-0.11	2.44*	-0.29	0.05	2.18***	-0.09
11	-0.20	-1.85***	-0.11	-0.24	-1.61	-0.05	-0.43*	-1.52***	-0.06
12	-0.03	-1.03*	-0.24	-0.24	-0.96	-0.12	-0.14	-1.17*	-0.10
13	0.26	-2.89***	-0.32	0.22	-2.50*	-0.33	0.39*	-3.43***	-0.20

MLN, maize lethal necrosis; GY, grain yield (tha^{-1}); AD, days to anthesis; ASI, anthesis-silking interval; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05

Table 6.8 General combining ability effects for testers across all environments

Tester	GY	GY_RANK	AD	ASI	MLN1	MLN2	MLN3	MLN4	MLN_AV	MLN_AUDPC	MLN_SC	TLB	MSV	GT
1	0.09	3	-0.04	0.03	-0.10	-0.10	-0.06	-0.06	-0.05	-2.20	-0.06	-0.08	0.07	0.23
2	0.09	2	-0.17	-0.03	0.15	0.14	0.23	0.20	0.11*	4.69*	0.11*	-0.15	0.02	0.18
3	-0.08	6	0.13	-0.04	-0.03	-0.10	-0.06	-0.12	-0.05	-2.12	-0.05	0.10	0.06	-0.22
4	0.07	4	0.33	0.03	0.00	0.01	-0.04	0.11	0.01	0.24	0.01	0.01	0.05	0.33*
5	0.09	1	-0.05	0.05	0.04	0.10	0.01	0.01	0.03	1.36	0.04	-0.07	-0.04	0.26*
6	-0.11	8	0.21	-0.07	0.05	-0.04	-0.02	-0.07	-0.01	-0.53	-0.02	0.17*	0.07	-0.17
7	-0.09	7	-0.17	0.06	-0.04	0.04	-0.14	-0.16	-0.05	-1.82	-0.05	0.01	-0.14*	-0.33*
8	-0.06	5	-0.24	-0.04	-0.06	-0.04	0.09	0.10	0.01	0.38	0.01	0.01	-0.09	-0.29*

GY, grain yield (t ha^{-1}); AD, days to anthesis; ASI, anthesis-silking interval; Maize lethal necrosis (MLN) ratings 1, 2, 3 and 4, subsequent MLN severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN symptoms; MLN_SC, calculated MLN susceptibility score; TLB, Turcicum leaf blight; MSV, Maize streak virus; GT grain texture; * $P \leq 0.05$

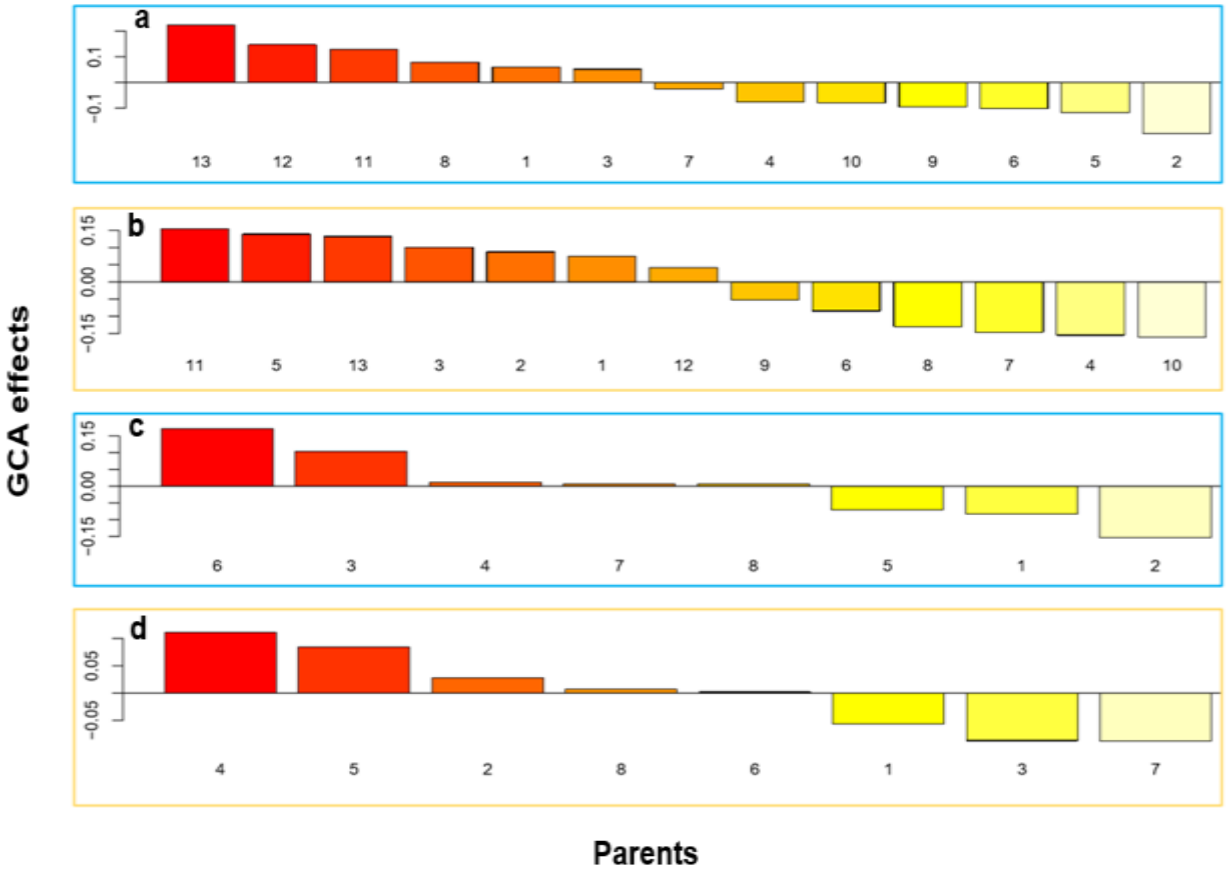

Figure 6.1 General combining ability (GCA) effects for lines and testers.

(a) Line GCA effects for Turcicum leaf blight (TLB) resistance; (b) Line GCA effects for gray leaf spot (GLS) resistance; (c) Tester GCA effect for TLB resistance; (d) Tester GCA effect for GLS resistance

6.3.5 Specific combining ability effects under MLN and non-MLN stress conditions

In this study, selected testcross hybrids with significant SCA effects for yield and resistance to MLN were identified, albeit with limited variance due to SCA for most traits. Four testcross hybrids had significant positive SCA effects for GY, 6x2 ($P \leq 0.001$), and 9x3, 12x4 and 13x1 ($P \leq 0.05$). Seven testcross hybrids, 2x7, 5x7, 6x3, 7x4, 8x2, 12x5 and 13x5, had significant SCA for resistance to MLN but non-significant SCA for grain yield while cross 9x3 had significant positive SCA effect for GY and consistent negative SCA for MLN resistance traits. For disease resistance, testcrosses 11x5 and 13x7 had highly significant ($P \leq 0.001$) and significant ($P \leq 0.05$) SCA effects for GLS, respectively (Table 6.9).

Table 6.9 Specific combining ability effects of selected testcrosses analysed across all environments

Testcross[+]	GY	MLN1	MLN2	MLN3	MLN4	MLN_AV	MLN_AUDPC	MLN_SC	GLS
2x7	0.09	-0.28	-0.71***	-0.55*	-0.34	-0.48***	-22.19***	-0.52***	0.32
4x7	-0.06	0.16	0.18	0.64*	0.24	0.29	14.42*	0.37	0.06
5x1	0.17	-0.04	0.42*	0.44	0.23	0.27	13.77*	0.34*	0.09
5x7	-0.25	-0.37	-0.23	-0.49	-0.17	-0.29	-13.56*	-0.33*	0.05
6x2	0.77***	-0.11	-0.03	0.12	0.21	0.06	2.12	0.05	-0.04
6x3	-0.36	0.04	-0.07	-0.34	-0.71***	-0.26	-10.44	-0.26	-0.08
6x4	-0.19	0.54***	0.07	0.14	0.52*	0.29	9.78	0.25	0.13
6x6	-0.66*	-0.27	0.36	0.37	0.23	0.17	9.65	0.24	-0.23
7x4	-0.09	-0.39*	-0.30	-0.14	-0.28	-0.27	-10.77	-0.25	-0.06
7x7	0.10	0.38*	0.16	0.20	0.20	0.22	8.95	0.22	0.16
8x2	-0.17	0.41*	0.54*	-0.16	-0.53*	0.06	4.61	0.12	-0.13
8x3	-0.27	-0.40*	0.07	0.12	0.28	0.00	1.93	0.03	0.31
9x3	0.53*	-0.06	-0.13	-0.28	-0.04	-0.13	-6.48	-0.16	-0.06
11x4	-0.29	-0.05	0.00	-0.61*	-0.26	-0.25	-10.42	-0.27	0.05
11x5	0.01	0.16	-0.08	0.58*	0.58*	0.30	12.12	0.31	-0.49***
12x4	0.59*	-0.06	0.14	0.14	-0.13	0.01	2.15	0.05	-0.29
12x5	-0.50	0.15	0.04	-0.17	-0.75**	-0.19	-5.93	-0.16	0.25
13x1	0.63*	-0.30	-0.01	0.22	0.10	-0.03	1.43	0.02	0.07
13x4	0.47	0.59***	0.35	0.20	-0.29	0.22	9.90	0.22	0.04
13x5	-0.26	-0.43*	-0.43*	-0.36	-0.47	-0.40	-17.02*	-0.43*	0.08
13x6	-0.54	0.02	-0.35	-0.57*	0.09	-0.21	-12.10	-0.30	0.20
13x7	-0.58	0.14	0.34	0.30	-0.08	0.18	8.96	0.22	-0.31*

GY, grain yield (tha^{-1}); MLN ratings 1, 2, 3 and 4, subsequent maize lethal necrosis (MLN) severity scores at 14, 28, 42 and 54 days after inoculation; MLN_AV, mean of MLN scores 1-4; MLN_AUDPC, area under disease progress curve for MLN symptoms; MLN_SC, calculated MLN susceptibility score; *** P ≤ 0.001, ** P ≤ 0.01, * P ≤ 0.05, [+]Testcross, refer to Table 6.1 for line and tester names

6.4 Discussion

Results of this study showed significant genetic variation among testcrosses for measured MLN resistance and agronomic traits, hence providing an opportunity for selecting testcross hybrids with combined disease resistance and desirable agronomic traits. In general, the GCA effects of lines were consistently significant for most traits, compared to GCA effects of testers. Compared to GCA, the magnitude of SCA variance was low for most traits. This is further supported by the high Baker's ratio (Baker 1978), which confirmed that GCA was predominant to SCA for MLN resistance and key agronomic traits.

The significant predominance of GCA over SCA implies that higher predictability of hybrid performance can be achieved from the estimated GCA effects of the parents and that the best performing hybrids may be obtained from crossing parents with the highest GCA effects. Beyene et al. (2017) obtained similar results from lines with the desirable combining ability that were selected as candidate testers in their study. Two MLN resistant lines (CKDHL140910 and CKDHL120312) with high GCA for yield were identified and could be used for making new hybrids and new pedigree populations with combined MLN, GLS and TLB resistance. Four testcross hybrids (6x2, 9x3, 12x4 and 13x1) with high SCA for yield could be further tested and released as varieties suitable for non-MLN areas while seven testcross hybrids (2x7, 5x7, 6x3, 8x2, 11x4, 12x5 and 13x5) with significant SCA for MLN resistance could be further tested and released in MLN-infested regions. Other disease resistant hybrids 11x5 and 13x7 with significant GLS resistance also need further testing in disease hotspots. The 13 hybrids above, identified with varying combining ability effects for disease resistance and agronomic traits, could form a base for improvement and formation of three-way hybrids. These hybrids could be improved for MLN-resistance using new resistant lines with favourable GCA for yield. Notably, Beckett et al. (2019) suggested that, in addition to test analysis for a wide range of newly developed inbred lines, genome-wide studies could be used to identify superior hybrid parents based on high mean performance and desirable genetic variance for target traits.

A good tester should effectively separate variances of lines under evaluation (Bernardo 2010). The candidate testers evaluated in this study were able to significantly contribute to high testcross variance and subsequently unveil the genetic variance for MLN resistance and key traits among the test lines. Among the eight single cross testers in this study, tester 2 (CKLTI0227/CKLMARSI0029) showed significant GCA effects for susceptibility to MLN, making it unsuitable for use in MLN resistance breeding. Based on GCA effects, there was similar yield

potential among candidate testers for grain yield. However, testers 7 and 8 could be used for selecting lines with flint grain types. The ideal tester should combine high testcross variance with a high mean performance for key agronomic traits, and resistance to MLN. It is suggested that when there is no dominance (d = 0), the tester has no effect on testcross variance, which results in no heterosis (Bernardo 2010). This phenomenon suggests that any future lines for the development of MLN resistant hybrids should first be fixed for MLN resistance alleles before crossing with elite lines or testers of opposite heterotic groups that have complementary alleles for MLN resistance. Identification of new disease resistant testers is possible by combing the HSGCA method and SNP marker-based methods (Annor et al. 2020; Chisaka et al. 2020). By combining heterotic informations and genetic diversity analysis, the use of a new tester will be more efficent in effectively seprating new disease resistant lines into heterotoc groups, resulting in higher combining ability for for both GY and disease reistance in new hybrid combinations.

Despite the predominance of additive gene action in testcross performance, there was some dominance variance for GY under MLN conditions, and across optimum and random abiotic stress environments. Unlike for GY, there was no dominance variance for most MLN parameters measured. These results further support the predominance of GCA over SCA for MLN resistance and agronomic performance. As further explained by Baker (1978), GCA is a result of additive gene action with some level of epistasis, while SCA variance is predominantly due to both dominance and epistasis (non-additive gene action). Besides direct production of high performing hybrids by crossing parents with high GCA for yield and disease resistance (such as lines 9 and 13), the predominance of additive genetic effects suggests that high genetic gains could be achieved by recombining the best lines followed by recurrent selection in populations derived from these lines.

6.5 Conclusions

This study identified two lines, CKDHL140910 and CKDHL120312 that consistently combined yield under stress and non-stress conditions, with MLN resistance. These lines will be useful for the formation of future superior populations. It is also suggested from this study that there is a need to create new hybrid combinations using inbred and DH lines with high GCA for yield and MLN resistance. Promising MLN resistant lines derived from genetically diverse populations at national programmes and CIMMYT should be included in new hybrid combinations. The similar performance of testers suggests that any of the candidate testers, except for tester 2, could be used in breeding MLN resistant hybrids. Perhaps another approach would be to further

testcross new MLN resistant segregating lines to more heterotically divergent lines to maximise tester variance and to identify the most desirable testers. This could further be aided by QTL mapping, genetic diversity and genomic prediction studies.

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CHAPTER 7

Performance and stability of maize lethal necrosis resistant hybrids under contrasting environments in East Africa

Abstract

Cost-effective management of MLN and prevailing biotic and abiotic constraints requires the development and deployment of stable, productive varieties with farmer-preferred traits. In this study, 104 candidate MLN resistant hybrids and 6 commercial checks were evaluated with two main objectives: (i) to determine the magnitude of GEI effects on three-way cross hybrids under both MLN pressure and MLN-free conditions and (ii) to identify productive and stable MLN resistant hybrids suitable for advancement in the variety release pipeline. Analysis of variance revealed significant genetic variation among hybrids for GY and AD under artificial MLN screening. Genotypic variation was highly significant for MLN severity and AUDPC. Significant variation was also observed for yield and other agronomic traits across MLN-free, random stress environments, except for ASI. Analysis across all testing environments revealed highly significant variation for GY and AD. Analysis of GEI using the AMMI model revealed that the effects of both genotype and environment were highly significant across MLN and non-MLN environments. The first two principal components accounted for 86% of the total variation in GEI. Analysis using GGE biplots identified three mega-environments with 10 vertex genotypes. Genotype 66 (CKHMLN170371) and 97 (CKHMLN170430) were suitable for MLN-free environments while genotypes, 100 (CKHMLN170433) and 105 (CKMLN150073) were best suited for MLN-stress conditions. Genotype 69 (CKHMLN170380) had overlapping suitability for both MLN and disease-free environments. Five genotypes with the highest stability across all environments included 69 (CKHMLN170380) ranking the highest, followed by 100 (CKHMLN170433), 66 (CKHMLN170371), 67 (CKHMLN170372) and 98 (CKHMLN170431). Results of this study demonstrated that MLN resistant hybrids identified could be advanced and validated for release for both MLN-endemic and MLN-free farming systems.

Key words: Genotype x environment interaction, three-way cross hybrids

7.1 Introduction

The recent outbreak and spread of MLN in various east African countries has posed a challenge to the region's maize production (Wangai et al. 2012; Adams et al. 2014; Lukanda et al. 2014;

Mahuku et al. 2015; Kagoda et al. 2016; Redinbaugh and Stewart 2018; Asiimwe et al. 2019; Terefe and Gudero 2019; Kiruwa et al. 2020; Prasanna et al. 2020). Field observations suggested that the disease greatly affected almost all commercial maize varieties, causing an estimated yield loss of 30 - 100%, depending on the stage of disease onset and severity. Within 2012, the first year of outbreak, MLN affected 77 000 ha of maize in Kenya alone, translating into an estimated yield loss of 126 million metric tons valued at US $52 million (Mahuku et al. 2015). An integrated approach, involving the deployment of different options to sustainably manage the disease, is widely accepted. Incorporating disease resistant varieties into farming systems together with routine crop management and improved crop intensification technologies is cost-effective in managing MLN (Marenya et al. 2018; Namikoye et al. 2018; Mengesha et al. 2019). The use of resistant varieties is vital because pesticides for disease vector control are costly to most farmers in SSA (Beyene et al. 2017a). Owing to the susceptibility of most commercial hybrids, the emergence of MLN could be viewed as a major setback to breeding efforts and gains made in developing and disseminating varieties with resistance to major biotic and abiotic stresses such as drought, low soil nitrogen, foliar diseases and ear rots (Semagn et al. 2015; Beyene et al. 2017b). To address this gap, first-generation MLN tolerant hybrids that were identified within the first three to five years of screening were released and commercialised in Uganda, Kenya and Tanzania (Prasanna et al. 2020). With extensive germplasm screening and rapid line selection, progress has been made in identifying more productive MLN resistant lines and varieties with desirable disease resistance levels, and with desirable agronomic and value-added traits (Beyene et al. 2017b; Prasanna et al. 2020).

Another important step in variety development is to identify hybrids with multiple stress tolerance that are suitable for farmers' conditions. Farmers are facing many production challenges such as erratic rainfall, low fertiliser application and prevailing biotic and abiotic stresses that limit the yield potential and on-farm productivity of maize varieties (Makumbi et al. 2018; Rezende et al. 2020). The current era of climate change and frequent disease and pest outbreaks requires rapid breeding, fast-tracking variety release and replacement of old, susceptible and less productive varieties to sustainably safeguard farmers' food security and livelihoods (Atlin et al. 2017).

In this study, 110 three-way cross hybrids were evaluated for productivity and stability under artificial MLN-infected and MLN-free environments. Three-way cross hybrids are preferred in SSA's seed systems due to ease of production and associated lower costs (Worku et al. 2020).

In developing new maize hybrids, new or elite inbred lines with high combining ability and high *per se* performance are crossed and resulting hybrids tested in replicated trials over various seasons and environments. Assessment using mean performance alone is not sufficient for selecting outstanding hybrids due to GEI, which is a result of differences in the relative performance of genotypes across test environments (Bernardo 2010). Cultivars are selected to suit a particular macro-environment, or bred for wider adaptation based on stability analysis. Stability analysis in this study was conducted to identify the most productive testcross hybrids with both wide adaptation and specific adaptation to MLN and non-MLN environments. Multiplicative models and biplots were used with the following specific objectives: (i) to determine the magnitude of GEI effects on testcross hybrids under both artificial MLN pressure and MLN-free conditions and (ii) to identify productive and stable MLN resistant hybrids suitable for advancement and variety release.

7.2 Materials and methods

7.2.1 Genetic material and experimental design

A total of 104 new MLN resistant three-way cross hybrids were evaluated alongside two MLN resistant pre-commercial checks, CKMLN150073 and CKMLN150077, and four commercial checks popular in SSA, H517, PHB30G19, WH505 and DK8031. The 110 hybrids were randomised in a 5 x 22 alpha-lattice design (Williams 1976) with two replications using CIMMYT Fieldbook-IMIS software (Bänziger and Vivek 2007; Vivek et al. 2007). Each experimental unit consisted of a 2-row plot of 5 m length, 0.75 m between rows and 0.25 m interplant spacing translating to a plant population density of 53 333 plants ha^{-1}.

7.2.2 Trial sites

Test hybrids were evaluated in MLN and diverse non-MLN sites over two seasons, 2017A (March - August) and 2017B (September - January 2018). Artificial MLN screening was conducted at the regional disease screening facility located in Naivasha in Kenya. Hybrids were further evaluated in two TLB hotspots in Uganda, Namulonge and Bulindi. Bulindi experienced optimum rainfall and management conditions. Namulonge and the additional two sites in eastern Uganda, Serere and Tororo, were considered as having random abiotic stress conditions as described by Makumbi et al. (2018), where a trial site was considered a random stress environment if average grain yield of entries was less than 3.3 t ha^{-1} and optimum if greater than this value. Trials sites are described in Table 6.2 and section 6.2.2.

7.2.3 Artificial screening for maize lethal necrosis resistance

Standard agronomic and pest management practices were applied uniformly to all locations. Di-ammonium phosphate was applied at a rate of 100 kg ha^{-1} at planting and urea was added at the same rate before flowering. At Naivasha, hybrids were artificially inoculated and screened for resistance to MLN, following an optimised protocol (Gowda et al. 2015) as described in section 3.2.3. The severity of MLN symptoms, AUDPC, susceptibility scores for MLN and rAUDPC were calculated as described in section 3.2.3

7.2.4 Assessment of natural infestation with common foliar diseases

Scoring for common foliar diseases, TLB, GLS and MSV was done as described in section 6.2.3.

7.2.5 Assessment of key agronomic traits

Agronomic data were collected on plot basis following the procedure described by and as described in section 6.2.3. Field weight was used to estimate GY from total plot weight adjusted to 12.5% moisture content and expressed in ton ha^{-1} as follows (CIMMYT 1999):

$$GY\ (t\ ha^{-1}) = \frac{(\text{Field weight (kg plot-1)} \times \text{Shelling percentage} \times (100 - MC) \times 10}{(100\text{-}12.5) \times \text{Plot area}}$$

Where MC is grain moisture content at harvest (%) and plot area is the harvested area per plot in m^2.

7.2.6 Statistical analysis

Descriptive statistics, variance and heritability estimates

Single and across-environment ANOVA were done for each management condition using the ReML method with the R program embedded in META-R software (Alvarado et al. 2016). Variance components were estimated following the linear mixed model:

$$Y_{ijko} = \mu + g_i + l_j + r_{kj} + b_{mjk} + e_{ijkm}$$

Where Y_{ijkm} is the *per se* performance of the i^{th} hybrid at the j^{th} environment in the k^{th} replication of the m^{th} incomplete block, μ is an intercept term (mean), g_i is the genotypic effect of the i^{th}

genotype, l_j is the effect of the j^{th} environment, r_{kj} is the effect of the k^{th} replication at the j^{th} environment, b_{mjk} is the effect of the m^{th} incomplete block nested in the k^{th} replication at the j^{th} environment, and e_{ijkm} is the residual effect. The effects of environments and replications were treated as random while genotypes were considered fixed. Broad-sense heritability (H^2) on an entry-mean basis was estimated from the variance components as the ratio of genotypic to phenotypic variance as follows:

$$H = \frac{\delta_g^2}{\delta_g^2 + \frac{\delta_{gl}^2}{l} + \frac{\delta_e^2}{lr}}$$

Where δ_g^2 is genotype variance, δ_{gl}^2 is genotype x location interaction variance, and δ_e^2 is the error variance for *l* locations and *r* replicates of the hybrids at each location.

Magnitude of genotype by environment and stability analysis

Analysis using the AMMI model was performed for GY to determine the contribution of genotype, environment major effects and the interaction of GEI on the total variation. Using GEA-R software (Pacheco et al. 2015), parametric and non-parametric stability parameters were estimated. The GGE biplot analysis was performed to determine the mega-environments, to display "which-won-where" patterns and to identify ideal genotypes for particular macro-environments and stable hybrids for all environments.

7.3 Results

7.3.1 Variance, heritability and mean performance for grain yield, other agronomic traits, and disease resistance

Effect of location and season

Under MLN screening, the effect of season was significant for GY and MOI ($P < 0.05$), moderately significant ($P < 0.01$) to highly significant ($P < 0.001$) for AD, EA, EH, PH and MLN severity scores. Location effect was not significant for ASI and ER infestation (Table 7.1). A significant effect of location was also observed for GY and AD across all MLN free environments. The effect of location on GY and ASI was not significant across the three random abiotic stress sites. Across-site analysis, therefore, implies that ASI was relatively consistent across environments. Mean performance of all hybrids showed that Bulindi had the highest yield potential (5.9 t ha^{-1}) while Tororo was the lowest potential non-MLN site. There was a large

difference in yield over the two seasons of MLN screening at Naivasha, with yield in season 2 dropping to half the mean yield of the first season. In general, mean yield at abiotic stress sites ranged from 2.5 - 3.8 t ha^{-1} although a maximum individual hybrid yield of up to 7.5 t ha^{-1} was seen, which suggests the existence of some exceptionally performing hybrids under stress (Figure 7.1).

Genotypic variation and heritability

Significant genotypic variance was evident for all traits under MLN and non-MLN conditions, except for ASI under MLN conditions. Highly significant genetic variation ($P < 0.001$) for GY and AD was observed when the analysis was conducted across MLN and non-MLN conditions. The variation for GY was, however only significant ($P \leq 0.05$) across the three non-MLN random abiotic stress sites. It was also observed that trait heritability estimates were moderate for yield and MLN rating at early disease development and high (0.68 - 0.77) for advanced disease development and AUDPC. Analysis across all sites (MLN pressure, optimum and abiotic stress) increased heritabilities for both GY and AD compared to the analysis within specific management environments. Lowest heritability for GY (0.37) was attained from the analysis across non-MLN abiotic stress environments (Table 7.1).

Genotype x environment effect

The GEI was highly significant for GY under MLN pressure and combined analysis across all trial environments. However, GEI was non-significant for GY and maturity traits when analysed across random abiotic stress sites only. A moderate to highly significant GEI effect was observed for the response to MLN. The GEI effect was non-significant for EH, ER and MOI and EA (Table 7.1).

7.3.2 Additive main effect and multiplicative interaction analysis for grain yield and other agronomic traits

Further analysis was conducted using the AMMI model (Table 7.2) for GY, six key agronomic traits and two common biotic constraints; TLB and ER. Main effects of genotypes (G), environment (E) and GEI were highly significant ($P \leq 0.001$) for GY, ASI, MOI, PH, EPP and ER. Variance due to GEI was further partitioned into two major interaction principal components (IPCA1 and IPCA2).

Table 7.1 Variance, heritability and mean performance of three-way hybrids under MLN and non-MLN optimum and random abiotic stress environments

Trait	Across two MLN screening seasons at Naivasha							
	δ^2_g	δ^2_{gxl}	δ^2_l	δ^2_e	Mean	H^2	LSD	CV (%)
GY (t ha^{-1})	0.46*	0.70***	2.95*	1.00	2.96	0.43	1.51	33.80
AD (days)	3.77***	0.64	253.16***	5.99	88.46	0.67	3.29	2.77
ASI (days)	0.05	0.10	0.09	0.36	2.35	0.26	0.58	25.64
PH (cm)	39.06**	29.71*	3147.54***	97.82	162.40	0.50	12.79	6.09
EH (cm	22.03**	12.61	1226.31**	68.68	99.22	0.48	9.80	8.35
ER (%)	112.58**	85.40	13.17	370.64	41.69	0.45	22.73	46.18
MOI (%)	1.24*	0.00	0.98*	5.11	19.83	0.49	2.40	11.40
EA (1 - 5)	0.12**	0.09*	0.65**	0.34	3.68	0.48	0.71	15.85
MLN1 (1 - 9)	0.06**	0.05**	0.96***	0.16	2.73	0.46	0.50	14.49
MLN2 (1 - 9)	0.15***	0.056*	1.77**	0.23	3.53	0.64	0.67	13.67
MLN3 (1 - 9)	0.24***	0.08**	1.85***	0.25	4.21	0.70	0.76	11.97
MLN4 (1- 9)	0.35***	0.16***	1.03**	0.32	4.75	0.68	0.95	11.91
MLN_AV (1 -9)	0.19***	0.05***	1.38***	0.11	3.83	0.78	0.59	8.70
MLN_AUDPC	336.42***	94.91***	2668.40***	214.15	160.79	0.77	25.44	9.10
MLN_SC (1 - 9)	0.203***	0.06***	1.62***	0.12	3.97	0.77	0.62	8.88
	Across four non-MLN abiotic stress and optimum sites							
GY (t ha^{-1})	0.09***	0.08*	2.09*	0.65	3.87	0.47	0.63	20.83
AD (days)	2.69***	0.00	2.93*	3.10	62.50	0.84	2.01	2.82
ASI (days)	0.16**	0.29**	0.12	1.39	1.54	0.40	0.88	76.87
	Across three non-MLN random abiotic stress sites							
GY (t ha^{-1})	0.06*	0.06	0.42	0.48	3.18	0.37	0.55	21.84
AD (days)	2.69***	0.00	2.93*	3.10	62.50	0.84	2.01	2.82
ASI (days)	0.41***	0.17	0.18	1.65	1.48	0.55	1.22	86.60
	Across all five sites (MLN, optimum and abiotic stress)							
GY (t ha^{-1})	0.17***	0.26***	1.94**	0.75	3.62	0.62	0.76	23.95
AD (days)	2.69***	0.00	2.93*	3.10	62.50	0.84	2.01	2.82

δ^2_g, genotypic variance; δ^2_{gxl}, genotype x location variance; δ^2_l, location variance; δ^2_e, residual variance; ***; **; * P ≤ 0.001, P ≤ 0.01, P ≤ 0.05; GY, grain yield; AD, days to anthesis; ASI, anthesis-silking interval; PH, plant height; EH, ear height; ER, percent ear rot infestation; MOI, grain moisture content at harvest; EA, ear aspect; MLN1, 2, 3, 4, first-fourth maize lethal necrosis (MLN) severity scores; MLN_AV, average of MLN severity scores; MLN_AUDPC, MLN area under disease progress curve, MLN_SC, computed MLN susceptibility score

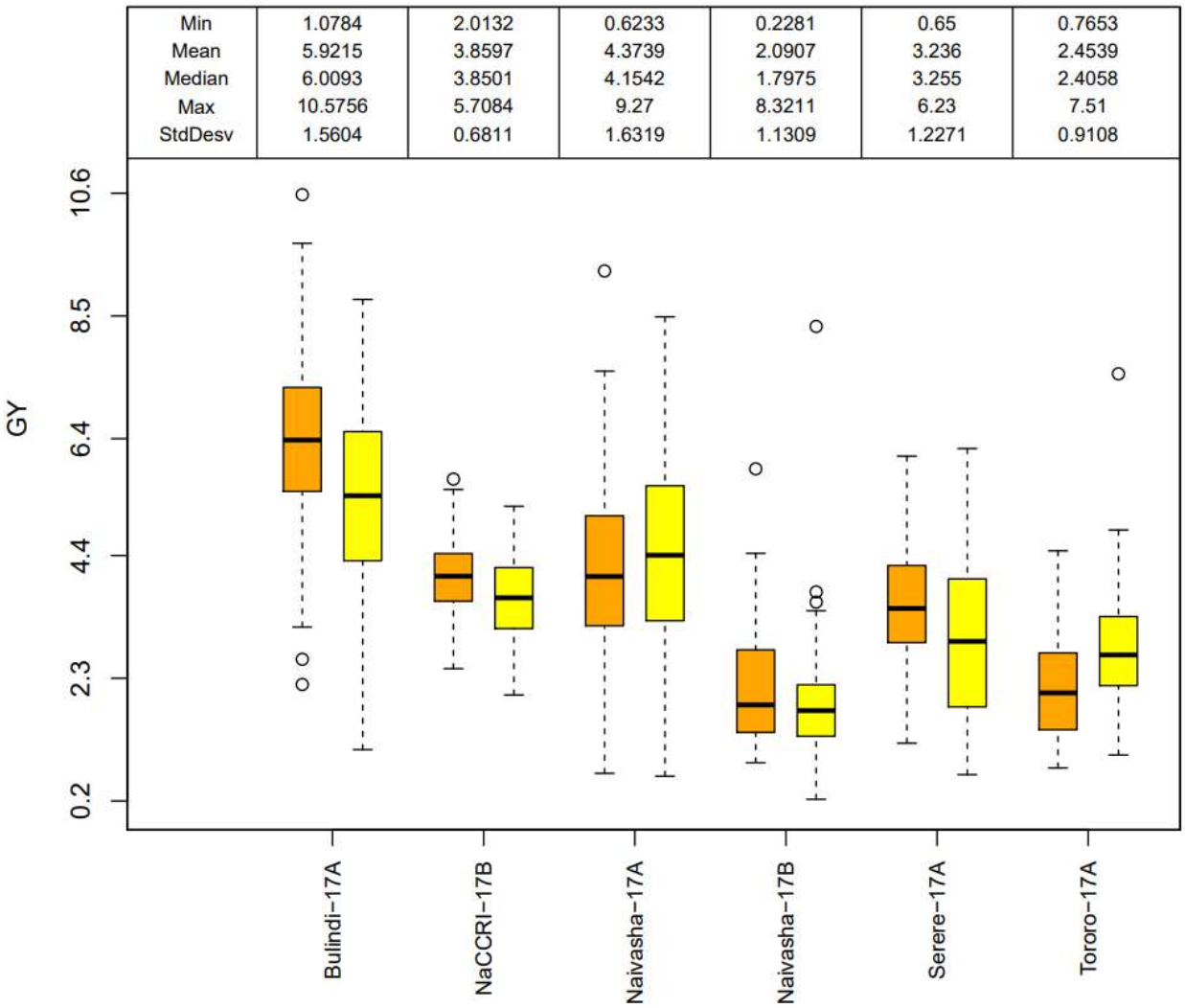

Figure 7.1 A box plot showing performance of hybrids for grain yield at individual test environments.

Testing season A (March **-** August)**;** testing season B (September **-** January of following year); **o**range box plots, median; yellow box plots, mean of entries

Results indicated IPCA1 accounted for 40.5% of the interaction sum of squares for GY while IPCA2 accounted for 30.5%. Both IPCAs explained 87% of the variation for AD and between 60% and 84% for other important traits (Table 7.2)

To visualise interactions and main effects of genotypes and environments, IPCA1 scores for both genotypes and environments were plotted against IPCA2 and then against the GY means (Figure 7.2). The graph space was divided into four quadrants displaying the lower-yielding environments in the quadrants to the left (Tororo, Serere and Namulonge) and higher-yielding to the right (Bulindi and Naivasha). AMMI biplot analysis identified up to 12 genotypes with high yield and high relative stability (Table 7.3). Among these high performing hybrids were two commercial checks, WH505 and PHB30G19.

7.3.3 Genotype and genotype by environment biplot analysis for grain yield

The GGE biplot analysis was conducted to provide a succinct visualisation of random stress and optimum mega-environments and to identify ideal genotypes suitable for all mega-environments and genotypes with specific adaptation. The GGE analysis was constructed using adjusted means across two seasons under MLN screening at Naivasha and genotype means at the individual non-MLN sites. The GGE scatter plot (Figure 7.3) presents the polygon of grain yield for the 110 genotypes across the five test environments (Namulonge, Serere, Tororo, Bulindi and Naivasha).

The first principal component axis (PCA1) explained 38.0% of the variation for GY while the second axis (PCA2) explained 19.9%, explaining 57.9% of the total variation. The line from the origin of the biplot and perpendicular to the polygon sides divided the biplot into sectors, with each sector having its winning cultivar corresponding to different sites. The biplot analysis of this study indicated 10 vertex genotypes (G107, G105, G100, G69, G66, G97, G24, G82, G84 and G110). The test locations appeared to fall in three mega-environments, two of which are overlapping. Tororo was a lower potential site compared to the other sites. Genotypes 66 (CKHMLN170371), and 97 (CKHMLN170430) won in three non-MLN environments, while genotypes 105 (CKMLN150073) and commercial check entry 107 (H517) won under random abiotic stress at Tororo. Two genotypes 100 (CKHMLN170433) and 105 (CKMLN150073) won under MLN conditions while 69 (CKHMLN170380) performed well under MLN and non-MLN environments (Figure 7.3).

Table 7.2 Additive main effects and multiplicative interaction (AMMI) analysis of variance for the three-way cross hybrids across maize lethal necrosis (MLN) and non-MLN environments

		AMMI mean squares								
Source	**Df**	**GY**	**AD**	**ASI**	**MOI**	**PH**	**EP**	**EPP**	**TLB**	**ER (%)**
Total	319	3.22	205.00	1.93	9.9	926	0.006	0.081	0.34	608
Treatments	659	5.38***	405.00***	2.59***	16.50***	1702***	0.011***	0.12***	0.47***	974***
Genotypes	109	3.67***	33.00***	3.82***	7.30***	812***	0.006***	0.09***	0.55***	690***
Sites	5	459.71***	51925.00***	74.80***	1492.90***	233177***	1.430***	9.84***	38.98***	66150***
Block	6	20.03***	82.00***	11.74***	6.90	4078***	0.018***	0.35***	0.58	115***
Interactions	509	1.67***	7.00*	1.81***	5.10***	232***	0.002*	0.08***	0.41**	436***
IPCA1	113	3.18***	10.00***	2.95***	7.70***	331***	0.004***	0.16***	0.74***	809***
IPCA2	111	2.03***	9.00***	2.18***	6.20***	287***	0.002	0.06***	0.264	646***
Residuals	285	0.92	5	1.21	3.6	149	0.001	0.032	0.208	230
Error	568	1.02	6	1.36	3.9	147	0.002	0.044	0.302	252
% IPCA1		40.51	73.98	36.31	33.60	37.27	49.94	57.1	62.0	38.7
% IPCA2		30.52	12.84	26.38	26.53	31.78	20.62	22.0	22.0	30.4

GY, grain yield (t ha^{-1}); AD, days to anthesis; ASI, Anthesis-silking interval; MOI, grain moisture; PH, plant height; EP, ear position; EPP, ears per plant; TLB, Turcicum leaf blight, ER, ear rots; ***P ≤ 0.001; **P ≤ 0.01; * P ≤ 0.05; IPCA, interaction principal component analysis

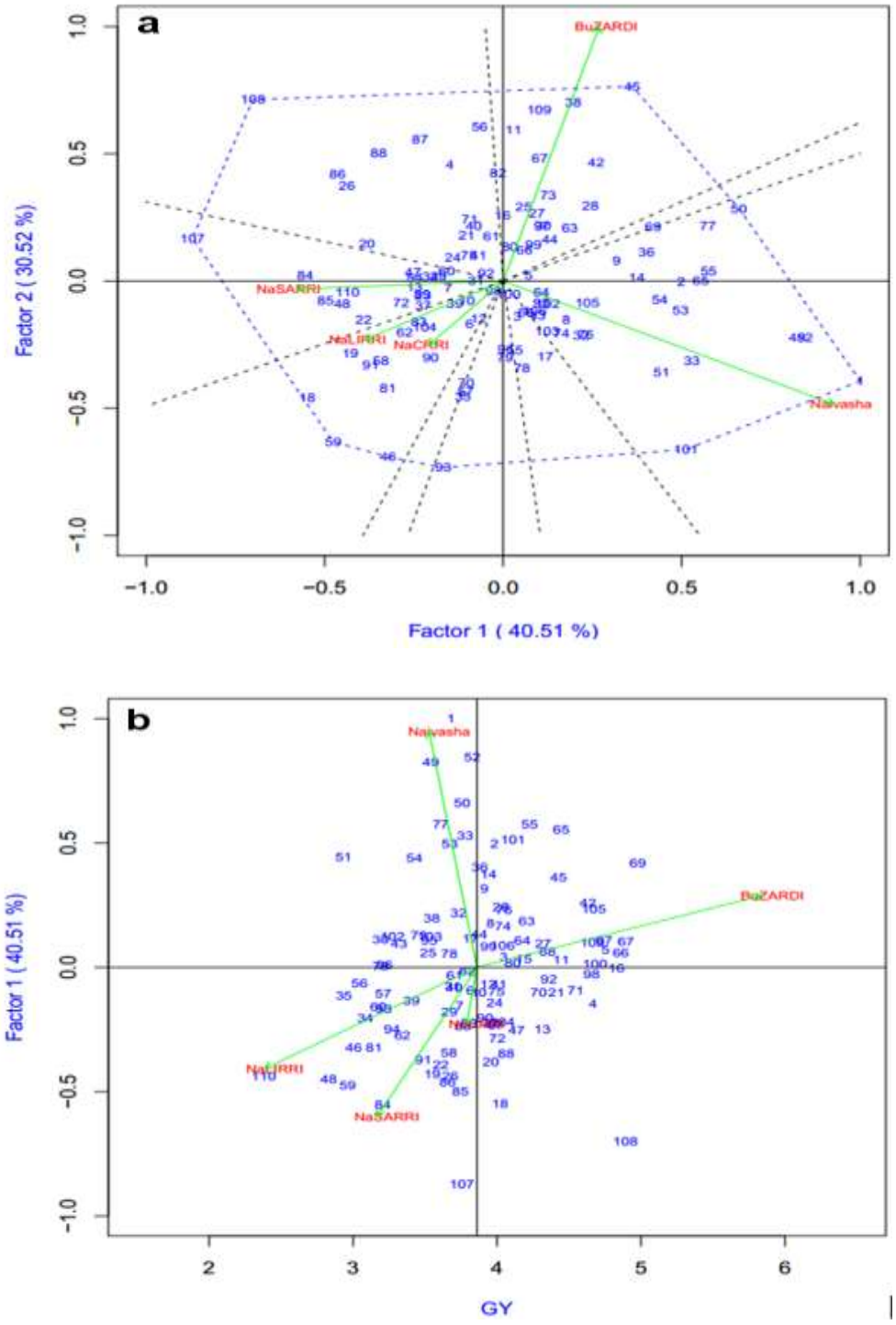

Figure 7.2 AMMI biplot showing IPCA1 vs IPCA2 for yield across five testing sites.

(a) AMMI biplot showing IPCA1 vs IPCA2 for yield across five testing sites; (b) AMMI biplot for IPCA1 scores and mean yield for 110 hybrids tested in five sites; AMMI, additive main effects and multiplicative interaction; IPCA, interaction principal component analysis

Table 7.3 Selected high yielding, stable hybrids across five test sites

Genotype	Name	Mean yield (T ha$^{-1)}$	Category
69	CKHMLN170380	4.98	Test genotype
67	CKHMLN170372	4.90	Test genotype
108	**PHB30G19**	**4.89**	**Commercial check**
66	CKHMLN170371	4.86	Test genotype
97	CKHMLN170430	4.74	Test genotype
05	CKHMLN170260	4.75	Test genotype
109	**WH505**	**4.66**	**Commercial check**
11	CKHMLN170267	4.44	Test genotype
68	CKHMLN170373	4.35	Test genotype
27	CKHMLN170297	4.32	Test genotype
15	CKHMLN170278	4.19	Test genotype
80	CKHMLN170399	4.11	Test genotype
N	110 (10% selected)		
Mean of entries	3.62		
CV%	23.95		
LSD	0.76		

CV (%) Percent coefficient of variation; LSD, least significant difference

Further GGE comparison biplot analysis was conducted to determine the ideal genotype with the highest stability across all environments as indicated by their position closest to the centre of the concentric rings. The two most ideal genotypes identified were 69 (CKHMLN170380) and 100 (CKHMLN170433), followed by 66 (CKHMLN170371), 67 (CKHMLN170372) and 98 (CKHMLN170431) (Figure 7.4, 7.5)

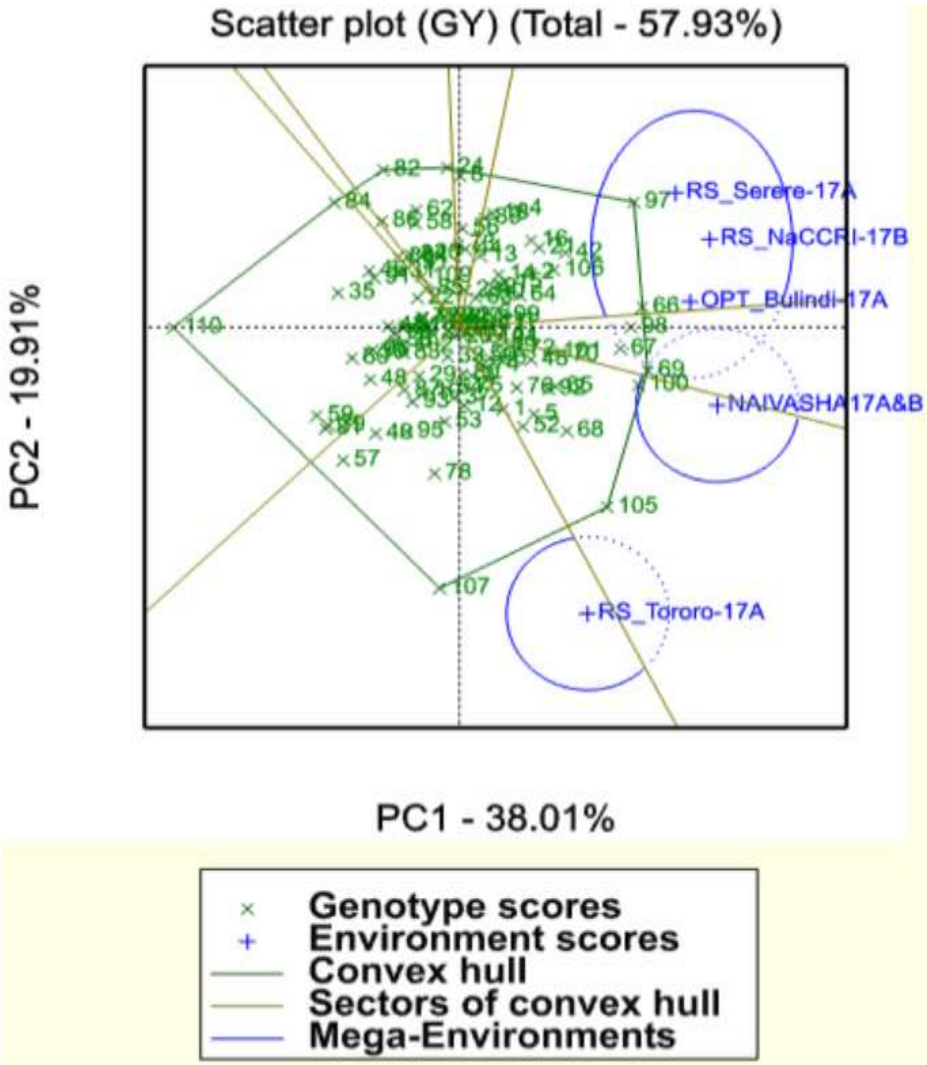

Figure 7.3 Scatter plot for grain yield analysed across five test environments.

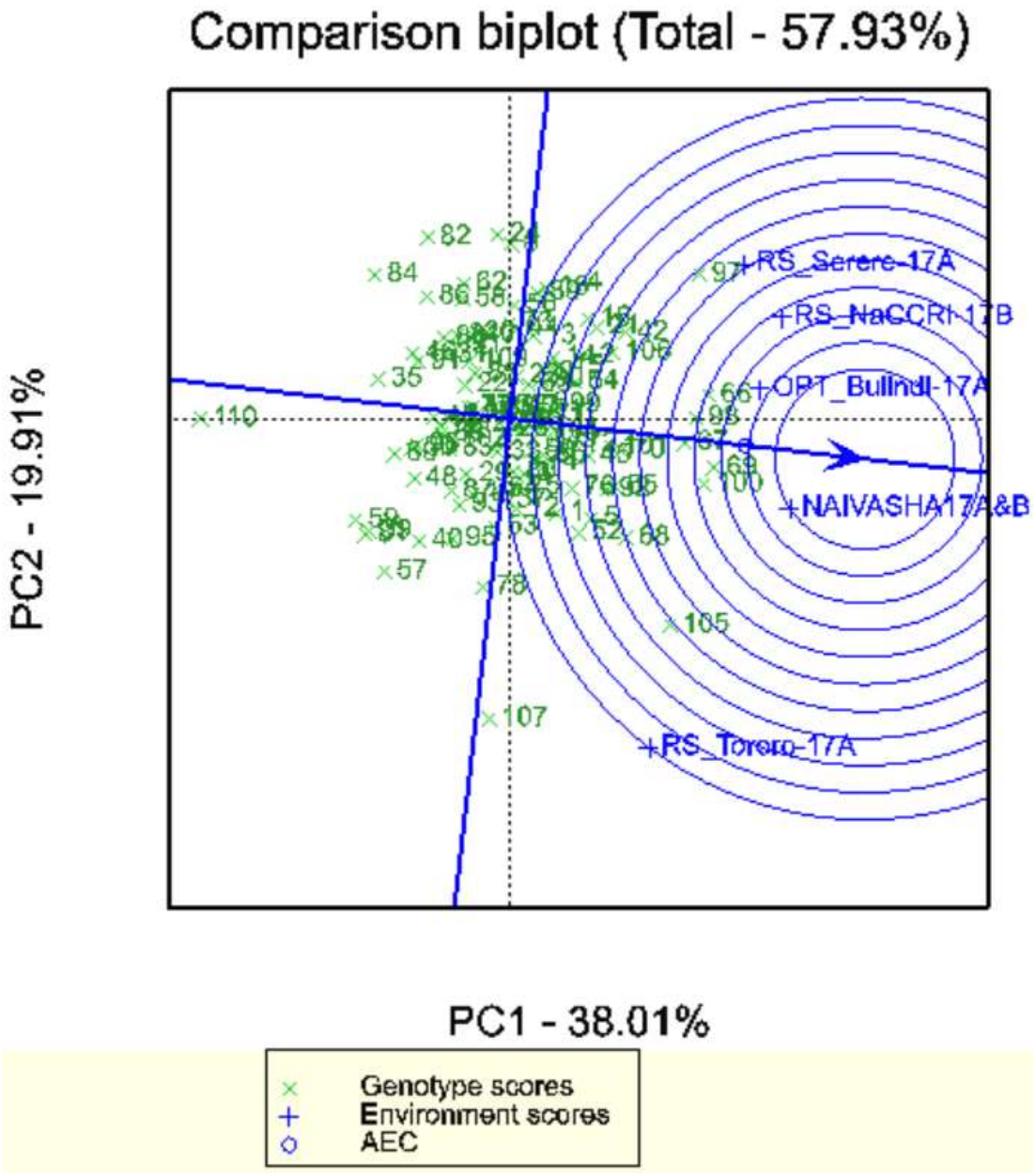

Figure 7.4 Comparison biplot for grain yield of 110 hybrids tested across five sites.

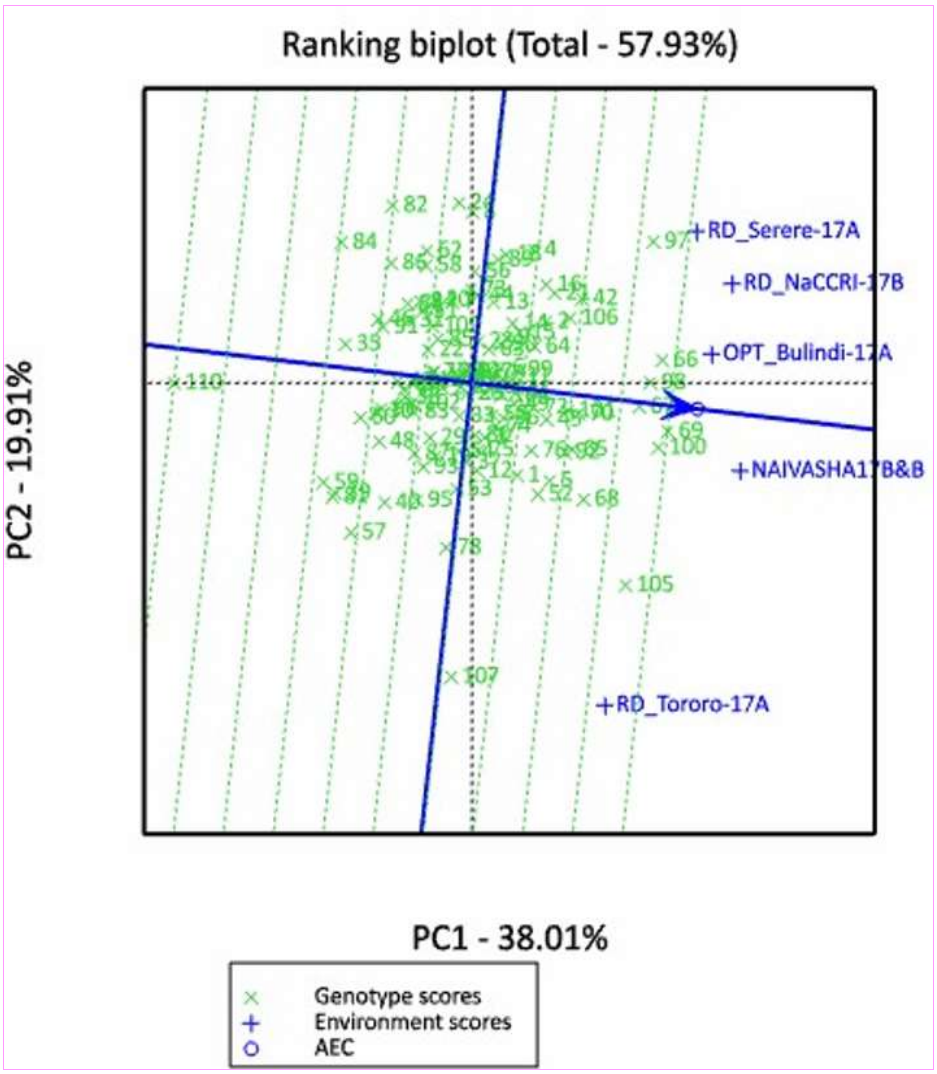

Figure 7.5 Ranking plot for grain yield of 110 hybrids tested across five sites.

7.4 Discussion

Evaluation of testcross hybrids for GY and other agronomic traits in five environments demonstrated the existence of adequate genetic variation for MLN resistance, GY and other desirable traits. Adequate genetic variation is necessary for crop improvement because it enables selection of target traits (Hallauer and Miranda 1981). Earlier developed MLN susceptible testcross hybrids evaluated by Beyene et al. (2017a), Sserumaga et al. (2018) and Rezende et al. (2020) under stress and non-stress conditions showed significant genotypic variation for yield and other key traits. Low GY in stress sites was used as an indicator of random stress conditions (Weber et al. 2012; Makumbi et al. 2018) due to the unavailability of deliberate artificial screening for drought and low soil nitrogen, which are common abiotic

stresses faced by farmers. Significant GEI effects of traits under investigation showed that genotypes had different relative performances across test environments. The significant GEI effects could be harnessed to select ideal genotypes that are superior and stable (Bernardo 2010). A simple method following the Francis and Kannenberg (1978) procedure, often used in preliminary trials, is to combine a favourable mean with low coefficient of variance to identify potential genotypes for advancement. This study, however, combined AMMI and GGE to select promising MLN tolerant hybrids, although a newer method of stability analysis based on multiple traits suggested by Olivoto et al. (2019) could be used in promising MLN tolerant hybrids. The AMMI analysis was conducted to partition the total variance into the main effects of genotype and environment and to measure the magnitude of GEI across MLN and non-MLN environments. The AMMI analysis differentiated three random stress sites (Serere, Tororo and Namulonge) from the optimum site (Bulindi) and MLN screening site, Naivasha. Results of this study are similar to others, where large variance due to the environment and GEI were reported under abiotic and optimum stress conditions (Beyene et al. 2016; Makumbi et al. 2018). Results, however, showed no significant GEI among the random stress sites, although there was a large significant environmental effect for yield across all stress and non-stress sites. This implies that similar varieties could be selected for production across random abiotic stress sites and it is more practical to conduct stability analysis across stress and non-stress mega-environments.

In addition to AMMI, GGE biplots often provide a good visualisation of the “which-won-where” pattern (Yan and Tinker 2006; Ding et al. 2008). Results of this study unveiled five genotypes with outstanding performance in specific macro-environments and five ideal genotypes with high performance and stability under MLN infestation, abiotic stress and optimum environments. The stable candidate hybrids, 69 (CKHMLN170380), 100 (CKHMLN170433), 66 (CKHMLN170371), 67 (CKHMLN170372) and 98 (CKHMLN170431), could be validated and released in MLN endemic and disease-free areas of East Africa and will complement the existing stress-tolerant maize product range in the region. Further testing of selected genotypes would therefore require multiple seasons across years, locations and replications to minimise inconsistencies due to the effect of GEI, to improve on repeatability and to decide whether the target region can be divided into different mega-environments (Yan et al. 2007). Farmer preference and seed productivity of selected hybrids need to be assessed to facilitate future adoption by farmers and seed companies, respectively. Worku et al. (2020) demonstrated that through extensive farmer

participation in region-wide on-farm trials, farmers' selection criteria combined with breeders' selections will increase the chance of releasing new stress-tolerant hybrids to SSA farmers.

7.5 Conclusions

New MLN tolerant hybrids evaluated in this study could provide a good base for the selection of new hybrid combinations for future release. The study also demonstrated the possibility of selecting multiple stress-tolerant hybrids that could combine MLN resistance with prevailing abiotic stress conditions. Selection of hybrids under random abiotic stress could lead to the identification of products for small-scale farmers who are often faced with unpredictable rainfall and low input conditions, making productivity low even in the absence of MLN.

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CHAPTER 8

General conclusions and recommendations

8.1 General conclusions

Since its outbreak in East Africa in 2011, MLN which is endemic in the Americas has become an economically important biotic constraint in SSA. Sustainable management of MLN and other biotic and abiotic stresses can be achieved through the development and deployment of resistant varieties in affected cropping systems. In the region, significant strides have been made in deployment of varieties with resistance to common biotic stresses including TLB, MSV, GLS, common rust, ER and abiotic stresses such as drought and low soil nitrogen. Recently, two major devastating biotic stress outbreaks have occurred in the region; MLN in 2011 followed by FAW in 2016, distorting the past achievements from breeding stress-tolerant maize varieties. These two challenges are currently being addressed sustainably by integrating resistant varieties with other proven pest and disease management practices.

Breeding for MLN resistance started with establishment of a regional artificial screening site in Kenya followed by massive screening of adapted and introduced inbred lines, hybrids and open-pollinated varieties. Initially, only < 5% of the thousands of entries showed tolerance to the disease. This available resistant germplasm base was used to develop new breeding populations and hybrids and to conduct genetic analyses from which this thesis forms part. Pre-breeding assessment, combining susceptible elite inbred lines, introduced virus resistant inbred lines form the USA, Asia and SSA and tropical lowlands in Mexico, revealed the existence of adequate available genetic variation necessary for breeding for MLN resistance. Existing elite lines with resistance to common foliar disease are a desirable base to pyramid MLN resistance into adapted elite backgrounds. Markers linked to major MLN resistance QTL could be validated and included in the MLN resistance marker panel for MAS. The combining ability of first-generation MLN tolerant inbred lines and potential testers was also determined. Promising results obtained indicates that these lines and single crosses could be used in developing MLN resistant varieties for further testing and release to farmers. The two lines (CKDHL140910 and CKDHL120312) with desirable combining ability both under MLN pressure and other stresses could be used in developing future breeding populations, parental lines and hybrids through trait introgression and forward breeding. The eight single cross female parents, except CKLTI0227/CKLMARSI0029, could be used as MLN resistant testers and for generating

potential MLN resistant three-way cross hybrids. Since these single cross hybrids had similar GCA effects, they need to be further crossed to diverse resistant lines to identify the ideal MLN resistant tester for routine maize breeding. While breeding for MLN resistance, GEI could be a hinderance to effective selection under both optimum and disease infested environments. Despite the existence of significant GEI, this study demonstrated the possibility of selecting productive and stable stress-tolerant hybrids that could combine MLN resistance with tolerance to prevailing abiotic stress conditions. In this study, at least 10% candidate hybrids out of 110 test hybrids were selected for further testing in national performance trials and on-farm for possible release. Several approaches to participatory variety selection approaches have been suggested. New and effective approaches such as the tricot method (van Etten et al. 2020) are recommended.

The significant genetic variation and high heritability estimates observed in this study could enabled formation of new pedigree and backcross populations by crossing MLN resistant introduction and tropical tolerant lines to tropical elite lines. Furthermore, significant expected gains from selection and potential for resistant line extraction were observed among nine biparental pedigree populations analysed in this study. Only one of the populations was significantly unsuitable for selection and advancement. However, using the usefulness criterion, three populations could be selected and prioritised for selection using limited resources. Whereas pedigree breeding remains popular among most breeding programmes in SSA, adoption of the DH technology combined with marker-assisted selection and genomic selection are viewed as the most reliable methods for increased genetic gains and cost-effectiveness. So far, at least two studies using CIMMYT populations have demonstrated the effectiveness of MAB and DH in breeding for MLN resistance.

To strengthen MAB for MLN resistance, progress to identify QTL and linked markers has been made. It was noted that MLN resistant QTL in tropical maize is polygenic in nature and controlled by a few major QTL and an array of minor QTL distributed throughout the genome. This diversity in MLN resistance QTL in tropical maize defies earlier studies that revealed tight clustering of virus resistance genes mainly on chromosomes 3 and 6, hence a limited number of loci are involved in conditioning MLN resistance. In case of clustered resistance genes, pedigree breeding could be appropriate for transferring virus resistance and marker-assisted selection may be straightforward. This study, confirmed the presence of a major resistance QTL from the KS23 genetic background that was earlier identified on the long arm of chromosome 6.

This QTL needs to be targeted for further fine mapping, for developing high quality diagnostic markers and for routine introgression of MLN resistance into elite genetic backgrounds in breeding programmes in SSA and globally. The major threat to the deployment of a single major-effect recessive QTL is the likelihood of resistance breakdown. Breeders therefore need to build durable MLN resistance by validation and deployment of both major and minor-effect QTL while retaining the background of other diffuse disease resistant QTL for TLB, GLS and MSV that have already been concentrated in elite tropical germplasm. This multiple QTL selection requires more efficient methods such as MARS and genomic selection, which is gaining ground in the region and globally.

As long as MLN continues to persist in SSA cropping systems, more effort is needed to strengthen integrated disease management approaches, best practices and scale-up resistant varieties to replace old susceptible varieties. Increased adoption of new MLN resistant varieties requires concerted efforts by both the public and private seed sector in areas of variety promotion and availability of seed through investment in seed production and efficient distribution networks. These efforts will be further strengthened by the implementation of regional policies and actions aimed at collective management of MLN and other transboundary pests and diseases. This should include awareness creation and engagement among policy makers and other key stakeholders on MLN management, regional disease monitoring and surveillance in collaboration with NPPOs and, seed sector partnerships for production and exchange of disease-free commercial maize and other cereal seed.

8.2 Recommendations for future research

Based on research findings of this study and other breeding efforts in East African breeding programmes, the following are future perspectives effective MLN resistance breeding and development of disease resistant farmer-preferred varieties for the region:

Utilization of genomic tools to efficiently pyramid MLN resistance genes from diverse background. The major recessive MLN resistance QTL identified in this work will need to be combined with multiple small-effect QTL from diverse backgrounds. Rather than utilising introduced founder lines, forward breeding using newly selected lines with better adaptability and resistance to common foliar diseases, MSV, TLB and GLS is more practical in developing durable disease resistance. A combination of both marker-assisted selection and genomic

section will be desirable. Marker-assisted selection will be useful in selecting lines with the major MLN resistant QTL and desirable alleles for other common foliar diseases while genomic selection will be handy for numerous minor-effect QTL for multiple diseases and high yield. Several genomic prediction models have been suggested. These and should be tested and validated using carefully constituted training populations.

Diversity analysis and heterotic grouping of newly identified inbred lines is needed. This will guide recombination of genetically distant lines to form new hybrids with the highest possible heterosis for yield. To effectively deploy the major recessive MLN resistant QTL, lines elite lines from the two the popular tropical maize heterotic groups, A and B should be introgressed by backcrossing and any two inbred bearing the QTL are crossed to for a resistant hybrid. Alternatively, one pool could be improve using marker-assisted backcrossing while the other is improved using pedigree or Marker-assisted selection on biparental populations.

As MLN resistance breeding and deployment of MLN-resistant varieties advances, there is need to conduct more virus-plant interaction studies to model future resistance breakdown that could result from virus mutation or multiple virus-virus and virus-plant interactions. Such studies will guide development of durable virus resistant germplasm for the future.

Appendices

Appendix 1. List of Kompetitive allele-specific polymerase chain reaction markers used in QTL mapping

MarkerID	Marker name	Chromosome	Position	Chi-Square	Pr > ChiSq	HetBand
	MLNR-P20: ((CM543/CML444//CM543)DH5-B-B-B/KS23-6)F_3					
1	PHM7616_35	1	0.00	21.47	0.0000	Codominant
2	PZA03020_8	1	21.88	97.12	0.0000	Codominant
3	PHM3563_17	1	33.87	4.50	0.1054	Codominant
4	d8_3	1	45.73	4.06	0.1315	Codominant
5	PHM14475_7	1	51.15	18.93	0.0001	Codominant
6	PHM4942_12	1	63.10	24.32	0.0000	Codominant
7	PHM1438_34	1	72.49	23.73	0.0000	Codominant
8	PZA03194_1	1	75.03	13.38	0.0012	Codominant
9	PZA01019_1	1	78.76	18.48	0.0001	Codominant
10	PHM5622_21	1	92.47	37.98	0.0000	Codominant
11	PHM3147_18	1	94.07	37.52	0.0000	Codominant
12	PHM4053_15	1	95.01	47.23	0.0000	Codominant
13	S1_46411896	1	115.28	30.89	0.0000	Codominant
14	PZA00447_8	1	137.82	51.55	0.0000	Codominant
15	PHM4997_11	1	142.76	30.09	0.0000	Codominant
16	S1_23047822	1	205.44	173.33	0.0000	Codominant
17	PZA02490_1	1	205.44	220.00	0.0000	Codominant
18	PZA02279_1	1	205.44	218.33	0.0000	Codominant
19	S1_36800023	1	205.44	213.33	0.0000	Codominant
20	PZA03742_1	1	205.44	220.00	0.0000	Codominant
21	umc128_2	1	205.44	218.33	0.0000	Codominant
22	S1_22744948	1	205.44	221.67	0.0000	Codominant
23	PHM2130_29	1	205.44	216.67	0.0000	Codominant
24	PZA03561_1	1	205.44	165.00	0.0000	Codominant
25	S1_2543968	1	205.44	221.67	0.0000	Codominant
26	S1_18838432	1	205.83	211.38	0.0000	Codominant
27	S1_26912154	1	206.21	221.67	0.0000	Codominant
28	PHM4752_14	1	206.60	214.71	0.0000	Codominant
29	PZB00648_5	1	206.60	211.37	0.0000	Codominant
30	PZA02269_4	1	206.60	207.80	0.0000	Codominant
31	PZA02269_3	1	206.60	207.80	0.0000	Codominant
32	PZA01254_2	1	206.98	204.32	0.0000	Codominant
33	PZA03200_2	1	207.35	204.45	0.0000	Codominant
34	S1_173654738	1	207.35	202.79	0.0000	Codominant
35	csu1138_4	1	207.73	211.20	0.0000	Codominant
36	S1_16470688	1	208.11	213.05	0.0000	Codominant
37	PHM3463_18	1	208.11	213.05	0.0000	Codominant

38	S1_36514510	1	208.11	209.54	0.0000	Codominant
39	PZA03404_1	1	208.49	202.79	0.0000	Codominant
40	PZA00939_1	1	208.87	209.54	0.0000	Codominant
41	PZA01978_23	1	209.24	216.38	0.0000	Codominant
42	PZA00030_11	1	209.24	218.33	0.0000	Codominant
43	S1_15353866	1	209.24	216.67	0.0000	Codominant
44	S1_85134292	1	209.24	221.67	0.0000	Codominant
45	PHM1932_51	1	209.62	214.72	0.0000	Codominant
46	PHM4695_5	1	210.00	221.67	0.0000	Codominant
47	PHM2187_34	1	210.00	220.00	0.0000	Codominant
48	S1_173421054	1	210.00	221.67	0.0000	Codominant
49	PHM5597_15	1	210.00	220.00	0.0000	Codominant
50	PHM12633_15	1	210.00	221.67	0.0000	Codominant
51	PZA02763_1	1	210.00	215.00	0.0000	Codominant
52	S1_25104820	1	210.00	215.00	0.0000	Codominant
53	PZA03037_2	1	210.00	220.00	0.0000	Codominant
54	S1_234122525	1	210.00	215.00	0.0000	Codominant
55	PHM175_25	1	210.00	194.33	0.0000	Codominant
56	PHM12693_8	1	210.00	195.99	0.0000	Codominant
57	PZA02467_10	1	210.00	195.99	0.0000	Codominant
58	PZA01315_1	1	210.00	199.31	0.0000	Codominant
59	PZA00175_2	1	210.38	199.20	0.0000	Codominant
60	PHM5306_16	1	210.38	190.87	0.0000	Codominant
61	PZA02284_1	1	210.38	197.54	0.0000	Codominant
62	PHM12323_17	1	211.15	187.51	0.0000	Codominant
63	PZA00620_3	2	0.00	38.15	0.0000	Codominant
64	PZA00613_22	2	21.32	20.15	0.0000	Codominant
65	PZA00680_3	2	34.68	38.22	0.0000	Codominant
66	PHM5817_15	2	34.68	47.10	0.0000	Codominant
67	PZB00772_7	2	70.11	15.35	0.0005	Codominant
68	PHM793_25	2	80.65	30.73	0.0000	Codominant
69	PHM3055_9	2	84.49	16.32	0.0003	Codominant
70	PZA03529_1	2	88.84	38.65	0.0000	Codominant
71	PZA00515_10	2	95.18	51.25	0.0000	Codominant
72	PZA00495_5	2	95.18	42.86	0.0000	Codominant
73	PZA03629_1	2	99.92	32.11	0.0000	Codominant
74	PZA01232_1	2	136.36	142.87	0.0000	Codominant
75	PZA02564_2	2	136.36	204.36	0.0000	Codominant
76	PHM1511_14	2	136.36	195.99	0.0000	Codominant
77	PZA01885_2	2	136.36	202.70	0.0000	Codominant
78	PZA02378_7	2	137.15	184.29	0.0000	Codominant
79	PZA02496_1	2	137.54	192.68	0.0000	Codominant
80	PZA03559_1	2	137.93	184.29	0.0000	Codominant
81	PZA02727_1	2	137.93	179.32	0.0000	Codominant

82	PHM10404_8	2	138.32	194.19	0.0000	Codominant
83	PHM13440_13	2	139.10	207.80	0.0000	Codominant
84	PZB00901_4	2	139.10	207.80	0.0000	Codominant
85	PHM635_23	2	139.48	220.00	0.0000	Codominant
86	S2_177750327	2	139.48	221.67	0.0000	Codominant
87	S2_185833014	2	139.48	215.00	0.0000	Codominant
88	S2_186160150	2	139.48	221.67	0.0000	Codominant
89	PZA02272_3	2	139.48	220.00	0.0000	Codominant
90	PHM499_19	2	139.48	218.33	0.0000	Codominant
91	PHM5535_8	2	139.48	218.33	0.0000	Codominant
92	PZA00365_2	2	139.48	218.33	0.0000	Codominant
93	PZA02681_8	2	139.48	216.67	0.0000	Codominant
94	PZA02890_4	2	139.48	214.72	0.0000	Codominant
95	PZA02450_1	2	139.48	214.72	0.0000	Codominant
96	PZA00538_15	3	0.00	11.21	0.0037	Codominant
97	PZB01457_1	3	4.24	19.68	0.0001	Codominant
98	PZA03527_1	3	44.13	18.39	0.0001	Codominant
99	PHM15475_27	3	49.27	11.72	0.0029	Codominant
100	S3_146602134	3	64.66	30.96	0.0000	Codominant
101	PZA00920_1	3	64.66	35.02	0.0000	Codominant
102	S3_133048570	3	65.26	32.47	0.0000	Codominant
103	S3_69767003	3	66.15	47.92	0.0000	Codominant
104	PZA02255_2	3	67.67	25.06	0.0000	Codominant
105	PZA00279_2	3	68.34	19.43	0.0001	Codominant
106	PZA01447_1	3	104.17	88.13	0.0000	Codominant
107	S3_150836832	3	115.80	216.67	0.0000	Codominant
108	PZA02402_1	3	115.80	213.05	0.0000	Codominant
109	S3_170110302	3	116.18	221.67	0.0000	Codominant
110	S3_156635016	3	116.18	220.00	0.0000	Codominant
111	S3_165911594	3	116.18	221.67	0.0000	Codominant
112	S3_50368120	3	116.18	220.00	0.0000	Codominant
113	S3_170370255	3	116.18	221.67	0.0000	Codominant
114	S3_170865263	3	116.18	220.00	0.0000	Codominant
115	S3_113429913	3	116.18	221.67	0.0000	Codominant
116	S3_53142920	3	116.18	220.00	0.0000	Codominant
117	PZB02044_1	3	116.18	218.33	0.0000	Codominant
118	S3_172668879	3	116.18	221.67	0.0000	Codominant
119	S3_101737919	3	116.18	220.00	0.0000	Codominant
120	S3_101903027	3	116.18	221.67	0.0000	Codominant
121	S3_154250438	3	116.18	165.00	0.0000	Codominant
122	PZA00088_3	3	116.18	220.00	0.0000	Codominant
123	PZA00402_1	3	116.18	220.00	0.0000	Codominant
124	PHM2919_23	3	116.18	215.00	0.0000	Codominant
125	PZA01688_3	3	116.18	213.05	0.0000	Codominant

126	PHM7672_7	3	116.18	213.05	0.0000	Codominant
127	PZA00316_10	3	116.18	216.67	0.0000	Codominant
128	S3_48493677	3	116.18	180.00	0.0000	Codominant
129	S3_68596995	3	116.18	213.04	0.0000	Codominant
130	S3_69321644	3	116.18	215.00	0.0000	Codominant
131	S3_90976749	3	116.57	202.63	0.0000	Codominant
132	PZA03733_1	3	117.37	194.21	0.0000	Codominant
133	PZA03735_1	3	117.37	189.23	0.0000	Codominant
134	PZA01931_17	3	117.37	184.23	0.0000	Codominant
135	PHM2343_25	3	117.37	195.99	0.0000	Codominant
136	S3_146363360	3	117.37	187.61	0.0000	Codominant
137	S3_146026612	3	117.37	185.94	0.0000	Codominant
138	S3_146966676	3	117.37	182.63	0.0000	Codominant
139	S3_146250249	3	117.37	184.28	0.0000	Codominant
140	PHM15449_10	3	117.75	187.51	0.0000	Codominant
141	S3_44062810	3	117.75	194.21	0.0000	Codominant
142	PZA00348_11	3	117.75	189.17	0.0000	Codominant
143	PZD00038_2	3	119.26	163.95	0.0000	Codominant
144	PZA01905_12	4	0.00	8.98	0.0112	Codominant
145	PZA03081_1	4	20.18	41.33	0.0000	Codominant
146	PZA00636_7	4	20.18	50.07	0.0000	Codominant
147	PZA00726_8	4	43.44	43.42	0.0000	Codominant
148	bt2_7	4	44.30	30.56	0.0000	Codominant
149	PZA00726_10	4	44.98	12.35	0.0021	Codominant
150	PHM1505_31	4	47.99	30.98	0.0000	Codominant
151	PZA02457_1	4	52.73	0.25	0.8841	Codominant
152	PHM687_25	4	58.08	17.20	0.0002	Codominant
153	S4_6544767	4	73.42	17.95	0.0001	Codominant
154	S4_9850443	4	76.59	35.09	0.0000	Codominant
155	S4_9741874	4	76.59	27.18	0.0000	Codominant
156	PHM16788_6	4	105.36	35.57	0.0000	Codominant
157	S4_229349149	4	105.36	3.33	0.1889	Codominant
158	PHM4117_14	4	105.36	189.21	0.0000	Codominant
159	PHM1971_20	4	105.75	187.51	0.0000	Codominant
160	PZA02027_1	4	105.75	210.00	0.0000	Codominant
161	S4_153520131	4	105.75	202.66	0.0000	Codominant
162	S4_229902280	4	106.14	213.33	0.0000	Codominant
163	S4_119182612	4	106.14	178.33	0.0000	Codominant
164	PZA03317_1	4	106.14	221.67	0.0000	Codominant
165	S4_79001676	4	106.14	221.67	0.0000	Codominant
166	S4_76048146	4	106.14	175.00	0.0000	Codominant
167	S4_17977654	4	106.14	220.00	0.0000	Codominant
168	S4_19430220	4	106.14	220.00	0.0000	Codominant
169	S4_149896839	4	106.14	220.00	0.0000	Codominant

170	PZA01187_1	4	106.14	214.72	0.0000	Codominant
171	PZD00030_2	4	106.14	216.38	0.0000	Codominant
172	S4_17742333	4	106.14	165.00	0.0000	Codominant
173	S4_15583336	4	106.14	210.00	0.0000	Codominant
174	S4_6601124	4	106.14	221.67	0.0000	Codominant
175	PZA01367_2	4	106.14	221.67	0.0000	Codominant
176	S4_155378923	4	106.14	170.00	0.0000	Codominant
177	S4_237313660	4	106.14	216.38	0.0000	Codominant
178	S4_233209591	4	106.14	165.00	0.0000	Codominant
179	S4_235381719	4	106.14	218.33	0.0000	Codominant
180	PZA02585_2	4	106.14	216.67	0.0000	Codominant
181	PZA03322_5	4	106.52	204.45	0.0000	Codominant
182	PZA03231_1	4	106.91	211.38	0.0000	Codominant
183	PHM18386_29	4	107.42	156.27	0.0000	Codominant
184	PZA03167_5	5	0.00	24.41	0.0000	Codominant
185	PHM563_9	5	7.05	36.98	0.0000	Codominant
186	S5_200938637	5	14.80	22.16	0.0000	Codominant
187	PHM662_27	5	47.98	43.33	0.0000	Codominant
188	PZA01294_1	5	74.14	49.76	0.0000	Codominant
189	PZA01796_1	5	78.21	35.55	0.0000	Codominant
190	PZA00148_3	5	78.21	54.15	0.0000	Codominant
191	PZA02164_16	5	82.37	32.87	0.0000	Codominant
192	PZA01693_1	5	82.70	11.49	0.0032	Codominant
193	PZA03677_1	5	85.14	26.38	0.0000	Codominant
194	PZA00522_12	5	87.04	29.40	0.0000	Codominant
195	PHM16854_3	5	89.44	33.03	0.0000	Codominant
196	PZA00996_1	5	89.44	26.23	0.0000	Codominant
197	PZA00934_2	5	90.30	30.80	0.0000	Codominant
198	S5_42339540	5	127.51	163.33	0.0000	Codominant
199	PHM533_46	5	127.51	197.65	0.0000	Codominant
200	PHM13675_18	5	127.51	194.33	0.0000	Codominant
201	PHM13675_17	5	127.51	195.99	0.0000	Codominant
202	S5_7153782	5	127.90	185.94	0.0000	Codominant
203	PZA01427_1	5	127.90	190.87	0.0000	Codominant
204	PZA02113_1	5	128.28	192.48	0.0000	Codominant
205	PZA01410_1	5	128.66	197.54	0.0000	Codominant
206	S5_170023977	5	129.05	213.33	0.0000	Codominant
207	S5_89094911	5	129.05	221.67	0.0000	Codominant
208	PHM7908_25	5	129.44	214.71	0.0000	Codominant
209	PHM6386_11	5	129.44	213.04	0.0000	Codominant
210	S5_202816906	5	129.44	170.00	0.0000	Codominant
211	S5_42297152	5	129.44	220.00	0.0000	Codominant
212	S5_201382475	5	129.44	220.00	0.0000	Codominant
213	S5_89288229	5	129.44	220.00	0.0000	Codominant

214	S5_217019076	5	129.44	218.33	0.0000	Codominant
215	PHM13639_13	5	129.44	221.67	0.0000	Codominant
216	S5_7240840	5	129.44	220.00	0.0000	Codominant
217	S5_170164477	5	129.44	220.00	0.0000	Codominant
218	S5_195686181	5	129.44	220.00	0.0000	Codominant
219	S5_196142088	5	129.44	158.33	0.0000	Codominant
220	PZA00273_5	5	129.44	221.67	0.0000	Codominant
221	PZA03226_3	5	129.44	216.67	0.0000	Codominant
222	PHM5484_22	5	129.44	211.20	0.0000	Codominant
223	PZA02818_6	5	129.44	216.67	0.0000	Codominant
224	PHM3762_18	5	129.44	216.38	0.0000	Codominant
225	PZA02792_26	5	129.44	216.38	0.0000	Codominant
226	PZB00765_1	5	129.44	216.67	0.0000	Codominant
227	PHM565_31	5	129.82	199.47	0.0000	Codominant
228	PZA02390_1	5	130.21	211.38	0.0000	Codominant
229	S5_201226926	5	130.60	204.54	0.0000	Codominant
230	PHM4468_13	6	0.00	39.58	0.0000	Codominant
231	S6_21007530	6	31.96	49.75	0.0000	Codominant
232	PZA00440_15	6	34.12	44.44	0.0000	Codominant
233	PHM8909_12	6	41.92	53.03	0.0000	Codominant
234	PZA00942_2	6	48.36	54.62	0.0000	Codominant
235	S6_125593444	6	63.35	22.90	0.0000	Codominant
236	S6_125019062	6	63.35	38.26	0.0000	Codominant
237	PZA01618_2	6	65.80	6.75	0.0342	Codominant
238	PZA01884_1	6	67.36	39.30	0.0000	Codominant
239	PZA02673_1	6	70.32	29.60	0.0000	Codominant
240	PZA00223_4	6	85.94	6.57	0.0375	Codominant
241	S6_156386857	6	117.66	170.00	0.0000	Codominant
242	PHM7922_8	6	117.66	220.00	0.0000	Codominant
243	PHM1572_17	6	117.66	221.67	0.0000	Codominant
244	S6_32967981	6	117.66	218.33	0.0000	Codominant
245	PZA00266_7	6	117.66	216.67	0.0000	Codominant
246	S6_87406549	6	117.66	218.33	0.0000	Codominant
247	S6_86345596	6	117.66	161.67	0.0000	Codominant
248	S6_18924381	6	117.66	221.67	0.0000	Codominant
249	PHM4503_25	6	117.66	208.33	0.0000	Codominant
250	S6_19369502	6	117.66	221.67	0.0000	Codominant
251	S6_89823772	6	117.66	220.00	0.0000	Codominant
252	PZA00427_3	6	117.66	216.37	0.0000	Codominant
253	PZB01009_1	6	118.05	206.13	0.0000	Codominant
254	PZA00910_1	6	118.43	211.20	0.0000	Codominant
255	PHM3466_69	6	118.43	206.39	0.0000	Codominant
256	PHM5529_4	6	118.43	207.87	0.0000	Codominant
257	PHM2658_129	6	118.81	201.13	0.0000	Codominant

258	S6_157568432	6	119.19	211.20	0.0000	Codominant
259	PHM4662_153	6	119.56	216.38	0.0000	Codominant
260	PZA03063_21	6	121.13	194.19	0.0000	Codominant
261	PZA01509_1	6	121.13	189.17	0.0000	Codominant
262	lac1_3	6	121.13	190.87	0.0000	Codominant
263	PZA02872_1	7	0.00	211.37	0.0000	Codominant
264	PZA02854_13	7	0.39	214.72	0.0000	Codominant
265	PZA00405_6	7	0.39	213.05	0.0000	Codominant
266	PZA00795_1	7	0.39	207.87	0.0000	Codominant
267	S7_137455469	7	0.77	206.21	0.0000	Codominant
268	PHM4818_15	7	1.15	214.72	0.0000	Codominant
269	S7_115310293	7	1.53	221.67	0.0000	Codominant
270	S7_128895684	7	1.53	215.00	0.0000	Codominant
271	PZA00986_1	7	1.53	220.00	0.0000	Codominant
272	S7_156215556	7	1.53	221.67	0.0000	Codominant
273	PHM1912_23	7	2.73	201.04	0.0000	Codominant
274	PHM7898_10	7	3.12	189.21	0.0000	Codominant
275	PHM9162_135	7	57.61	21.02	0.0000	Codominant
276	PZA00424_1	7	137.89	23.97	0.0000	Codominant
277	S8_141802902	8	0.00	216.38	0.0000	Codominant
278	PHM4677_11	8	0.38	221.67	0.0000	Codominant
279	PZB02155_1	8	0.38	221.67	0.0000	Codominant
280	PHM1978_111	8	0.38	220.00	0.0000	Codominant
281	PZA00951_1	8	0.38	216.38	0.0000	Codominant
282	PHM4552_6	8	0.38	216.38	0.0000	Codominant
283	PHM765_24	8	0.38	220.00	0.0000	Codominant
284	PHM4968_10	8	0.38	216.67	0.0000	Codominant
285	PHM4786_9	8	0.38	220.00	0.0000	Codominant
286	PZA01301_1	8	0.38	214.72	0.0000	Codominant
287	PZA01257_1	8	0.38	211.20	0.0000	Codominant
288	PHM14104_23	8	0.38	213.05	0.0000	Codominant
289	PHM15278_6	8	0.38	214.72	0.0000	Codominant
290	PHM4560_54	8	0.38	218.33	0.0000	Codominant
291	PZA00739_1	8	0.38	221.67	0.0000	Codominant
292	PHM3312_23	8	1.55	204.36	0.0000	Codominant
293	PHM9126_15	8	1.55	195.99	0.0000	Codominant
294	PZA01290_1	8	1.55	201.04	0.0000	Codominant
295	PZA02019_1	8	1.55	195.99	0.0000	Codominant
296	PHM5235_8	8	1.55	199.20	0.0000	Codominant
297	PZA00368_1	8	1.93	189.17	0.0000	Codominant
298	PZA00758_1	8	1.93	190.82	0.0000	Codominant
299	PZA01600_2	8	2.40	144.60	0.0000	Codominant
300	PZA01857_1	8	3.06	116.91	0.0000	Codominant
301	PHM12749_13	8	42.24	28.95	0.0000	Codominant

302	PZA02746_2	8	47.54	36.48	0.0000	Codominant
303	PHM4203_11	8	58.69	39.42	0.0000	Codominant
304	PZA01049_1	8	60.92	22.10	0.0000	Codominant
305	PHM5805_19	8	64.12	12.74	0.0017	Codominant
306	PZA00498_5	8	69.45	37.36	0.0000	Codominant
307	PHM5158_13	8	72.80	29.49	0.0000	Codominant
308	PZA01691_1	8	82.81	16.96	0.0002	Codominant
309	PZA02388_1	8	106.25	34.40	0.0000	Codominant
310	PZA01715_2	9	0.00	184.29	0.0000	Codominant
311	PZD00036_2	9	0.00	189.26	0.0000	Codominant
312	PHM4604_18	9	0.00	197.65	0.0000	Codominant
313	PZB00221_3	9	1.60	221.67	0.0000	Codominant
314	S9_113056055	9	2.36	209.54	0.0000	Codominant
315	PZA02648_2	9	2.74	218.33	0.0000	Codominant
316	PZA02235_14	9	2.74	213.05	0.0000	Codominant
317	S9_145906361	9	2.74	215.00	0.0000	Codominant
318	S9_108521912	9	2.74	221.67	0.0000	Codominant
319	PHM5185_13	9	2.74	221.67	0.0000	Codominant
320	S9_69569894	9	2.74	220.00	0.0000	Codominant
321	S9_114980354	9	2.74	220.00	0.0000	Codominant
322	PZB01358_1	9	2.74	220.00	0.0000	Codominant
323	PHM4689_49	9	2.74	221.67	0.0000	Codominant
324	S9_37149685	9	3.12	214.72	0.0000	Codominant
325	PZA00708_3	9	68.37	10.26	0.0059	Codominant
326	PZA00832_1	9	72.34	13.74	0.0010	Codominant
327	PHM7916_4	9	93.23	64.05	0.0000	Codominant
328	S9_109549230	9	103.53	20.75	0.0000	Codominant
329	PHM229_15	9	108.67	28.95	0.0000	Codominant
330	PZB00761_1	9	108.97	28.82	0.0000	Codominant
331	PHM697_21	9	112.00	37.63	0.0000	Codominant
332	sh1_11	9	125.71	40.87	0.0000	Codominant
333	PHM4066_11	10	0.00	151.26	0.0000	Codominant
334	PHM15868_56	10	0.98	194.34	0.0000	Codominant
335	PZA03713_1	10	0.98	189.37	0.0000	Codominant
336	PZA02961_6	10	1.38	194.33	0.0000	Codominant
337	PZA00814_1	10	3.41	199.47	0.0000	Codominant
338	PZA01642_1	10	4.16	209.54	0.0000	Codominant
339	PZA00866_2	10	4.94	194.30	0.0000	Codominant
340	PZA01313_2	10	6.12	214.72	0.0000	Codominant
341	S10_91086489	10	6.12	209.72	0.0000	Codominant
342	PHM1576_25	10	6.12	216.67	0.0000	Codominant
343	S10_132088679	10	6.12	221.67	0.0000	Codominant
344	S10_105877132	10	6.12	211.67	0.0000	Codominant
345	S10_113832226	10	6.12	220.00	0.0000	Codominant

346	S10_120670943	10	6.12	220.00	0.0000	Codominant
347	PHM557_21	10	6.12	220.00	0.0000	Codominant
348	PHM3631_47	10	6.12	220.00	0.0000	Codominant
349	S10_91685820	10	6.12	220.00	0.0000	Codominant
350	PZA02221_20	10	6.12	220.00	0.0000	Codominant
351	PHM5740_9	10	7.65	147.95	0.0000	Codominant
352	S10_97796845	10	9.08	171.67	0.0000	Codominant
353	PHM15331_16	10	45.89	22.95	0.0000	Codominant
354	PZA01877_2	10	52.39	20.28	0.0000	Codominant
355	PHM537_22	10	53.89	31.18	0.0000	Codominant
356	S10_113702444	10	54.72	38.26	0.0000	Codominant
357	PZA01456_2	10	63.70	34.34	0.0000	Codominant
358	PZA03605_1	10	73.12	20.48	0.0000	Codominant
359	PZA01001_2	10	86.60	34.36	0.0000	Codominant
360	PHM3736_11	10	90.82	23.44	0.0000	Codominant
361	S10_148638187	10	94.36	27.18	0.0000	Codominant
	MLNR-P16: ((CM543/CML444//CM543)DH6-B-B-B/KS23-5)F_3					
1	PHM1438_34	1	0.00	160.30	0.0000	Codominant
2	S1_2543968	1	5.79	243.33	0.0000	Codominant
3	PZA03037_2	1	5.79	245.00	0.0000	Codominant
4	PHM3563_17	1	5.79	243.33	0.0000	Codominant
5	PHM3463_18	1	5.79	245.00	0.0000	Codominant
6	PZA01019_1	1	5.79	245.00	0.0000	Codominant
7	PHM4695_5	1	5.79	245.00	0.0000	Codominant
8	PZA00030_11	1	5.79	243.33	0.0000	Codominant
9	csu1138_4	1	5.79	245.00	0.0000	Codominant
10	PHM1932_51	1	5.79	243.33	0.0000	Codominant
11	PHM12633_15	1	5.79	243.33	0.0000	Codominant
12	PZA03200_2	1	5.79	238.33	0.0000	Codominant
13	PZA00447_8	1	5.79	245.00	0.0000	Codominant
14	PZA02490_1	1	5.79	243.33	0.0000	Codominant
15	PZA00939_1	1	5.79	241.67	0.0000	Codominant
16	S1_26912154	1	5.79	243.33	0.0000	Codominant
17	PHM5597_15	1	5.79	245.00	0.0000	Codominant
18	PZA02279_1	1	5.79	245.00	0.0000	Codominant
19	S1_18838432	1	5.79	243.33	0.0000	Codominant
20	S1_25104820	1	5.79	236.67	0.0000	Codominant
21	PZA02467_10	1	5.79	215.92	0.0000	Codominant
22	PHM5306_16	1	5.79	219.24	0.0000	Codominant
23	PHM175_25	1	6.13	214.13	0.0000	Codominant
24	d8_3	1	6.47	215.92	0.0000	Codominant
25	S1_234122525	1	6.47	235.00	0.0000	Codominant
26	S1_16470688	1	6.47	240.00	0.0000	Codominant
27	S1_15353866	1	6.47	236.67	0.0000	Codominant

28	PZA02269_3	1	6.47	241.67	0.0000	Codominant
29	PZA01978_23	1	6.47	236.38	0.0000	Codominant
30	S1_23047822	1	6.82	227.74	0.0000	Codominant
31	S1_173654738	1	7.16	232.85	0.0000	Codominant
32	PZA02763_1	1	7.16	236.67	0.0000	Codominant
33	S1_85134292	1	7.16	245.00	0.0000	Codominant
34	S1_22744948	1	7.16	245.00	0.0000	Codominant
35	S1_173421054	1	7.16	245.00	0.0000	Codominant
36	PZA03561_1	1	7.16	243.33	0.0000	Codominant
37	PHM2130_29	1	7.16	241.67	0.0000	Codominant
38	PHM3147_18	1	7.16	243.33	0.0000	Codominant
39	PHM5622_21	1	7.16	245.00	0.0000	Codominant
40	PZA03742_1	1	7.16	245.00	0.0000	Codominant
41	S1_36800023	1	7.16	216.67	0.0000	Codominant
42	S1_36514510	1	40.74	37.10	0.0000	Codominant
43	S1_46411896	1	51.65	94.40	0.0000	Codominant
44	PHM12323_17	1	51.65	102.38	0.0000	Codominant
45	PZA01315_1	1	55.26	60.22	0.0000	Codominant
46	PZA01254_2	1	61.25	46.59	0.0000	Codominant
47	PHM2187_34	1	62.91	17.30	0.0002	Codominant
48	PHM4053_15	1	65.57	38.17	0.0000	Codominant
49	PZA03194_1	1	81.68	21.49	0.0000	Codominant
50	PHM12693_8	1	87.58	25.02	0.0000	Codominant
51	umc128_2	1	92.89	18.47	0.0001	Codominant
52	PHM4942_12	1	93.71	28.83	0.0000	Codominant
53	PZA03404_1	1	103.02	34.21	0.0000	Codominant
54	PZA02269_4	1	103.91	17.78	0.0001	Codominant
55	PHM14475_7	1	106.74	46.39	0.0000	Codominant
56	PZA03020_8	1	129.00	132.17	0.0000	Codominant
57	PHM7616_35	1	155.27	21.41	0.0000	Codominant
58	PHM4752_14	1	156.11	28.60	0.0000	Codominant
59	PZB00648_5	1	189.46	49.23	0.0000	Codominant
60	PZA00175_2	1	201.28	41.18	0.0000	Codominant
61	PZA02284_1	1	202.49	17.96	0.0001	Codominant
62	PHM4997_11	1	209.24	28.22	0.0000	Codominant
63	PZA00680_3	2	0.00	9.55	0.0085	Codominant
64	PHM13440_13	2	4.35	8.73	0.0127	Codominant
65	PZB00901_4	2	22.35	36.53	0.0000	Codominant
66	PZA00620_3	2	25.07	29.38	0.0000	Codominant
67	PZA03559_1	2	33.26	32.69	0.0000	Codominant
68	PZA02378_7	2	48.60	27.32	0.0000	Codominant
69	PHM10404_8	2	49.91	43.42	0.0000	Codominant
70	PZA02496_1	2	49.91	76.89	0.0000	Codominant
71	PZA00495_5	2	55.37	75.04	0.0000	Codominant

72	PZA00515_10	2	55.37	70.79	0.0000	Codominant
73	PZA03529_1	2	61.22	53.87	0.0000	Codominant
74	PHM3055_9	2	66.72	45.10	0.0000	Codominant
75	PHM793_25	2	69.94	67.70	0.0000	Codominant
76	PZB00772_7	2	78.18	54.86	0.0000	Codominant
77	PZA02727_1	2	94.19	43.23	0.0000	Codominant
78	PZA03629_1	2	144.11	127.89	0.0000	Codominant
79	PZA01232_1	2	150.45	159.46	0.0000	Codominant
80	PZA01885_2	2	150.45	210.94	0.0000	Codominant
81	PHM1511_14	2	150.45	220.92	0.0000	Codominant
82	PZA02564_2	2	150.45	219.24	0.0000	Codominant
83	PZA00613_22	2	150.45	222.58	0.0000	Codominant
84	PHM5535_8	2	151.88	245.00	0.0000	Codominant
85	S2_177750327	2	151.88	245.00	0.0000	Codominant
86	S2_185833014	2	151.88	243.33	0.0000	Codominant
87	S2_186160150	2	151.88	243.33	0.0000	Codominant
88	PZA02681_8	2	151.88	241.67	0.0000	Codominant
89	PHM499_19	2	151.88	243.33	0.0000	Codominant
90	PHM5817_15	2	151.88	236.38	0.0000	Codominant
91	PZA00365_2	2	151.88	243.33	0.0000	Codominant
92	PZA02272_3	2	151.88	243.33	0.0000	Codominant
93	PHM635_23	2	151.88	241.67	0.0000	Codominant
94	PZA02450_1	2	151.88	240.00	0.0000	Codominant
95	PZA02890_4	2	151.88	232.85	0.0000	Codominant
96	S3_150836832	3	0.00	83.27	0.0000	Codominant
97	PZA01447_1	3	5.54	146.95	0.0000	Codominant
98	S3_146602134	3	7.25	166.91	0.0000	Codominant
99	PZA00279_2	3	7.25	168.80	0.0000	Codominant
100	PZA00920_1	3	7.25	162.10	0.0000	Codominant
101	S3_146250249	3	7.25	159.68	0.0000	Codominant
102	S3_146363360	3	7.25	166.21	0.0000	Codominant
103	S3_133048570	3	7.25	172.04	0.0000	Codominant
104	PZA02255_2	3	7.25	167.18	0.0000	Codominant
105	S3_146026612	3	7.25	174.72	0.0000	Codominant
106	S3_69767003	3	7.25	160.75	0.0000	Codominant
107	S3_146966676	3	7.25	172.32	0.0000	Codominant
108	S3_154250438	3	10.44	193.33	0.0000	Codominant
109	PZA01688_3	3	10.44	238.05	0.0000	Codominant
110	PHM2919_23	3	10.44	241.67	0.0000	Codominant
111	PZA02402_1	3	10.44	236.38	0.0000	Codominant
112	PZA00088_3	3	10.44	240.00	0.0000	Codominant
113	PZA00402_1	3	10.44	241.67	0.0000	Codominant
114	PHM7672_7	3	10.44	245.00	0.0000	Codominant
115	S3_165911594	3	10.44	245.00	0.0000	Codominant

116	PZB02044_1	3	10.44	241.67	0.0000	Codominant
117	S3_156635016	3	10.44	243.33	0.0000	Codominant
118	PZA00316_10	3	10.44	245.00	0.0000	Codominant
119	S3_68596995	3	10.44	241.67	0.0000	Codominant
120	S3_172668879	3	10.44	245.00	0.0000	Codominant
121	S3_50368120	3	10.44	206.67	0.0000	Codominant
122	S3_53142920	3	10.44	241.67	0.0000	Codominant
123	S3_48493677	3	10.44	236.67	0.0000	Codominant
124	PHM2343_25	3	10.44	217.58	0.0000	Codominant
125	PHM15475_27	3	10.44	214.26	0.0000	Codominant
126	S3_44062810	3	10.44	207.50	0.0000	Codominant
127	PZA03527_1	3	10.44	214.13	0.0000	Codominant
128	PZD00038_2	3	12.49	182.13	0.0000	Codominant
129	PZA01931_17	3	14.59	215.92	0.0000	Codominant
130	S3_69321644	3	14.59	236.67	0.0000	Codominant
131	S3_170865263	3	14.59	245.00	0.0000	Codominant
132	S3_170370255	3	14.59	245.00	0.0000	Codominant
133	S3_170110302	3	14.59	245.00	0.0000	Codominant
134	S3_101737919	3	14.59	243.33	0.0000	Codominant
135	S3_101903027	3	14.59	245.00	0.0000	Codominant
136	S3_113429913	3	14.59	198.33	0.0000	Codominant
137	S3_90976749	3	16.39	135.88	0.0000	Codominant
138	PHM15449_10	3	41.72	21.41	0.0000	Codominant
139	PZA00348_11	3	43.28	35.59	0.0000	Codominant
140	PZA03733_1	3	55.66	28.99	0.0000	Codominant
141	PZA03735_1	3	55.66	35.27	0.0000	Codominant
142	PZA00538_15	3	75.11	28.27	0.0000	Codominant
143	PZB01457_1	3	81.41	42.91	0.0000	Codominant
144	bt2_7	4	0.00	178.86	0.0000	Codominant
145	PZA00726_8	4	0.00	173.95	0.0000	Codominant
146	PZA00726_10	4	0.00	186.16	0.0000	Codominant
147	S4_119182612	4	1.50	235.00	0.0000	Codominant
148	S4_17977654	4	1.50	243.33	0.0000	Codominant
149	PZA03081_1	4	1.50	243.33	0.0000	Codominant
150	PZD00030_2	4	1.50	241.67	0.0000	Codominant
151	PZA03317_1	4	1.50	245.00	0.0000	Codominant
152	S4_149896839	4	1.50	245.00	0.0000	Codominant
153	PZA01187_1	4	1.50	238.33	0.0000	Codominant
154	S4_155378923	4	1.50	191.67	0.0000	Codominant
155	PZA01367_2	4	1.50	243.33	0.0000	Codominant
156	S4_79001676	4	1.50	243.33	0.0000	Codominant
157	S4_76048146	4	1.50	193.33	0.0000	Codominant
158	PHM16788_6	4	1.50	243.33	0.0000	Codominant
159	S4_15583336	4	1.50	236.67	0.0000	Codominant

160	PZA02457_1	4	1.50	243.33	0.0000	Codominant
161	S4_19430220	4	1.50	243.33	0.0000	Codominant
162	S4_6544767	4	1.50	243.33	0.0000	Codominant
163	PZA02585_2	4	1.50	238.33	0.0000	Codominant
164	S4_237313660	4	1.50	245.00	0.0000	Codominant
165	S4_235381719	4	1.50	245.00	0.0000	Codominant
166	S4_6601124	4	1.50	245.00	0.0000	Codominant
167	S4_233209591	4	1.50	198.33	0.0000	Codominant
168	S4_229902280	4	1.50	235.00	0.0000	Codominant
169	S4_153520131	4	1.50	229.45	0.0000	Codominant
170	S4_229349149	4	1.50	178.33	0.0000	Codominant
171	S4_9741874	4	1.50	215.92	0.0000	Codominant
172	PHM18386_29	4	1.94	156.40	0.0000	Codominant
173	PHM1971_20	4	2.79	212.47	0.0000	Codominant
174	PZA02027_1	4	3.14	236.67	0.0000	Codominant
175	S4_17742333	4	3.14	193.33	0.0000	Codominant
176	S4_9850443	4	10.75	116.04	0.0000	Codominant
177	PHM687_25	4	45.60	42.73	0.0000	Codominant
178	PHM1505_31	4	51.07	54.87	0.0000	Codominant
179	PZA03231_1	4	56.98	16.04	0.0003	Codominant
180	PZA01905_12	4	80.86	28.39	0.0000	Codominant
181	PZA03322_5	4	86.02	45.18	0.0000	Codominant
182	PZA00636_7	4	102.79	38.87	0.0000	Codominant
183	PHM4117_14	4	103.59	57.37	0.0000	Codominant
184	PHM662_27	5	0.00	16.01	0.0003	Codominant
185	S5_7153782	5	24.86	33.24	0.0000	Codominant
186	PZA00934_2	5	42.43	24.31	0.0000	Codominant
187	PZA01427_1	5	43.22	26.10	0.0000	Codominant
188	PHM565_31	5	43.22	25.12	0.0000	Codominant
189	PZA03677_1	5	47.35	24.47	0.0000	Codominant
190	PZA00273_5	5	47.87	24.55	0.0000	Codominant
191	PZA01796_1	5	49.79	21.69	0.0000	Codominant
192	PHM563_9	5	86.31	33.02	0.0000	Codominant
193	S5_202816906	5	124.82	191.67	0.0000	Codominant
194	PZA02390_1	5	124.82	243.33	0.0000	Codominant
195	PHM13639_13	5	124.82	240.00	0.0000	Codominant
196	PZA01294_1	5	124.82	241.67	0.0000	Codominant
197	S5_89094911	5	124.82	245.00	0.0000	Codominant
198	S5_195686181	5	124.82	245.00	0.0000	Codominant
199	PZA03226_3	5	124.82	241.67	0.0000	Codominant
200	S5_170164477	5	124.82	245.00	0.0000	Codominant
201	PZA00522_12	5	124.82	241.67	0.0000	Codominant
202	S5_89288229	5	124.82	176.67	0.0000	Codominant
203	S5_42339540	5	124.82	231.38	0.0000	Codominant

204	PZA01410_1	5	125.18	219.24	0.0000	Codominant
205	S5_200938637	5	125.18	217.58	0.0000	Codominant
206	PZA01693_1	5	125.18	217.58	0.0000	Codominant
207	PHM13675_18	5	125.18	215.92	0.0000	Codominant
208	PZA02164_16	5	125.18	217.58	0.0000	Codominant
209	PHM13675_17	5	125.18	215.92	0.0000	Codominant
210	PZA00996_1	5	125.18	215.92	0.0000	Codominant
211	PHM16854_3	5	125.18	210.82	0.0000	Codominant
212	PZA03167_5	5	125.52	210.73	0.0000	Codominant
213	PZA02113_1	5	125.86	207.46	0.0000	Codominant
214	PHM533_46	5	125.86	215.92	0.0000	Codominant
215	S5_170023977	5	125.86	235.00	0.0000	Codominant
216	S5_217019076	5	125.86	245.00	0.0000	Codominant
217	PHM6386_11	5	125.86	241.67	0.0000	Codominant
218	PHM7908_25	5	125.86	245.00	0.0000	Codominant
219	PZB00765_1	5	125.86	238.33	0.0000	Codominant
220	S5_42297152	5	125.86	245.00	0.0000	Codominant
221	S5_201382475	5	125.86	245.00	0.0000	Codominant
222	S5_201226926	5	125.86	240.00	0.0000	Codominant
223	S5_7240840	5	125.86	245.00	0.0000	Codominant
224	S5_196142088	5	125.86	190.00	0.0000	Codominant
225	PZA02818_6	5	125.86	240.00	0.0000	Codominant
226	PHM3762_18	5	125.86	227.85	0.0000	Codominant
227	PZA02792_26	5	125.86	240.00	0.0000	Codominant
228	PHM5484_22	5	126.21	229.41	0.0000	Codominant
229	PZA00148_3	5	131.60	162.67	0.0000	Codominant
230	S6_21007530	6	0.00	96.22	0.0000	Codominant
231	S6_125019062	6	7.56	162.23	0.0000	Codominant
232	PHM2658_129	6	14.00	173.72	0.0000	Codominant
233	PZA03063_21	6	16.57	214.26	0.0000	Codominant
234	PHM8909_12	6	16.92	212.39	0.0000	Codominant
235	PZA00942_2	6	18.69	238.05	0.0000	Codominant
236	S6_156386857	6	19.14	181.39	0.0000	Codominant
237	PZA00266_7	6	19.14	241.67	0.0000	Codominant
238	PZA01884_1	6	19.14	241.67	0.0000	Codominant
239	PHM7922_8	6	19.14	243.33	0.0000	Codominant
240	PHM4662_153	6	19.14	245.00	0.0000	Codominant
241	S6_18924381	6	19.14	245.00	0.0000	Codominant
242	S6_87406549	6	19.14	241.67	0.0000	Codominant
243	S6_86345596	6	19.14	243.33	0.0000	Codominant
244	PHM4503_25	6	19.14	245.00	0.0000	Codominant
245	PZA00440_15	6	19.14	243.33	0.0000	Codominant
246	PHM1572_17	6	19.14	243.33	0.0000	Codominant
247	S6_32967981	6	19.14	241.67	0.0000	Codominant

248	PZA00427_3	6	19.14	243.33	0.0000	Codominant
249	S6_89823772	6	19.14	208.33	0.0000	Codominant
250	PZA02673_1	6	43.77	40.56	0.0000	Codominant
251	lac1_3	6	58.87	32.92	0.0000	Codominant
252	S6_125593444	6	69.93	10.55	0.0051	Codominant
253	PZA00223_4	6	84.74	7.38	0.0250	Codominant
254	S6_157568432	6	87.36	54.14	0.0000	Codominant
255	PZA01618_2	6	104.42	172.75	0.0000	Codominant
256	PZB01009_1	6	126.48	61.19	0.0000	Codominant
257	PZA01509_1	6	129.72	61.19	0.0000	Codominant
258	S6_19369502	6	136.41	20.47	0.0000	Codominant
259	PHM4468_13	6	159.23	28.69	0.0000	Codominant
260	PHM3466_69	6	162.22	24.04	0.0000	Codominant
261	PHM5529_4	6	162.22	40.65	0.0000	Codominant
262	PZA00910_1	6	163.22	39.94	0.0000	Codominant
263	PHM4818_15	7	0.00	104.06	0.0000	Codominant
264	PZA00795_1	7	11.97	238.33	0.0000	Codominant
265	PZA00986_1	7	11.97	245.00	0.0000	Codominant
266	PHM1912_23	7	13.77	217.58	0.0000	Codominant
267	PZA00424_1	7	13.77	210.82	0.0000	Codominant
268	S7_156215556	7	15.95	245.00	0.0000	Codominant
269	S7_115310293	7	15.95	245.00	0.0000	Codominant
270	PZA00405_6	7	15.95	245.00	0.0000	Codominant
271	S7_128895684	7	15.95	183.33	0.0000	Codominant
272	PZA02872_1	7	33.68	26.89	0.0000	Codominant
273	S7_137455469	7	68.34	17.59	0.0002	Codominant
274	PHM9162_135	7	79.38	48.06	0.0000	Codominant
275	PZA02854_13	7	79.38	14.73	0.0006	Codominant
276	PHM7898_10	7	109.49	41.07	0.0000	Codominant
277	PHM4203_11	8	0.00	23.29	0.0000	Codominant
278	PHM4552_6	8	9.46	28.31	0.0000	Codominant
279	PZA00498_5	8	9.46	36.04	0.0000	Codominant
280	PZA01691_1	8	26.27	16.95	0.0002	Codominant
281	PZA00368_1	8	32.35	47.92	0.0000	Codominant
282	PHM9126_15	8	61.15	92.00	0.0000	Codominant
283	PZA00758_1	8	77.05	215.78	0.0000	Codominant
284	PHM5235_8	8	77.39	219.24	0.0000	Codominant
285	PZA02019_1	8	77.39	217.58	0.0000	Codominant
286	PZA01049_1	8	77.39	214.13	0.0000	Codominant
287	PHM5158_13	8	77.39	212.47	0.0000	Codominant
288	PZA01290_1	8	77.39	214.12	0.0000	Codominant
289	PHM5805_19	8	77.39	209.16	0.0000	Codominant
290	PZA02388_1	8	77.39	209.07	0.0000	Codominant
291	PZA01857_1	8	77.39	214.26	0.0000	Codominant

292	PZA01600_2	8	77.39	164.50	0.0000	Codominant
293	PHM3312_23	8	77.39	229.48	0.0000	Codominant
294	PZA00739_1	8	78.10	245.00	0.0000	Codominant
295	PZA01301_1	8	78.10	240.00	0.0000	Codominant
296	PHM14104_23	8	78.10	234.71	0.0000	Codominant
297	PHM12749_13	8	78.10	241.67	0.0000	Codominant
298	PHM15278_6	8	78.10	236.38	0.0000	Codominant
299	PHM765_24	8	78.10	238.05	0.0000	Codominant
300	PHM4560_54	8	78.10	235.00	0.0000	Codominant
301	PHM4786_9	8	78.10	243.33	0.0000	Codominant
302	S8_141802902	8	78.10	243.33	0.0000	Codominant
303	PZA02746_2	8	78.10	245.00	0.0000	Codominant
304	PZA01257_1	8	78.10	241.67	0.0000	Codominant
305	PHM4968_10	8	78.10	243.33	0.0000	Codominant
306	PHM1978_111	8	78.10	243.33	0.0000	Codominant
307	PHM4677_11	8	78.10	175.00	0.0000	Codominant
308	PZB02155_1	8	78.10	241.67	0.0000	Codominant
309	PZA00951_1	8	78.10	236.38	0.0000	Codominant
310	PZA00832_1	9	0.00	53.11	0.0000	Codominant
311	PZD00036_2	9	31.26	62.56	0.0000	Codominant
312	sh1_11	9	40.05	49.02	0.0000	Codominant
313	S9_109549230	9	55.34	54.53	0.0000	Codominant
314	PHM229_15	9	67.01	24.60	0.0000	Codominant
315	PZA01715_2	9	96.85	207.62	0.0000	Codominant
316	PZB00761_1	9	96.85	214.26	0.0000	Codominant
317	PHM4604_18	9	96.85	219.24	0.0000	Codominant
318	PHM697_21	9	97.21	205.84	0.0000	Codominant
319	PHM7916_4	9	97.57	209.07	0.0000	Codominant
320	PZB00221_3	9	99.74	239.71	0.0000	Codominant
321	PZA00708_3	9	99.74	234.71	0.0000	Codominant
322	PZA02235_14	9	100.09	238.05	0.0000	Codominant
323	PHM4689_49	9	100.43	245.00	0.0000	Codominant
324	S9_113056055	9	100.77	236.38	0.0000	Codominant
325	PHM5185_13	9	101.12	245.00	0.0000	Codominant
326	S9_145906361	9	101.12	243.33	0.0000	Codominant
327	S9_114980354	9	101.12	240.00	0.0000	Codominant
328	S9_108521912	9	101.12	241.67	0.0000	Codominant
329	S9_37149685	9	101.12	243.33	0.0000	Codominant
330	PZB01358_1	9	101.12	245.00	0.0000	Codominant
331	S9_69569894	9	101.46	236.38	0.0000	Codominant
332	PZA02648_2	9	113.11	102.17	0.0000	Codominant
333	PHM15868_56	10	0.00	150.26	0.0000	Codominant
334	PZA01456_2	10	1.69	127.46	0.0000	Codominant
335	PZA02961_6	10	8.41	214.26	0.0000	Codominant

336	PZA03713_1	10	8.41	210.94	0.0000	Codominant
337	PZA01001_2	10	10.26	234.51	0.0000	Codominant
338	PHM3736_11	10	10.26	236.38	0.0000	Codominant
339	PHM4066_11	10	10.26	189.35	0.0000	Codominant
340	PZA01313_2	10	10.26	240.00	0.0000	Codominant
341	PZA00814_1	10	10.26	243.33	0.0000	Codominant
342	PZA02221_20	10	10.26	245.00	0.0000	Codominant
343	S10_132088679	10	10.26	245.00	0.0000	Codominant
344	PHM1576_25	10	10.26	245.00	0.0000	Codominant
345	PHM557_21	10	10.26	245.00	0.0000	Codominant
346	S10_120670943	10	10.26	240.00	0.0000	Codominant
347	S10_113832226	10	10.26	245.00	0.0000	Codominant
348	S10_113702444	10	10.26	243.33	0.0000	Codominant
349	S10_105877132	10	10.26	245.00	0.0000	Codominant
350	PZA01877_2	10	10.26	245.00	0.0000	Codominant
351	PHM537_22	10	10.26	243.33	0.0000	Codominant
352	S10_97796845	10	10.26	195.00	0.0000	Codominant
353	S10_91685820	10	10.26	241.67	0.0000	Codominant
354	PHM3631_47	10	10.26	193.04	0.0000	Codominant
355	PHM15331_16	10	44.53	36.15	0.0000	Codominant
356	PHM5740_9	10	51.38	28.04	0.0000	Codominant
357	PZA01642_1	10	54.84	40.71	0.0000	Codominant
358	S10_91086489	10	60.77	51.50	0.0000	Codominant
359	PZA00866_2	10	63.43	29.23	0.0000	Codominant
360	PZA03605_1	10	84.26	26.52	0.0000	Codominant
361	S10_148638187	10	104.90	29.07	0.0000	Codominant
	MLNR-P13: (CM543/KS23-5)F_3					
1	PHM4752_14	1	0.00	18.26	0.0001	Codominant
2	PHM7616_35	1	3.46	14.86	0.0006	Codominant
3	PHM12323_17	1	26.68	16.76	0.0002	Codominant
4	S1_46411896	1	30.84	18.99	0.0001	Codominant
5	PZA01315_1	1	40.13	19.02	0.0001	Codominant
6	PZA01254_2	1	52.61	22.98	0.0000	Codominant
7	PHM2187_34	1	55.95	7.01	0.0301	Codominant
8	PHM4053_15	1	58.86	38.03	0.0000	Codominant
9	PZA03194_1	1	79.41	9.58	0.0083	Codominant
10	PHM4942_12	1	86.87	15.14	0.0005	Codominant
11	PHM12693_8	1	92.12	18.90	0.0001	Codominant
12	umc128_2	1	96.19	13.37	0.0013	Codominant
13	PZA03404_1	1	101.98	30.40	0.0000	Codominant
14	PZA02269_4	1	103.02	2.43	0.2961	Codominant
15	PHM14475_7	1	105.28	23.20	0.0000	Codominant
16	PZA03020_8	1	120.59	125.57	0.0000	Codominant
17	PZA00175_2	1	135.53	83.35	0.0000	Codominant

18	PZA02284_1	1	137.79	26.93	0.0000	Codominant
19	PHM4997_11	1	141.88	40.07	0.0000	Codominant
20	PZB00648_5	1	155.78	25.05	0.0000	Codominant
21	S1_36514510	1	177.62	14.02	0.0009	Codominant
22	S1_36800023	1	203.24	138.33	0.0000	Codominant
23	PHM3563_17	1	203.24	163.33	0.0000	Codominant
24	S1_25104820	1	203.24	158.33	0.0000	Codominant
25	S1_16470688	1	203.24	149.74	0.0000	Codominant
26	PHM3463_18	1	203.24	153.07	0.0000	Codominant
27	PZA01978_23	1	203.24	146.28	0.0000	Codominant
28	PHM2130_29	1	203.24	154.74	0.0000	Codominant
29	PZA03200_2	1	203.76	147.95	0.0000	Codominant
30	PHM5306_16	1	204.31	134.43	0.0000	Codominant
31	d8_3	1	204.31	129.48	0.0000	Codominant
32	PHM175_25	1	204.86	141.18	0.0000	Codominant
33	PZA02467_10	1	204.86	141.18	0.0000	Codominant
34	S1_234122525	1	204.86	158.33	0.0000	Codominant
35	S1_173421054	1	204.86	161.67	0.0000	Codominant
36	S1_26912154	1	204.86	163.33	0.0000	Codominant
37	S1_22744948	1	204.86	163.33	0.0000	Codominant
38	PZA02279_1	1	204.86	161.67	0.0000	Codominant
39	PHM4695_5	1	204.86	161.67	0.0000	Codominant
40	PZA03742_1	1	204.86	160.00	0.0000	Codominant
41	PZA00030_11	1	204.86	153.07	0.0000	Codominant
42	PZA00939_1	1	204.86	151.40	0.0000	Codominant
43	PHM1932_51	1	204.86	156.67	0.0000	Codominant
44	S1_85134292	1	204.86	163.33	0.0000	Codominant
45	PZA03561_1	1	204.86	161.67	0.0000	Codominant
46	PZA02490_1	1	204.86	163.33	0.0000	Codominant
47	PZA02763_1	1	204.86	158.33	0.0000	Codominant
48	S1_18838432	1	204.86	154.74	0.0000	Codominant
49	PHM5622_21	1	205.39	142.96	0.0000	Codominant
50	PZA00447_8	1	205.92	133.42	0.0000	Codominant
51	S1_173654738	1	206.96	128.42	0.0000	Codominant
52	PZA02269_3	1	206.96	142.96	0.0000	Codominant
53	csu1138_4	1	206.96	144.62	0.0000	Codominant
54	PHM1438_34	1	208.04	151.27	0.0000	Codominant
55	PZA03037_2	1	208.04	155.00	0.0000	Codominant
56	S1_2543968	1	208.04	161.67	0.0000	Codominant
57	S1_15353866	1	208.04	163.33	0.0000	Codominant
58	PHM5597_15	1	208.04	161.67	0.0000	Codominant
59	PHM12633_15	1	208.04	161.67	0.0000	Codominant
60	PZA01019_1	1	208.04	163.33	0.0000	Codominant
61	PHM3147_18	1	208.04	161.67	0.0000	Codominant

62	S1_23047822	1	210.83	123.49	0.0000	Codominant
63	PZA02727_1	2	0.00	130.50	0.0000	Codominant
64	PZA01885_2	2	2.13	132.79	0.0000	Codominant
65	PHM1511_14	2	2.68	156.67	0.0000	Codominant
66	PZA02681_8	2	2.68	163.33	0.0000	Codominant
67	PZA02890_4	2	2.68	163.33	0.0000	Codominant
68	PHM635_23	2	2.68	158.33	0.0000	Codominant
69	PZA02450_1	2	2.68	158.33	0.0000	Codominant
70	S2_177750327	2	2.68	163.33	0.0000	Codominant
71	S2_185833014	2	2.68	161.67	0.0000	Codominant
72	S2_186160150	2	2.68	163.33	0.0000	Codominant
73	PHM5535_8	2	2.68	161.67	0.0000	Codominant
74	PZA02272_3	2	3.21	147.95	0.0000	Codominant
75	PZA00365_2	2	3.75	151.40	0.0000	Codominant
76	PHM499_19	2	4.29	144.63	0.0000	Codominant
77	PHM5817_15	2	4.81	147.95	0.0000	Codominant
78	PZA00613_22	2	4.81	153.33	0.0000	Codominant
79	PZA02564_2	2	4.81	147.82	0.0000	Codominant
80	PZA01232_1	2	4.81	108.33	0.0000	Codominant
81	PZA02496_1	2	24.01	34.27	0.0000	Codominant
82	PZA02378_7	2	30.88	21.73	0.0000	Codominant
83	PHM10404_8	2	46.60	17.75	0.0001	Codominant
84	PZA03629_1	2	49.47	22.96	0.0000	Codominant
85	PZA00495_5	2	57.53	20.54	0.0000	Codominant
86	PZA00515_10	2	58.32	22.79	0.0000	Codominant
87	PZA03529_1	2	63.35	10.62	0.0049	Codominant
88	PHM3055_9	2	66.54	11.94	0.0026	Codominant
89	PHM793_25	2	69.05	25.85	0.0000	Codominant
90	PZB00772_7	2	76.92	29.21	0.0000	Codominant
91	PZA03559_1	2	100.85	38.77	0.0000	Codominant
92	PZA00620_3	2	107.94	40.36	0.0000	Codominant
93	PZB00901_4	2	110.32	37.78	0.0000	Codominant
94	PHM13440_13	2	123.04	28.19	0.0000	Codominant
95	PZA00680_3	2	127.40	23.51	0.0000	Codominant
96	PZB01457_1	3	0.00	17.73	0.0001	Codominant
97	PZA00538_15	3	4.94	18.17	0.0001	Codominant
98	PZA03733_1	3	21.10	23.11	0.0000	Codominant
99	PZA03735_1	3	21.10	25.50	0.0000	Codominant
100	PHM15449_10	3	37.59	46.95	0.0000	Codominant
101	PZA00348_11	3	40.29	40.09	0.0000	Codominant
102	S3_90976749	3	76.76	61.94	0.0000	Codominant
103	S3_113429913	3	78.24	120.00	0.0000	Codominant
104	S3_101737919	3	78.24	158.33	0.0000	Codominant
105	S3_101903027	3	78.24	163.33	0.0000	Codominant

106	S3_48493677	3	78.24	160.00	0.0000	Codominant
107	S3_156635016	3	78.24	163.33	0.0000	Codominant
108	S3_165911594	3	78.24	163.33	0.0000	Codominant
109	PHM7672_7	3	78.24	161.67	0.0000	Codominant
110	S3_170110302	3	78.24	161.67	0.0000	Codominant
111	S3_170370255	3	78.24	161.67	0.0000	Codominant
112	S3_170865263	3	78.24	163.33	0.0000	Codominant
113	S3_172668879	3	78.24	163.33	0.0000	Codominant
114	PZA00402_1	3	78.24	161.67	0.0000	Codominant
115	PZB02044_1	3	78.24	156.67	0.0000	Codominant
116	S3_69321644	3	78.24	160.00	0.0000	Codominant
117	S3_154250438	3	78.24	130.00	0.0000	Codominant
118	S3_50368120	3	78.24	120.00	0.0000	Codominant
119	PZA00316_10	3	78.24	160.00	0.0000	Codominant
120	S3_53142920	3	78.24	163.33	0.0000	Codominant
121	S3_146966676	3	78.79	148.07	0.0000	Codominant
122	PZA00088_3	3	79.33	152.94	0.0000	Codominant
123	S3_146602134	3	79.85	154.74	0.0000	Codominant
124	S3_146250249	3	79.85	154.74	0.0000	Codominant
125	S3_146026612	3	79.85	152.94	0.0000	Codominant
126	S3_133048570	3	80.36	151.27	0.0000	Codominant
127	PZA00279_2	3	80.87	142.96	0.0000	Codominant
128	PZA02402_1	3	80.87	131.77	0.0000	Codominant
129	PZA00920_1	3	80.87	139.65	0.0000	Codominant
130	S3_69767003	3	80.87	135.07	0.0000	Codominant
131	S3_68596995	3	80.87	146.41	0.0000	Codominant
132	S3_146363360	3	80.87	146.11	0.0000	Codominant
133	PZA02255_2	3	80.87	149.72	0.0000	Codominant
134	S3_150836832	3	81.56	105.04	0.0000	Codominant
135	PZA01447_1	3	83.57	134.44	0.0000	Codominant
136	PZA01931_17	3	83.57	134.44	0.0000	Codominant
137	PZD00038_2	3	84.64	117.13	0.0000	Codominant
138	S3_44062810	3	86.24	131.13	0.0000	Codominant
139	PHM2343_25	3	87.30	147.82	0.0000	Codominant
140	PZA03527_1	3	87.30	144.50	0.0000	Codominant
141	PHM15475_27	3	87.30	149.56	0.0000	Codominant
142	PHM2919_23	3	87.30	151.40	0.0000	Codominant
143	PZA01688_3	3	88.88	127.70	0.0000	Codominant
144	PHM18386_29	4	0.00	69.52	0.0000	Codominant
145	PHM1971_20	4	1.59	129.45	0.0000	Codominant
146	S4_9741874	4	2.16	127.79	0.0000	Codominant
147	PZA02027_1	4	2.74	158.33	0.0000	Codominant
148	S4_79001676	4	2.74	163.33	0.0000	Codominant
149	S4_149896839	4	2.74	161.67	0.0000	Codominant

150	PZA03231_1	4	3.29	144.62	0.0000	Codominant
151	PHM16788_6	4	3.83	144.62	0.0000	Codominant
152	S4_9850443	4	4.37	142.96	0.0000	Codominant
153	S4_6544767	4	4.90	146.29	0.0000	Codominant
154	PZA02457_1	4	5.40	151.27	0.0000	Codominant
155	S4_19430220	4	6.43	139.78	0.0000	Codominant
156	S4_153520131	4	8.03	136.47	0.0000	Codominant
157	PZA02585_2	4	8.60	146.41	0.0000	Codominant
158	S4_229349149	4	8.60	116.67	0.0000	Codominant
159	PZA01187_1	4	8.60	154.74	0.0000	Codominant
160	PZD00030_2	4	8.60	160.00	0.0000	Codominant
161	PZA03081_1	4	8.60	161.67	0.0000	Codominant
162	PZA01367_2	4	8.60	163.33	0.0000	Codominant
163	S4_17977654	4	8.60	163.33	0.0000	Codominant
164	S4_17742333	4	8.60	125.00	0.0000	Codominant
165	S4_15583336	4	8.60	161.67	0.0000	Codominant
166	S4_229902280	4	8.60	163.33	0.0000	Codominant
167	S4_233209591	4	8.60	123.33	0.0000	Codominant
168	S4_237313660	4	8.60	163.33	0.0000	Codominant
169	S4_6601124	4	8.60	163.33	0.0000	Codominant
170	S4_235381719	4	8.60	161.67	0.0000	Codominant
171	PZA03322_5	4	8.60	149.74	0.0000	Codominant
172	PZA01905_12	4	8.60	158.33	0.0000	Codominant
173	S4_76048146	4	8.60	128.33	0.0000	Codominant
174	S4_155378923	4	8.60	131.67	0.0000	Codominant
175	PZA03317_1	4	8.60	163.33	0.0000	Codominant
176	S4_119182612	4	8.60	130.00	0.0000	Codominant
177	PZA00726_10	4	39.06	8.66	0.0132	Codominant
178	PZA00726_8	4	39.98	35.83	0.0000	Codominant
179	bt2_7	4	40.38	21.66	0.0000	Codominant
180	PHM1505_31	4	40.38	23.60	0.0000	Codominant
181	PHM687_25	4	46.30	22.20	0.0000	Codominant
182	PHM4117_14	4	73.09	27.93	0.0000	Codominant
183	PZA00636_7	4	74.84	20.59	0.0000	Codominant
184	PZA02113_1	5	0.00	141.05	0.0000	Codominant
185	PZA00996_1	5	0.52	139.40	0.0000	Codominant
186	PZA01693_1	5	0.52	142.90	0.0000	Codominant
187	PZA01410_1	5	0.52	144.50	0.0000	Codominant
188	PHM13675_18	5	0.52	141.18	0.0000	Codominant
189	PZA01796_1	5	0.52	129.45	0.0000	Codominant
190	PHM13675_17	5	0.52	141.18	0.0000	Codominant
191	PZA02164_16	5	0.52	129.63	0.0000	Codominant
192	PHM662_27	5	1.04	117.13	0.0000	Codominant
193	PHM16854_3	5	1.04	132.75	0.0000	Codominant

194	PHM533_46	5	1.04	131.13	0.0000	Codominant
195	S5_200938637	5	1.04	131.10	0.0000	Codominant
196	PHM565_31	5	1.04	131.21	0.0000	Codominant
197	PZA03167_5	5	2.09	133.42	0.0000	Codominant
198	PZA00522_12	5	3.16	146.28	0.0000	Codominant
199	PHM7908_25	5	3.16	142.96	0.0000	Codominant
200	PHM6386_11	5	3.16	138.12	0.0000	Codominant
201	PZA01294_1	5	3.16	131.77	0.0000	Codominant
202	PZA00148_3	5	3.16	138.12	0.0000	Codominant
203	S5_201226926	5	3.16	155.00	0.0000	Codominant
204	PHM3762_18	5	3.16	151.40	0.0000	Codominant
205	PZA02792_26	5	3.16	154.74	0.0000	Codominant
206	PHM5484_22	5	3.16	151.27	0.0000	Codominant
207	PZB00765_1	5	3.16	149.74	0.0000	Codominant
208	S5_7153782	5	3.16	158.33	0.0000	Codominant
209	PHM13639_13	5	3.16	160.00	0.0000	Codominant
210	PZA02390_1	5	3.16	161.67	0.0000	Codominant
211	PZA02818_6	5	3.16	163.33	0.0000	Codominant
212	S5_202816906	5	3.16	126.67	0.0000	Codominant
213	S5_89094911	5	3.16	161.67	0.0000	Codominant
214	S5_170023977	5	3.16	156.67	0.0000	Codominant
215	S5_170164477	5	3.16	163.33	0.0000	Codominant
216	PZA03226_3	5	3.16	161.67	0.0000	Codominant
217	S5_42339540	5	3.16	118.33	0.0000	Codominant
218	S5_195686181	5	3.16	161.67	0.0000	Codominant
219	S5_196142088	5	3.16	125.00	0.0000	Codominant
220	S5_7240840	5	3.16	163.33	0.0000	Codominant
221	S5_201382475	5	3.16	163.33	0.0000	Codominant
222	S5_217019076	5	3.16	163.33	0.0000	Codominant
223	S5_89288229	5	3.16	131.67	0.0000	Codominant
224	PZA00273_5	5	40.76	26.10	0.0000	Codominant
225	PZA03677_1	5	42.36	27.18	0.0000	Codominant
226	S5_42297152	5	42.36	11.43	0.0033	Codominant
227	PZA00934_2	5	43.24	22.60	0.0000	Codominant
228	PZA01427_1	5	43.63	24.82	0.0000	Codominant
229	PHM563_9	5	66.62	15.53	0.0004	Codominant
230	S6_157568432	6	0.00	123.03	0.0000	Codominant
231	PZA00223_4	6	1.10	137.74	0.0000	Codominant
232	PHM2658_129	6	1.67	127.79	0.0000	Codominant
233	S6_21007530	6	2.49	83.84	0.0000	Codominant
234	PHM8909_12	6	4.00	135.07	0.0000	Codominant
235	PZA00942_2	6	5.56	151.27	0.0000	Codominant
236	S6_18924381	6	6.59	163.33	0.0000	Codominant
237	PHM4503_25	6	7.12	153.07	0.0000	Codominant

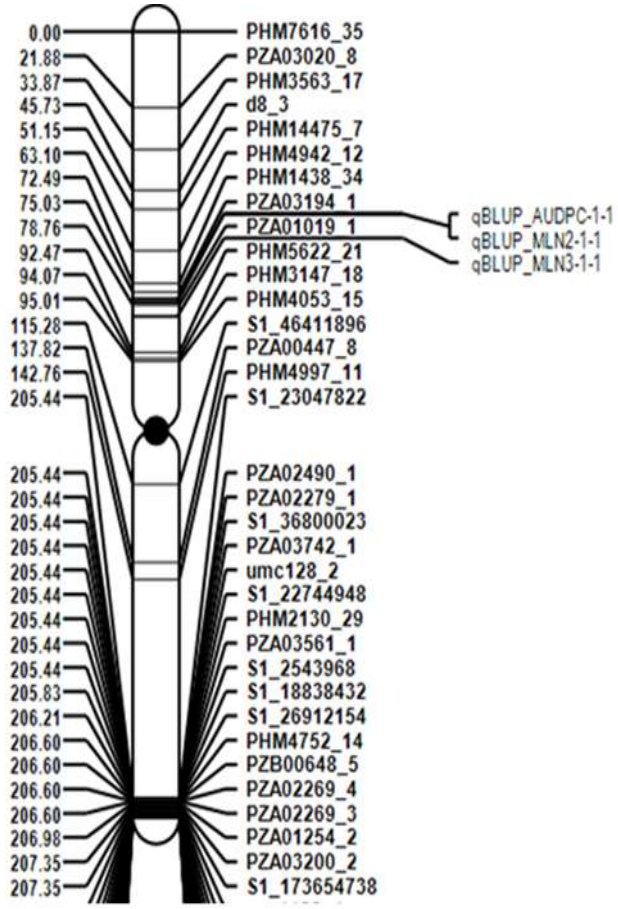
238	PZA00266_7	6	7.12	160.00	0.0000	Codominant
239	S6_87406549	6	7.12	158.33	0.0000	Codominant
240	S6_86345596	6	7.12	136.67	0.0000	Codominant
241	S6_89823772	6	7.12	123.33	0.0000	Codominant
242	PHM4662_153	6	7.12	160.00	0.0000	Codominant
243	S6_156386857	6	7.12	113.09	0.0000	Codominant
244	S6_125019062	6	7.12	149.74	0.0000	Codominant
245	PZA01884_1	6	7.12	142.96	0.0000	Codominant
246	PZA00427_3	6	7.12	149.61	0.0000	Codominant
247	PHM7922_8	6	7.12	151.40	0.0000	Codominant
248	S6_19369502	6	7.12	154.74	0.0000	Codominant
249	PHM1572_17	6	7.64	163.33	0.0000	Codominant
250	PZA00440_15	6	7.64	161.67	0.0000	Codominant
251	S6_32967981	6	7.64	136.67	0.0000	Codominant
252	PZA01509_1	6	47.95	35.14	0.0000	Codominant
253	PZA03063_21	6	49.83	30.44	0.0000	Codominant
254	PZB01009_1	6	54.37	31.46	0.0000	Codominant
255	PZA01618_2	6	72.26	116.44	0.0000	Codominant
256	lac1_3	6	82.58	33.26	0.0000	Codominant
257	S6_125593444	6	86.72	17.14	0.0002	Codominant
258	PZA02673_1	6	92.75	35.26	0.0000	Codominant
259	PZA00910_1	6	118.95	26.09	0.0000	Codominant
260	PHM5529_4	6	121.32	22.59	0.0000	Codominant
261	PHM3466_69	6	121.32	8.54	0.0140	Codominant
262	PHM4468_13	6	121.32	22.37	0.0000	Codominant
263	PHM7898_10	7	0.00	30.15	0.0000	Codominant
264	PZA02872_1	7	33.55	12.62	0.0018	Codominant
265	PZA00424_1	7	84.40	131.27	0.0000	Codominant
266	PHM1912_23	7	85.47	147.89	0.0000	Codominant
267	S7_156215556	7	86.55	163.33	0.0000	Codominant
268	PHM4818_15	7	86.55	161.67	0.0000	Codominant
269	S7_115310293	7	86.55	161.67	0.0000	Codominant
270	S7_128895684	7	86.55	123.33	0.0000	Codominant
271	PZA00986_1	7	86.55	155.00	0.0000	Codominant
272	PZA00795_1	7	86.55	151.40	0.0000	Codominant
273	PZA00405_6	7	86.55	149.51	0.0000	Codominant
274	PZA02854_13	7	86.55	151.40	0.0000	Codominant
275	PHM9162_135	7	86.55	156.67	0.0000	Codominant
276	S7_137455469	7	86.55	146.41	0.0000	Codominant
277	PZA01600_2	8	0.00	99.63	0.0000	Codominant
278	PHM5158_13	8	0.00	139.57	0.0000	Codominant
279	PZA01049_1	8	0.00	127.98	0.0000	Codominant
280	PZA02388_1	8	0.00	123.19	0.0000	Codominant
281	PZA01857_1	8	0.56	118.28	0.0000	Codominant

282	PHM5805_19	8	0.56	127.83	0.0000	Codominant
283	PZA02019_1	8	1.12	146.23	0.0000	Codominant
284	PHM5235_8	8	1.12	147.73	0.0000	Codominant
285	PHM3312_23	8	1.12	151.40	0.0000	Codominant
286	PZA01301_1	8	1.12	144.62	0.0000	Codominant
287	PZA00758_1	8	1.66	131.77	0.0000	Codominant
288	PZA01290_1	8	2.20	146.28	0.0000	Codominant
289	PHM12749_13	8	2.73	137.99	0.0000	Codominant
290	PHM14104_23	8	3.26	142.96	0.0000	Codominant
291	PHM765_24	8	4.35	149.74	0.0000	Codominant
292	PZA02746_2	8	4.89	134.81	0.0000	Codominant
293	PHM15278_6	8	5.43	142.96	0.0000	Codominant
294	PHM4560_54	8	5.43	148.07	0.0000	Codominant
295	PHM1978_111	8	5.43	156.67	0.0000	Codominant
296	PZA01257_1	8	5.43	154.74	0.0000	Codominant
297	PZB02155_1	8	5.43	156.40	0.0000	Codominant
298	PZA00951_1	8	5.43	158.07	0.0000	Codominant
299	PZA00739_1	8	5.43	160.00	0.0000	Codominant
300	S8_141802902	8	5.43	163.33	0.0000	Codominant
301	PHM4786_9	8	5.43	160.00	0.0000	Codominant
302	PHM4968_10	8	5.43	160.00	0.0000	Codominant
303	PHM4677_11	8	5.43	130.00	0.0000	Codominant
304	PHM9126_15	8	14.92	77.58	0.0000	Codominant
305	PZA00368_1	8	46.48	35.15	0.0000	Codominant
306	PZA01691_1	8	53.35	15.00	0.0006	Codominant
307	PZA00498_5	8	65.41	20.65	0.0000	Codominant
308	PHM4552_6	8	66.74	8.25	0.0161	Codominant
309	PHM4203_11	8	77.25	43.61	0.0000	Codominant
310	PZB00221_3	9	0.00	147.95	0.0000	Codominant
311	PZA02235_14	9	1.04	153.07	0.0000	Codominant
312	PZB01358_1	9	1.04	160.00	0.0000	Codominant
313	PZA00708_3	9	1.57	149.51	0.0000	Codominant
314	S9_37149685	9	2.11	160.00	0.0000	Codominant
315	S9_145906361	9	2.11	160.00	0.0000	Codominant
316	PHM4689_49	9	2.11	161.67	0.0000	Codominant
317	S9_69569894	9	2.11	163.33	0.0000	Codominant
318	S9_108521912	9	2.11	161.67	0.0000	Codominant
319	S9_113056055	9	2.11	160.00	0.0000	Codominant
320	PZA02648_2	9	2.11	163.33	0.0000	Codominant
321	PHM5185_13	9	2.11	163.33	0.0000	Codominant
322	S9_114980354	9	2.11	160.00	0.0000	Codominant
323	PHM4604_18	9	2.65	151.22	0.0000	Codominant
324	PZA01715_2	9	2.65	139.52	0.0000	Codominant
325	PZB00761_1	9	2.65	136.20	0.0000	Codominant

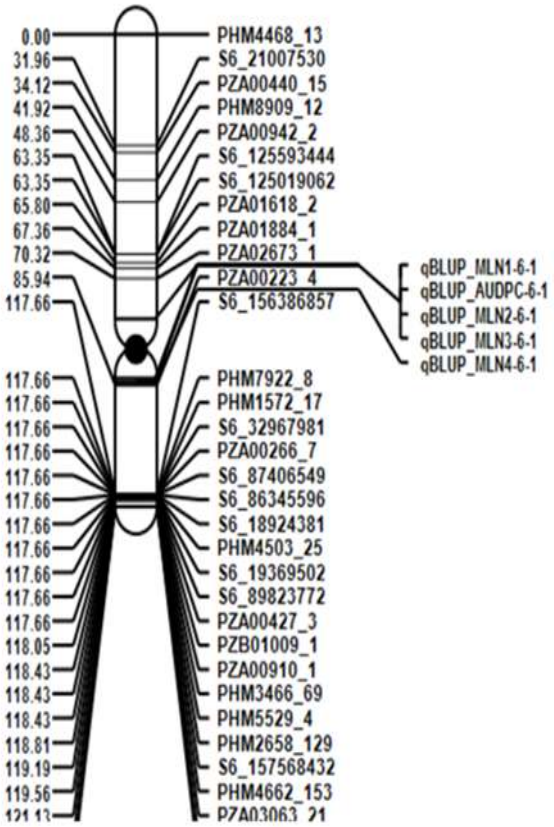
326	PHM7916_4	9	3.83	116.92	0.0000	Codominant
327	PHM697_21	9	4.95	123.19	0.0000	Codominant
328	PHM229_15	9	39.04	29.31	0.0000	Codominant
329	S9_109549230	9	49.92	21.16	0.0000	Codominant
330	PZD00036_2	9	71.72	25.41	0.0000	Codominant
331	sh1_11	9	79.14	50.06	0.0000	Codominant
332	PZA00832_1	9	113.02	22.89	0.0000	Codominant
333	S10_148638187	10	0.00	26.86	0.0000	Codominant
334	PZA03605_1	10	13.83	38.08	0.0000	Codominant
335	PZA00866_2	10	37.11	41.16	0.0000	Codominant
336	S10_91086489	10	38.57	44.39	0.0000	Codominant
337	PZA01642_1	10	42.03	29.02	0.0000	Codominant
338	PHM15331_16	10	44.54	42.49	0.0000	Codominant
339	PHM5740_9	10	45.27	35.49	0.0000	Codominant
340	PHM4066_11	10	87.11	113.33	0.0000	Codominant
341	PZA03713_1	10	89.14	123.03	0.0000	Codominant
342	PZA01456_2	10	90.23	127.98	0.0000	Codominant
343	PZA02961_6	10	91.31	142.84	0.0000	Codominant
344	S10_91685820	10	92.96	161.67	0.0000	Codominant
345	S10_97796845	10	92.96	138.33	0.0000	Codominant
346	PHM537_22	10	92.96	161.67	0.0000	Codominant
347	PZA02221_20	10	92.96	163.33	0.0000	Codominant
348	S10_113702444	10	92.96	156.67	0.0000	Codominant
349	S10_113832226	10	92.96	161.67	0.0000	Codominant
350	PZA01877_2	10	92.96	160.00	0.0000	Codominant
351	S10_120670943	10	92.96	161.67	0.0000	Codominant
352	PHM557_21	10	92.96	160.00	0.0000	Codominant
353	S10_105877132	10	92.96	163.33	0.0000	Codominant
354	S10_132088679	10	93.48	154.74	0.0000	Codominant
355	PZA00814_1	10	93.48	147.95	0.0000	Codominant
356	PHM15868_56	10	94.02	139.51	0.0000	Codominant
357	PZA01001_2	10	95.08	123.94	0.0000	Codominant
358	PHM1576_25	10	96.73	153.07	0.0000	Codominant
359	PHM3736_11	10	96.73	151.40	0.0000	Codominant
360	PHM3631_47	10	96.73	130.00	0.0000	Codominant
361	PZA01313_2	10	99.51	94.52	0.0000	Codominant

Appendix 2. Major and minor QTL across mapping populations

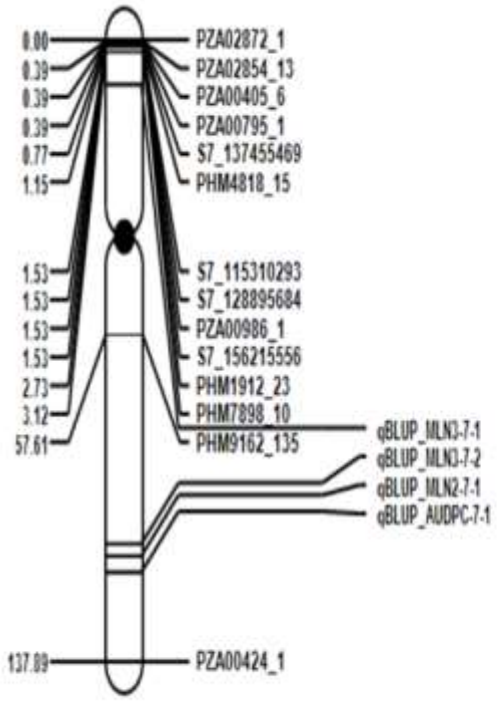
Appendix 2.1 Major and minor- effect QTL linked to MLN resistance in MLNR-P20



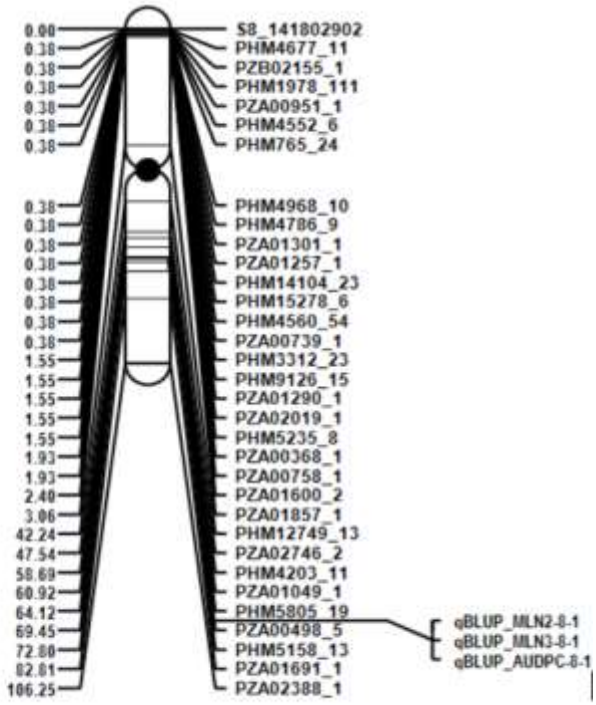
Chromosome 1



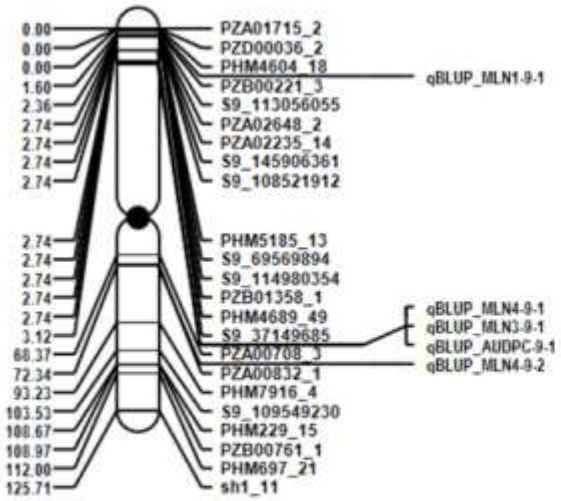
Chromosome 6



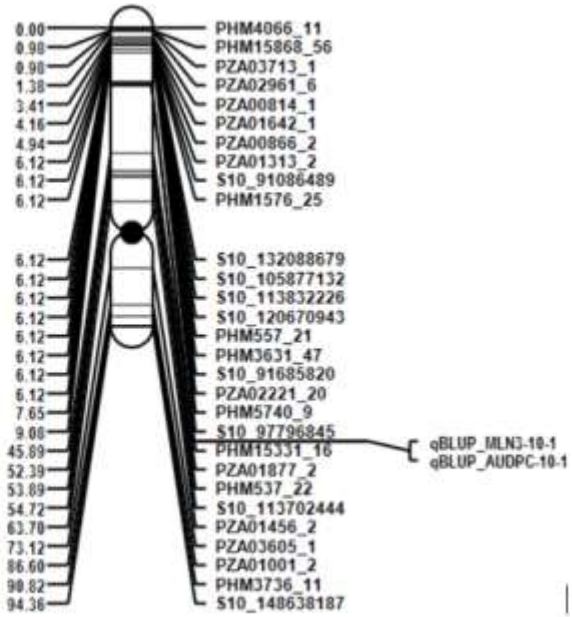
Chromosome 7



Chromosome 8



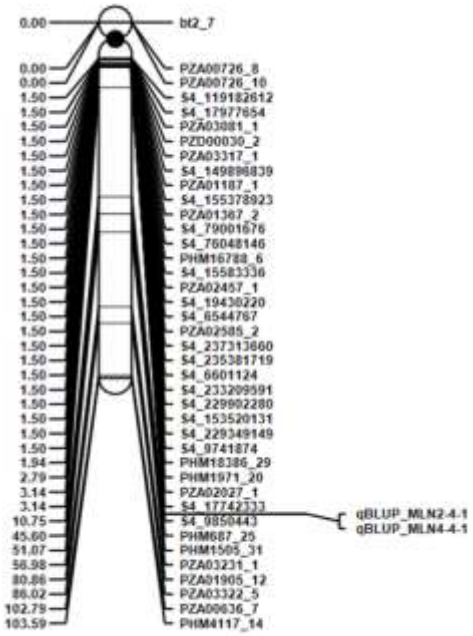
Chromosome 9



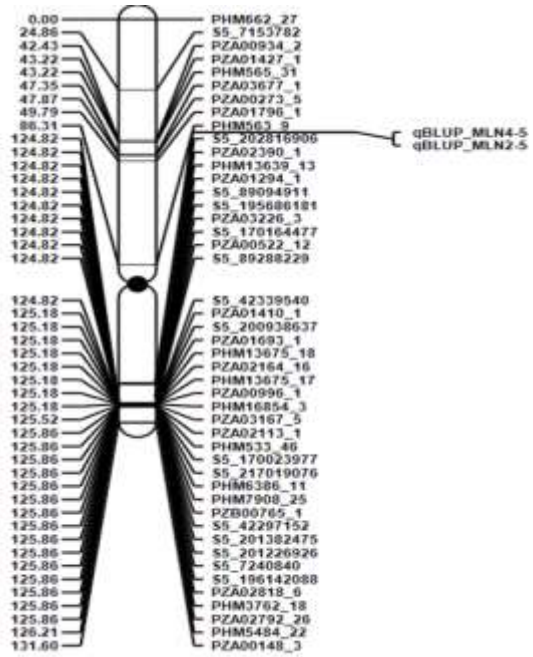
Chromosome 10

Values to the left of the chromosome represent marker position; to the right are SNP markers and identified QTL

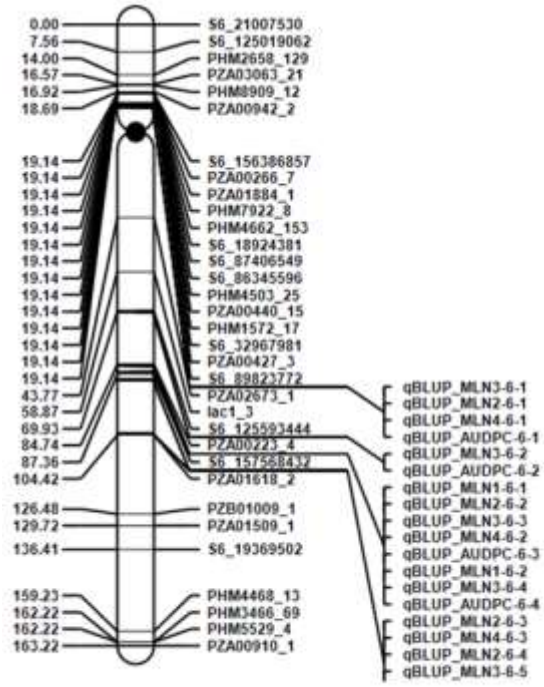
Appendix 2.2 Major and minor- effect QTL linked to MLN resistance in MLNR-P16



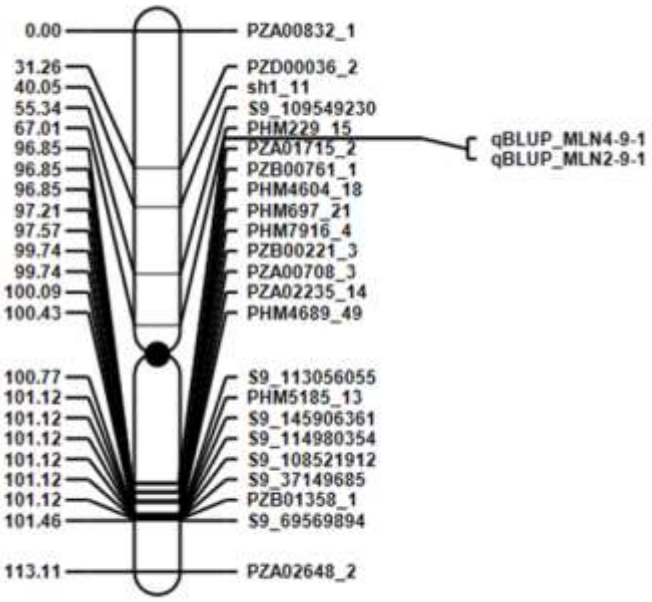
Chromosome 4



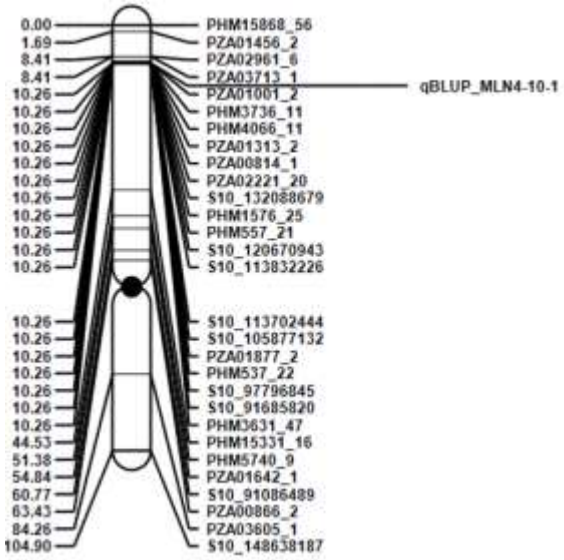
Chromosome 5



Chromosome 6



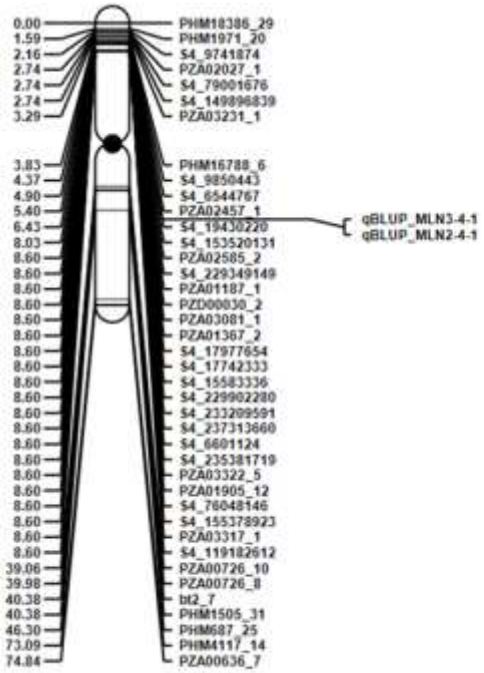
Chromosome 9

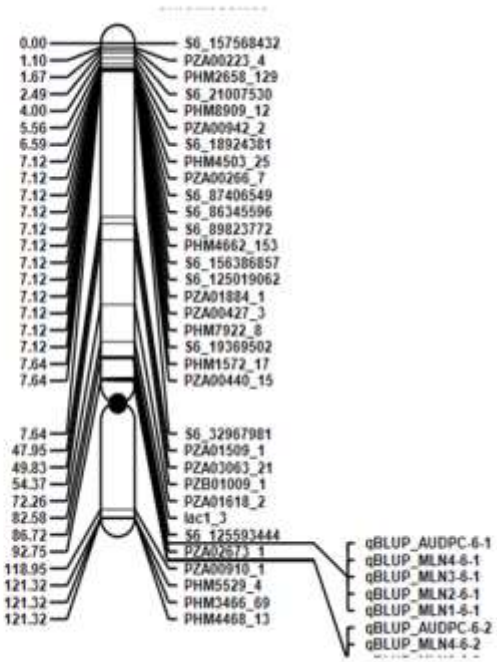


Chromosome 10

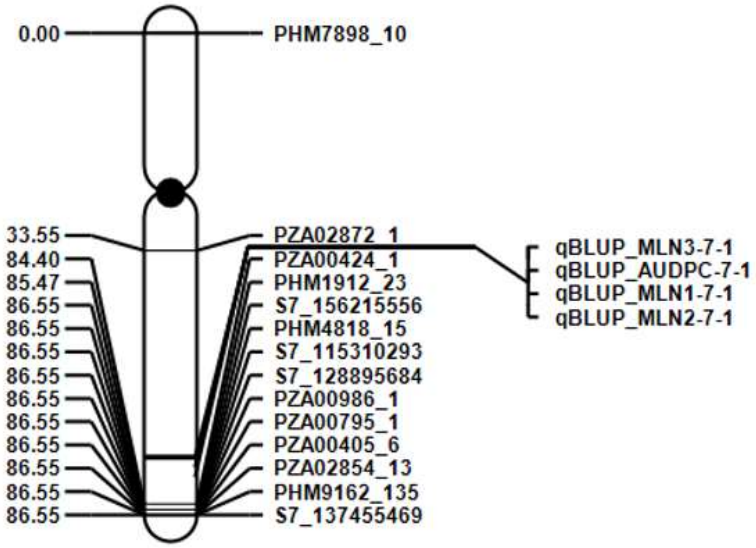
Values to the left of the chromosome represent marker position; to the right are SNP markers and identified QTL

Appendix 2.3 Major and minor- effect QTL linked to MLN resistance in MLNR-P13





Chromosome 4 **Chromosome 6**



Chromosome 7

Values to the left of the chromosome represent marker position; to the right are SNP markers and identified QTL