IMAGERY PHENOTYPING AND MAPPING QUANTITATIVE TRAIT LOCI FOR NORTHERN LEAF BLIGHT (NLB) RESISTANCE IN MAIZE

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DECLARATION

This MSc. research thesis is my original work and has not been presented for a degree award in this or any other University.

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DEDICATION

To my dear parents Mr. and Mrs. Kuria Mwangi and my daughter Stephanie for their efforts and support throughout the course

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ACRONYMS

ANOVA	Analysis of Variance
CV	Coefficient of Variation
CIMMYT	International Maize and Wheat Improvement Center
CML	CIMMYT maize inbred line
DH	Doubled haploids
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significance Difference
SAS	Statistical Analysis Software
GWAS	Genome wide association studies
LD	Linkage disequilibrium
MAS	Marker Assisted Selection
MLN	*Maize Lethal Necrosis*
MSV	*Maize Streak Virus*
TLB	*Turcicum leaf blight*
QTL	Quantitative Trait Loci
SNP	Single Nucleotide Polymorphism
SSA	Sub Saharan Africa

ABSTRACT

Northern leaf blight (NLB) is a major foliar disease caused by fungus *Exserohilum turcicum* that leads to limited production of cereals in the Sub-Saharan Africa. Maize is normally susceptible to NLB from the seedling stage to maturity making it expensive in the management and control. The disease lowers production of maize up to 80%, threatening food security in the region. However, to achieve increased food production, improved agricultural technologies should be adopted, whereby research institutions and breeders have continued assessing the breeding values and using advanced technologies for phenotyping diseases. Currently, new technologies have been incorporated where digital imagery tools are used for detecting foliar diseases in the field earlier enough before the severity is high. To curb this major problem of foliar diseases in maize quantitative trait loci (QTL) mapping is recommended and adopted to assist as an effective and efficient tool in breeding to generate resistant host plants. QTL mapping enhances in identification and evaluation of potential sources of resistance followed by introgression of favorable alleles into susceptible variety. This study was implemented to; i) compare the visual scoring method of phenotyping foliar diseases with the digital imagery methodology under a high disease pressure area. ii) Identifying the genomic regions associated with resistance to Northern leaf blight disease through quantitative trait loci (QTL) mapping. One hundred and ninety-two double haploid (DH) lines obtained from International maize and wheat improvement Center (CIMMYT) were test crossed to 2 single cross parents (CML539 x Laposta Seq F64) and (CML 312 x Laposta Seq F64). An alpha lattice design with two replications was used to evaluate the 192DH hybrids with three commercial local checks across two locations in Kenya under high disease pressure area condition during 2016-2017 growing seasons. Each plot measured 4m long spaced at 0.75m between rows and 0.25m

between hills. Data was collected on days to anthesis, grain yield, plant and ear aspect, number of ears, plant and ear height and northern corn leaf blight where the disease severity was scored using a CIMMYT scoring scale of 1-5 where 1-there are no infections, the plant is fully clean, 2- light infection with moderate number of lesions on the lower leaves, 3-moderate infection with abundant lesions on the lower leaves and a few lesions on the middle leaves, 4- heavy infection with lesion abundant on all leaves, 5- very heavy infection with lesions on all leaves. At flowering stage, image analysis was conducted using a Nikon camera where images of the maize plot were taken; scanners were also used where maize leaves from every plot were scanned to obtain a clear view of the damaged lesions. All data collected was analyzed using Meta-R software to obtain the analysis of variance. It was concluded from the studies that digital imagery analysis led to more efficient and effective breeding since it gives accurate and precise information on the field data and also it consumes less time. To identify genomic loci associated with NLB resistance, double haploid (DH) lines from two bi-parental mapping populations were genotyped and marker trait association analysis carried out. Genome-Wide Association Study (GWAS) revealed a major quantitative trait locus (QTL) on chromosome 5 and chromosome 7 that were significantly associated with NLB resistance. This study provides important insights into the genetic architecture underlying resistance to NLB, and identified a useful set of polymorphic single nucleotide polymorphism (SNPs) to be used in breeding for NLB resistance.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize (*Zea mays*) originated from Balsas River basin of southwestern Mexico about 9000 years ago (Matsuoka, *et al*., 2002) Since then maize has spread geographically and economically becoming one of the most important food crops adapted globally (CIMMYT, 1999- 2000). It's also the second largest crop adapted in the world after rice. Maize can be grown over a range of agro ecological zones defined by the total rainfall received, elevation, maturity period and the length of the growing season (FAO Statistics, 2000). Maize is grown from 50°N to 40°S and a sea level of up to 4000m altitude in areas with 250 mm to 5000mm of rainfall per year (Doswell *et al*., 1996). The optimum temperature for maize growth and development is 18°C to 32°C with temperature of 35°C. It has a growing cycle ranging from 3 months to 13 months (CIMMYT, 1999- 2000). However, the continuous diversification and high demand for maize production has led to the need for genetic improvement of various agricultural and economical important traits.

In sub-Saharan countries, maize has accounted for 22 to 25 percent of starchy staple consumption from 1980, representing the largest single source of calories, followed closely by cassava. It also ranks the first among rice and wheat due to its diverse uses and relatively lower price. Maize is used directly for human consumption since it has great nutritional value as it contains 10% protein, 73% starch, 8.5% fibre, 4% oil, 3.0% sugar and 1.7% ash (Ranum *et al*., 2014). It also contains 1.2 to 5.7 % edible oil; this oil is widely used for cooking and for manufacturing hydrogenated oil. The oil has the quality of reducing cholesterol in the human blood like sunflower oil.

White maize which is in two types mainly dented and flint is associated with different food products (FAOSTAT, 1997), the dent maize is soft and floury therefore it's used for porridges, while flint maize has a hard, vitreous endosperm used primarily for gruel or couscous maize flour which is easily stored after drying or milling it. In some parts of Sub-Saharan Africa such as Malawi, flint maize has been preferred to dent because of smaller losses incurred in traditional storage and processing practices (VIB, 2017).

Maize is a multi-purpose food crop which may be consumed fresh as green roasted cobs, boiled separately or mixed with legumes and other foods. In industries, maize is used for processing foods such as corn meal, sweetener and starch, recently there has been interest in using maize for production of fermentation products such as ethanol which is a substitute for petroleum based fuels; the combs and stalks are used to provide domestic fuel especially in rural areas. Maize stalks, leaves and remains from the cobs are used to feed animals directly or making silage which is very nutritious particularly to dairy cattle thus enhancing high milk production. Processed feeds such as bran are given to poultry and pigs (VIB, 2017).

1.2 Problem statement

Cereals are the most important sources of food in the world whereby millions of consumers in both developing and developed countries rely on as their preferable staple food. Production of cereals globally is facing serious challenges since the current production rates cannot provide enough food to meet the rising demand of the world's population by 2050, thus affecting the global food security (Conway and Barbier, 2013). Constraints that mainly affect crop production negatively are abiotic stresses, biotic stress and socio-

economic factors which include poor soils, low-yielding varieties, inadequate access to farm inputs like fertilizers and improved seeds (VIB, 2017). Recent study showed that Sub-Saharan Africa crop losses and low yields are highly attributed to biotic stresses such as foliar diseases, weeds and insect pests (Bekeko, 2013).

1.3 Justification

Few decades ago, screening of crop diseases to identify resistant germplasm has always depended on traditional ways which are confounded with high error rate due to biasness. The use of high throughput digital imagery tools is currently replacing the traditional phenotyping methods since in most crops growing regions digital imagery tools have been introduced to enhance proper phenotyping of field crops on various traits such as disease severity, insect attack and nutrient levels. Additionally, these digital imagery technologies provide new opportunities to plant researchers to study a wider range of physiological and developmental plant processes with greater efficiency. Digital imagery tools such as unmanned aerial vehicle (UAV) have therefore been proposed for use in this study to collect disease data on fields to test its efficacy in obtaining precise and accurate information (Xu, et al., 2020). Breeding for resistance requires quantifying and genotyping of the plant population to identify the genetic bases in the traits (Goggin, Argelia, & Christopher, 2015).

1.4 Objectives

1.4.1 Main objective

To improve disease monitoring, in maize fields through use of high-throughput tools for high precision data and mapping of Quantitative trait loci (QTL) related to Northern leaf blight.

1.4.2 Specific objectives

1) To compare high-throughput phenotyping with the visual crop evaluation of Northern leaf blight diseases among Kenyan and International maize germplasm.
2) To identify the genomic region associated with resistance to Northern leaf blight disease through genome- wide association selection (GWAS) in tropical germplasm.

1.4.3 Hypotheses

1) Visual crop evaluation of foliar diseases may lead to biased or inaccurate results unlike high-throughput phenotyping platforms.

2) There are sources of resistance to NLB among Kenyan and international maize germplasm.

CHAPTER TWO
LITERATURE REVIEW

2.1 Maize taxonomy, botany, growth and development

Maize belongs to the family of grasses (Poaceae) and genus Zea, which comprises of `both perennial and annual species. The genus comprises of several wild species; Teosinte and the cultivated maize (Zea mays). Maize is a diploid crop with 2n = 20 chromosome number (Poehman and Sleper, 2006). Although there are some major differences between the two, various morphological and genetic studies show the relationship within the genus (Buckler and Steven, 2005).

Maize is a tall, determinate annual C_4 plant varying in height from 1 to 4m. It has two growth stages namely the vegetative and reproductive stages. The vegetative stage occurs in three distinct growth levels. By the end of the first one week after planting, 2- 4 leaves appear. At 35-45 days after planting, Knee- height stage occurs, lastly the male flowers appear which is tasseling stage. The reproductive stage occurs 2-3 days after tasseling stage where female flowers and combs are formed. At this stage silk is identified outside the husk enhancing pollination through trapping falling pollen grains. After fertilization is over, grains start developing in the cob. Finally, it reaches maturity stage when harvesting is done after the leaves and silk get dry completely and become very brittle (Tripathi, 2011). Maize have a male inflorescence with loose panicles which produces pairs of free spikelet each enclosing a fertile and a sterile floret. The female inflorescences have a spike, which produces pairs of spikelet on the surface of a highly condensed rachis. The female flower is tightly covered over by several layers of leaves, and so closed in by them to the stem that they don’t show themselves easily until emergence of the pale yellow silks from the leaf

whorl at the end of the ear. The silks are the elongated stigmas that look like tufts of hair initially and later turn green or purple in color. Each of the female spikelet encloses two fertile florets, one of whose ovaries will mature into a maize kernel once sexually fertilized by wind-blown pollen (Tripathi, 2011). Maize grain is botanically a caryopsis, a dry fruit containing a single seed fused to the inner tissues of the fruit case. The seed contains two sister structures, a germ which includes the plumule and radical from which a new plant will develop. Endosperms provide nutrients for that germinating seedling until the seedling establishes sufficient leaf area to become autotrophy.

2.2 Production of maize in the world

Maize is produced widely throughout the world, in temperate and tropical zones. In the year 2014, more than 1,022 million tons of maize was produced in more than 170 countries on about 181 million hectares of land (FAO, 2016). The high maize producers were United States of America with about 361 million tons, followed by China with 216 million tons, Brazil with 80 million tons, lastly Argentina and Ukraine with 33 and 28million tons respectively. India is the sixth – largest producing country with about 24 million tons, followed by Mexico and Indonesia both with about 23 million tons and South Africa with 14 million tons. These ten regions accounts for 80% of the world's total maize production (FAO, 2016). Global cereal production is expected to increase in the next decade reflecting a growth of 15% by 2023 (OECD-FAO, 2014), while in developing regions, there will be more than 75% agricultural outputs over the next decade (OECD-FAO, 2014).

Maize production in the world can be divided into two categories namely the white maize production and yellow maize production (Meyer, 2006). Both the white maize and yellow

maize are genetically the same but differs in their appearance; the yellow maize has no carotin oil pigments in the kernel thus causing the yellow color of the grain. In the world, a larger area in the tropical highland and sub-tropical/mid-altitude environments is planted white maize, as opposed to yellow maize. White maize occupies about 40 percent of the lowland tropical maize (Mosisa *et al*., 2007). Areas which mostly grow white maize are Central America excluding the Caribbean sub-region, where it represents about 90 percent of total maize output of the region, and the northern part of South America, Colombia and Venezuela. Yellow maize is considerably more important in their total cereal production than white maize. However, yellow maize is becoming un-popular nowadays in Africa because its associated with food aid programs therefore people are perceived that it only consumed by the poor also its associated with animal feeds (Doebley, 2004).

2.2.1 Maize Production in the Sub-Saharan Africa

Maize was first introduced into Africa by the Portuguese who came as explorers and traders in the 16th to 18th century (Vollbrecht and Sigmon, 2005). Since then, maize has become Africa's most preferred staple food and feed system. Besides being a major staple food for most of the households in sub-Saharan Africa, it dominates the diet of the rural and urban people. Much of the maize in Africa is produced in Eastern and Southern Africa region namely, Tanzania, Uganda, Zambia and Swaziland under 17.4 million hectares which is about 12.5% of the global production (FAOSTAT, 2014). In Eastern Africa, 3.9% of the cultivated land is under maize which yields 700 to 1800 kg /ha as opposed to7437 kg /ha in the USA (Mosisa *et al*., 2007). Generally, maize in Africa is grown by small-scale farmers for local consumption. By 2020, maize production in both sub-Saharan countries and developed countries will surpass that of wheat and rice (FAO, 2016).

Many African countries, the average maize yield per hectare is very low. The reason being, maize production is continuously affected by a number of biotic and abiotic stresses. Biotic stresses occur as a result of damage done to the plant by other living organisms such like bacterial, nematodes, fungus, weeds and viruses while the abiotic stresses are the negative impacts of non-living factors on plants in a given area. For maize abiotic stresses are poor fertility, drought and poor post-harvest management.

2.3 Maize production constraints

Maize suffers from about 110 diseases on a global basis caused by fungus, bacteria and viruses. In Sub Saharan Africa the widely spread fungal disease is Northern leaf blight.

2.3.1 Northern Leaf Blight

2.3.1.1 The causal agent

Northern leaf blight (NLB) is caused by the hemibiotrophic ascomycete fungus *Exserohilum turcicum*(Leonard and Suggs, 1974). The *Exserohilum* fungus belongs to division Eumycota, sub-division Deuteromycotina, order Moniliales and family Dematiaceae. This fungus spreads biotrophically during the initial infection stage before changing to a necrotrophic lifestyle.

2.3.1.2 Pathogenesis

Exserohilum turcicum fungus lowers yields up to 70% in maize by forming necrotic lesions, thus interfering with photosynthesis (Tillahum, *et al*., 2001). This fungus forms an aspersorium which penetrates through the maize leaf cell using an infection hypha which produces infection pegs to get in the epidermal cell wall. After penetration, the fungus produces intracellular vesicle to obtain nutrients from the cell resulting in necrotic spots that cause epidermal cell to collapse within 48 hours (Lexy, *et al*., 1983).

2.3.1.3 Epidemiology and infection

During warm, humid conditions, mycelia and conidia, of *E. turcicum* are produced on infested maize residues (Leach, *et al.*, 1977). These forms the primary source of inoculums and spread when splashed by rain or carried by wind from distant areas and deposited on the surfaces of maize leaves.

Once deposited, conidia germinate when free water mainly in the form of dew is present on the leaf surface for 6 to 16 hours and temperature of 18 to 27°C (Levy and Cohen, 1983). Conidia develop a germ tube which penetrates through stomata, and invades the parenchyma cells (Gowda, *et al.*, 1994). In moist conditions, the fungus overwinters as mycelium and conidia produced on maize residues are left on the ground surface. These conidia can also develop into spores, thick-walled resting spores that remain viable for long periods of time. Northern Leaf Blight incidence is influenced by temperature of 22 to 32°C and relative humidity of 70% (Misra and Singh, 2005).

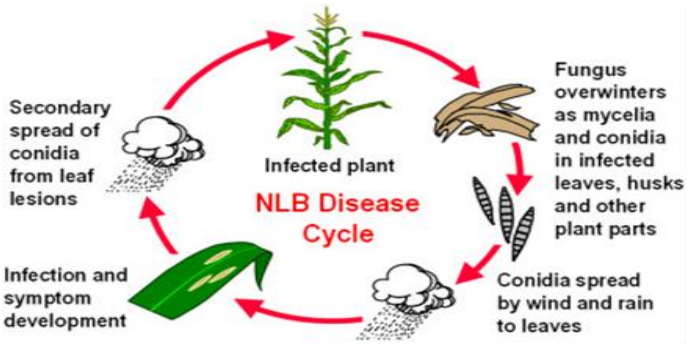

Figure 1: Disease cycle of Northern leaf blight (obtained from www.pioneer.com)

2.3.1.4 Symptoms

Northern leaf blight is identified as one of the most devastating foliar disease of maize in Sub-Saharan Africa (Harlapur, 2005). It is also a main problem in the North eastern United States, areas of China, Latin America and India (Adipala, *et al.*, 1993). The disease is more aggressive in young susceptible plants but the fungus is capable of infecting maize plants

at all the stages of crop growth right from seedlings to maturity. The fungus can survive for years in an infected maize residue, thus making it a problem once it develops in an area (Muiru, 2008).

Northern leaf blight disease is more prevalent in humid areas with moderate temperatures that favor fungal spore production and germination (Muriithi and Mutinda, 2001). Spores produced on the residue or the diseased plants can be splashed to new leaves or brown by wind over distances to nearby fields. If the weather conditions become warmer or drier, disease progression may be slower but the fungus remain viable and can resume activity once conditions become more favorable.

Northern leaf blight disease infection shows by long elliptical lesions that run parallel to the leaf margin. It begins on lower leaves and progress up the plant (Muiru, 2008) (Fig 2). It takes 7- 12days for the lesions to develop after infection but it highly depends on hybrid susceptibility and high nitrogen levels in the soils. The infection may also begin in the upper plant canopy where spore loads are high since the lesions produce olive green or black fungal spores. Northern leaf blight lesions may also appear on the leaf sheaths and husks of susceptible maize plants leading to reduction of maize yield caused by destruction of photosynthetic activity on leaf area due to blighting of the leaf surface during the period of grain filling (Hooker, 1981).

NLB disease first starts as a small elliptical spot on the leaves, grayish green in colour with water soaked lesions. The spot turns greenish with age and increase in size and finally

attains a spindle shape. Spores of the fungus develop abundantly on both sides of the spot. Heavily infected field shows scorched appearance (Chenulu and Hora, 1962). When fully expanded, the lesions may be 5- 10 cm length. These lesions first appear on the lower leaves and as the season progresses, the lesion number increases abundantly with time and then all the leaves are covered, the plants look dead and grey (Ullstrup, 1966).

Figure 2: Maize plant showing Northern Leaf Blight lesions (picture taken by Arnet)

Infection occurs from the lower part of the leaves and gradually extends to the upper side. The size and color of the disease lesions differ with variety, when the weather is damp and rainy, grey black mold layer occurs on the lesions (Zhao and Wang, 2009). NLB disease causes extensive leaf damage and defoliation during grain filling period. It also reduces the sugar content and viability of the seed predisposing the crop to stalk rot (Harlapur, 2005).

2.3.1.5 Management of NLB disease in maize

Various ways are used to control the disease. The basic management strategy to reduce northern leaf blight incidences and severity is planting of resistant varieties because it fits Africans condition. This method is cheap, effective and gives high results (Dunn and Namn, 1977).

Northern leaf blight has two types of resistance that exist in maize, the polygenic resistance that is expressed as a reduction in lesions size, number of lesions, and sporulation. Monogenic resistance is controlled by four single dominant genes Ht1, Ht2, Ht3 and HtN. Both resistances monogenic and polygenic can be incorporated together to lower TLB severity (Monsanto, 2014).

Cultural methods such as crop rotation using non-host plant crops like beans, soybean and sunflower have been adopted. It lower incidence of the disease by allowing the conidia that survive in the residue to desiccate before maize is planted again (Harlapur, 2005). Burying residue and conventional tillage ensures proper handling of infested crop remains, this helps reduce infection levels by decreasing the amount of primary inoculum of the pathogens and incidences of diseases developing early in the season (Madden *et al*., 1993). Additionally, in a reduced tillage or no till field with NLB history it requires a two-year rotation (Monsanto, 2014). Multiple fungicides are registered for use on maize for NLB control. The commonly used fungicide includes Dithine, Difolatan, Zinc ethiylenebis and O-Ethyl-S.S-diphenyl. Spraying schendule begins when the first lesion appear on the leaf below the ear. However, before deciding to use fungicides one should consider cropping practices since the application cost of fungicide leads to an additional cost in maize production (PANNAR Seed Ltd, 2009), and can represent a risk to the farmers and to the environment when not handled in the right ways.

Various biological control agents such as *Bacillus subtilis* and Enterococcus species can be used to lowers the growth of *S.turcica* effectively. These biological controls have several advantages in that they are environmental friendly, do not develop resistance to pathogens

and do not require industry processes (James-Cook, 2003). However, this method is slow and unpredictable therefore it requires a specialized person for rearing and releasing them to the field. This is due to the species multiplying in excess and turning to be pathogens in other crops (Jutsum *et al*., 1988).

2.3.2 Source of resistance

Qualitative and quantitative resistances to Northern leaf blight (NLB) have been identified by maize breeders (Pataky, *et al*., 1986). In maize Quantitative disease resistance (QDR) has widely been used in breeding programs, it provides more durable disease resistance that remains effective over a long period in a large area (Brown, 2015) although it is difficult to test (St Clair, 2010).

Two important reasons for wide use of quantitative disease resistance in maize are; maize is naturally outcrossing species which makes the genetic architecture controlling quantitative traits more complex in maize than in self-pollinating plants such as rice and wheat (Buckler, *et al*., 2014). Secondly, the most economical maize disease is caused by necrotrophic pathogens where resistance to necrotrophic disease is almost exclusively quantitative rather than qualitative (Govrin and Levine, 2000). Quantitative disease resistance is a polygenic; it's controlled by many genes each with a small effect which is often affected by the environmental factors (Pataky, *et al*., 1986).

Researchers in Uganda used variety Babungo3, Ev8342-SR, Mo 17, and H99 as sources of northern corn leaf blight resistance; they reported that the results were successful (Lipps, *et al*., 1997). Other researchers recorded that Mo17 provides polygenic TLB resistance to maize plants (Freymark, *et al*., 1993). CML104 and CM105 from CIMMYT were used to analyze the mechanism of northern leaf blight disease in India where it was identified that

they conferred durable resistance to TLB disease (Sharma and Payak, 1990). Further it was reported that, durable resistance can be obtained by improving population through recurrent selection (Campana and Pataky, 1995).

2.3.3 Plant immune system

In nature, plants are highly affected by various destructive pathogens and pests, including viruses, bacteria, fungi, nematodes and insect herbivores. Each of these disease causing organisms attacks vital features so as to develop a parasitic relationship with its host plant. Plant pathogens are generally divided according to their way of living, the necrotrophs and biotrophs (Glazebrook, 2005). Necrotrophs first destroy plant cells mainly through production of phytotoxins, and then later it feed on the contents. Biotrophs obtain nutrients from living plant tissues, mainly through specialized feeding structures that invade the plant cell without disrupting it.

For plants to defend themselves against all these different types of pathogens, they have a number of structural barriers and preformed antimicrobial metabolites to prevent or to stop invasion by the pathogens. Despite the plant having much defense, numerous microbes fortunately succeed in getting through. However, a broad spectrum of inducible plant defenses can be introduced to control further pathogen invasion.

2.3.3.1 Systemic acquired resistance and induced systemic resistance in maize

Most of the maize Quantitative Trait Loci and genetic inheritance studies of disease resistance have assessed foliar pathogens, thus leading to many breeders progressing successively against foliar pathogens. Plants which are initially infected by a microorganism may become systemically more resistant to any other pathogen attack (Balint and Johal, 2009).

There are two types of systemic responses namely systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is a response to necrotizing pathogens that gives a broad-spectrum resistance (Hunt and Ryais, 1996) and is related to introgresion of pathogenic- related (PR) gene. Salicylic acid seems to be the inducer of systemic acquired resistance and Methyl salicylate (MS) acts as the moving signal which induces SAR systemically (Park, *et al*., 2007). Methyl salicylic is produced at the point with an infection, carried though the whole plant in the phloem and is converted to salicylic acid at the point of action. Induced systemic resistance (ISR) is induced by symbiotic micro-organisms in the rhizosphere (Vallad and Goodman, 2004). It gives broad-spectrum resistance but the pathway is controlled by jasmonate and ethylene instead of salicylic acid (SA) and pathogenesis- related (PR) genes. The SAR and ISR pathways have widely been described in dicotyledonous systems but the presence of analogous pathways in monocotyledonous systems has not been scientifically shown. Systemic acquired resistance has also been identified to work in a wheat field (Calonec, *et al*., 1996) while an induced systemic resistance has recently been published in maize for response to the fungal root colonizing fungus *Trichoderma*(Djonovic*et al*., 2007).

2.3.4 Genetics of Northern leaf blight resistance

In maize, resistance to Northern leaf blight is located on chromosome 3, 5, and 8 (Welz and Geiger, 2000). According to (Brewster, *et al*., 1992) MO17 maize line had NLB disease resistances linked to chromosome 3 and chromosome 6. However, northern leaf blight disease resistance is controlled by six dominant Ht1, Ht2, Ht3, HtN, NN, HtM and a recessive gene Ht4 (Ferguson and Carson, 2004).

All these provide qualitative resistance either dominant or partial dominant. For the HtN gene it provides partial resistance to NLB disease (Pataky, *et al*., 1986). In the past, both qualitative and quantitative disease resistances were important for controlling NLB, comparing with Ht genes quantitative trait loci (QTL) is highly adopted in maize breeding because they show durable resistance and are less likely to be overcome by evolution of novel resistant pathogen races. A number of studies have been conducted to map QTL for resistance to NLB, these QTL seem to be distributed throughout the genome (Ali and Yan, 2012).

2.3.5 Breeding for Northern Leaf Blight resistance

Host plant resistance breeding is the most feasible approach in combating the major stresses hindering optimum maize production. Many breeding strategies have been used to contribute to genetic gains in grain yield and other traits of economic significance (Welz and Geiger, 2000). The use of pedigree breeding combined with extensive multi-location testing, has been used to assess the phenotypic performance of new genotypes across a large sample of environments in Sub-Saharan Africa.

Breeding for resistance have been implemented by national programs, Kenya agricultural and livestock research organization –KALRO, International institute for tropical Agriculture IITA, and the International Center for the Improvement of Maize and Wheat (CIMMYT) (Pratt, *et al*.,1997). These breeding programs have managed to improve multiple populations and inbred lines (Kim, *et al*, 1989), also has improved the performance of the germplasm for agronomic traits as well as for quantitative resistance to maize diseases.

In some breeding programs breeders are trying to incorporate durable resistance into maize germplasm. Numerous crop varieties with the resistance have been achieved from the R gene that introgress strong resistance (Dang, *et al*., 2013). However, this has been a success in few areas but due to the rapid evolution of pathogens the resistance is often lost quickly (Dang, *et al*., 2013).

Resistance to foliar diseases such as southern and northern leaf blights, gray leaf spot and maize streak virus are all highly controlled through conventional breeding, where by susceptible genotypes are eliminated before recombining the germplasm. This is achieved by introducing the germplasm under a high disease pressure area. Additionally, in modern molecular breeding, PCR based markers can be used for developing disease resistant varieties and hybrids. This is achieved since it gives a complete insight into the diseases and it's cost effective. It also offers an effective summary on gene mapping, genetic transformation and quantitative genetics. Recently, breeders have reported identifying disease resistance quantitative trait loci and exploring the mechanism through molecular breeding such as; SCMV (*Sugarcane Mosaic Virus*) resistance (Zhang, *et al*., 2003) and MDMV (*Maize Dwarf Mosaic Virus*) resistance (Liu, *et al*., 2006).

All disease QTLs are not easy to identify and clone, due to their limited capacity to identify small effect QTLs, large genotype × environment interactions thus preventing scientists from achieving an effective breeding program through molecular assisted selection. High throughput genotyping platforms are available at the moment and when joined with precision phenotyping in the field can provide the information needed to effectively use marker assisted selection in a breeding program for complex traits. Due to current high costs in genotyping this method may be highly adapted for use in breeding programs. All

these will lead to upgrading the economic value of maize and ensure global food safety (Zhang, *et al*., 2003).

2.4 Plant phenotyping

Plant phenotyping is an assessment of complex traits in plants such as resistance, growth and development, tolerance and architecture (Lei, *et al*., 2014). Traditionally, assessment of phenotypic traits for plant diseases resistance or stress in breeding research highly depend on visual scoring by qualified experts, who are capable of identifying disease severity in a discrete scale (Bock, *et al*., 2010).

Unfortunately, these visual assessment methods are time consuming, confounded with high error rate due to temporal variation arising from (a) the raters being prone to various illusions for example lesions number and (b) rater may tire and lose concentration thus lacking accuracy and reproducibility (Lei, *et al*., 2014).Phenotyping of disease symptoms can be divided into two (a) collection of data (b) analyzing the data, they both requires one to be keen.

2.4.1 Image based methods for assessment of plant disease symptoms

Variety of quantitative high throughput image-based methods for assessing plant growth and development are currently being developed. These methods range from simple analysis of a single plant, to broad assessment of crop canopy in a field (Andrew, *et al*., 2015).

Image-based phenotyping methods have numerous advantages; (a) Phenotypic data can be obtained from one particular plant population in the entire experiment. (b)Produces correct and specific data compared to the visual crop assessment (c) The phenotyping tools have

the ability to collect data from large experimental fields due to their increased statistical power (Andrew, *et al*., 2015). In a particular study on imaging, strains of the fungus *Zymoseptoria tritici* on wheat leaves was compared to the visual crop evaluation method (Stewart and McDonald, 2014). The fungus leads to wheat blotching which is identified by necrotic lesion, fruiting fungus known as *pycnidia* and chlorosis of leaves.

Basically visual evaluation of diseases depends on estimates of the leaf area affected by *pycnidia* or lesions. Estimation of these fungus *pycnidia* is not easy due to its size and numbers when they multiple. For reliable and objective diagnosis of plant diseases, new methods have been introduced and incorporated in the rating system. This improves the precision, accuracy and reproducibility of diseases and insect damage (Nilsson, 1995). The use of high-throughput imagery tools in phenotyping crop diseases allows data collection at various time, it also generate quantitative data from the generated images, therefore these improves high outcome of the experiment. (Andrew, *et al*., 2015).

High-throughput image analysis is an improved method used in extraction of useful information from photographs, screens and infra-red photograph. Raw data obtained from image-based phenotyping tools like camera and the scanner is not directly usable. It requires processing to extract information which is normally done by humans, electronic and also by chemicals means (Christian, 2012). The statistical method by which the data information is analyzed is important. It was suggested that any data on image analysis can be grouped into three stages; computational- these describes the process done, algorithm-

is the steps used by the process to implement the computational theory and mechanism is the physical systems and software that carry out the process (Primore, *et al*., 2012).

However, these stages should be adopted when generating phenotypic experiments. To note the appropriate approach on the computational theory and algorithm, one chooses the appropriate method to implement. This is because plant breeders may lack skills in computers and tends to focus on methods depending on hardware and software (Primore, *et al*., 2012). The use of the digital sensing systems has enhanced opportunities for plant researchers. They study a wide range of physiology and developmental plant processes, with greater efficiency hence their integration in seed companies and National Agricultural Research System (NARS) (Araus and Cairns, 2014).

2.5 Quantitative trait loci mapping

Quantitative trait loci analysis has really improved knowledge on genetic constitution of different traits. These include resistance of disease in maize crops (Wiser, *et al*., 2006). Various quantitative trait loci for important traits are mapped in maize. Majority of agronomic important traits of plants have a complicated inheritance pattern and are under the control of many genes.

Quantitative trait loci mapping shows information about the inheritance of disease resistance generating information such as genome location of each genetic factor, gene action, gene effect and the direction of effects. A couple of traditional methodologies with QTL mapping in disease resistance could result in more high and precise population improvement (Pereira, *et al*., 2000). However, QTL mapping has brought about new

approaches for understanding and exploiting both qualitative and quantitative resistance factors. Analyses have also certainly helped in understanding the genetic constitution of various traits in a greater way, including resistance of diseases (Wisser *et al*., 2006) and tolerance in drought (Tuberosa *et al*., 2007) in maize crops. Quantitative trait loci (QTL) mapping of bi-parental populations is an appropriate method of identifying the genetic constitution of crops.

Quantitative trait loci is mapped in maize for a number of important traits including plant height (Zhang, 2007) downy mildew resistance (Agrama *et al*., 1999), resistance to sugar cane mosaic virus (SCMV) (Zhang, *et al*., 2003) resistance to common smut (Ding, *et al*., 2008), resistance to head smut (Tian, *et al*., 2008), yield under drought stress at flowering time (Lu, 2006), and popping ability (Li, *et al*., 2006). QTLs for Northern Leaf Blight resistance have been identified in different populations and they are distributed throughout the genome showing insensitivity to light and temperature variations (Carson and Van Dyke, 1994).

2.5.1 Genome – wide association mapping

Genome wide association selection (GWAS) is a powerful tool to effectively and efficiently identify genomic regions. However, this is achieved by enhancing analysis of genetic make-up of complex traits in plants. It also gives effective output for identifying the genetic loci responsible for a particular trait of interest, therefore saving time and cost (Yu and Buckler, 2006).

Association analysis has been successfully carried out to identify the genomic regions related to diseases such as Northern leaf blight (Poland, *et al*., 2011). It has the ability to identify a single polymorphism within a gene that is responsible for phenotypic variation of a specific trait. Association mapping is faster and cost effective compared to other mapping methodologies. Association mapping can map quantitative traits in a very powerful statistical way. It has been identified to be useful in locating major QTL in maize (Ingheland *et al*., 2012) whereby diverse inbred lines are incorporated in several association mapping studies in maize. Additionally, it helps in identifying molecular markers associated with complex traits. To ensure success in association mapping, germplasm chosen should be composed of elite inbred lines, diverse inbred lines or land races (Yang, *et al*., 2010). However, the best association mapping population should have a wide genetic diversity as possible and be used to identify complex genetic traits (Yang, *et al*., 2010). Recently, 32 and 29 QTLs for the two most threatening foliar diseases in maize in the world, southern leaf blight and northern leaf blight have been observed (Poland, *et al*., 2011).

Another method is association mapping based on linkage disequilibrium concept which exploits the diversity observed in existent cultivar and in breeding line without developing new populations. Quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) are beneficial tools for genetic analysis since they give valuable information about genomes in various plant studies. They have been broadly adopted for gene mapping (Topp *et al*., 2013).

Recently, scientists have been using modern molecular technologies such as association mapping and joint linkage to confirm genes for different traits. These techniques will help the scientists to improve on the basic understanding of plant disease resistance, and improving the genetic constitution of plants. However, very little is known about the genetic make-up of many plant traits (Mackay, 2001). This is because the phenotypic variation in most traits is an outcome of several genes involved in the biological system, where each gene has a small to moderate effect on phenotype. Several studies involving exploring the mechanisms of disease resistance showed that different genes are involved in controlling the plant growth or activation of defense responses against pathogens (Esquerre, *et al.*, 2000).

2.5.2 Application of Molecular markers for Northern leaf blight

Molecular markers use has been identified in maize for characterization of germplasm, linkage disequilibrium analysis and mapping quantitative trait loci for different traits (Prakash, 2014). These markers are 100% heritable, fast, efficient, not influenced by environmental factors, have an advantage in selection of simple inherited traits which is more effective and less expensive than phenotypic selection for that trait. They have the ability of improving efficient selection for complex traits following the concept of correlated traits selection (Falconer, 1960). Markers were identified in 1980s with great chances in breeding because they meet some objectives including identification of QTLs, genetic diversity analysis and prediction of hybrid performances of different plant species (Christian, 2012). Markers are polymorphic and show in different ways that the chromosome consisting the mutant gene can be identified from a chromosome with normal genes.

In most cases plant breeders monitor the physical make up of a plant through the use of molecular marker instead of waiting for a plant to reach maturity.

2.5.2.1 Low-Throughput marker system

Restricted fragment length polymorphism molecular markers were first used in 1975 to identify DNA sequence polymorphisms for genetic mapping of a temperature-sensitive mutation of adeno-virus serotypes (Grodicker *et al*., 1975). In 1980s and 1990s RFLP markers were the most popular and widely adopted in plant breeding as they were the first generation molecular markers (Jones, 2009).

The main advantages of RFLPs markers are reproducible, high locus- specificity and co-dominance. Restricted fragment length polymorphism markers were successfully incorporated in constructing genetic maps in several crops such as wheat, maize and rice (Cho, 1998). However, in the past years the use of RFLPs markers in genetic research and breeding has been reported to be low because most plant breeders think that RFLP markers are time consuming, requires the presence of high quantity and quality of DNA and the experimental procedure is tiresome (Edward and Mc.couch, 2007).

2.5.2.2 Medium –Throughput Marker Systems

PCR-based markers- invented in the beginning of 1990s they include random amplification of polymorphic DNA (RAPDs) these markers were invented by two laboratories (Welsh and Mc Cleland, 1990). RAPD markers have been used widely in plant species for assessing genetic variation in population and fingerprinting.

Amplified fragment length polymorphism (AFLPs) is anonymous while the level of their reproducibility is very high but their detection method was laborious and not amenable to automation.

Simple sequence repeat (SSRs) markers were established in 1990s and provided a choice for many genetic researchers since they are highly polymorphic thus highly informative in plants, are co-dominant markers, abundant and uniformly dispersed in plant genome. SSRs markers are time consuming and expensive to develop because genomic regions carrying SSRs must be identified and sequenced.

2.5.2.3 High-Throughput Marker Systems

2.5.2.3.1 Single nucleotide polymorphism

Single nucleotide polymorphism is a single nucleotide base change between two DNA sections. It can either be a deletion or insertion of nucleotides. SNPs are used as molecular markers in plant breeding program and especially in genetic analysis which is gaining interest and displacing other form of molecular markers due to their increasing availability in the genome sequence and reduction in cost (Rafalski, 2002). SNP markers are identified to have several recommendable features of an ideal marker (Yang, *et al*., 2010) and their abundance availability in plant genomes enhances construction of very high density genetic maps. This enhances SNPs to become markers of choice in construction of linkage maps, analysis of genetic diversity, mapping quantitative trait loci and in marker-assisted selection (Ching and Rafalski, 2002). SNPs can work as powerful tool for markers assisted selection because they are highly stable and have low mutation rate that makes them good markers for studying complex genetic traits (Syvanen, 2001). Recently SNPs have been

used to assess diversity within genomic region for a number of years where maize genetic diversity was studied using SNPs at 21 loci along chromosome 1 (Tenallon *et al*., 2002). The study enhanced understanding of the forces contributing to genetic diversity in maize. A number of genotyping methods are available but most are expensive for academic laboratories and breeding purposes like Illumina Golden Gate® genotyping and Biosciences Allele Specific PCR technologies (Comai, 2004). It has been identified that SNP frequency in maize are higher compared to other crops such as rice that has a SNP frequency of 0.5-0.78%, soybean 0.36% and wheat with 0.5% (Vroh *et al*., 2006).

2.5.2.3.2 The K Bioscience Competitive Allele-specific PCR

K Bioscience Competitive Allele-specific is a SNP genotyping system that enhances detection of SNPs without separating and also gives a strong system for determination of SNP insertion or deletion. It originated from K Bioscience where it was used in laboratories for some years while undergoing improvement.

K Bioscience Competitive Allele-specific genotyping help researchers and breeders who are interested in analyzing small quantity of specified SNPs in a large number of samples. However, this makes it the most efficient, cost effective, simplest and flexible way to determine SNP genotypes. Although KASPar system has been introduced in the market recently they have started to be used for large number of species. In maize 695 highly polymorphic gene-based SNPs from 13,882 validated SNPs were selected and converted into KASPar genotyping system with successive rate of 98% (Mammdow *et al*., 2012).

2.5.2.3.3 Genotyping by sequencing

Genotyping -by - sequencing is an important platform for constructing libraries for next generation sequencing, also help to study a single gene marker to a whole genome (Poland, 2012). GBS allows detection of a number of genetic variations that is capable to contain small indels and also large-mega- base indels (Kiani *et al*., 2013). Genotyping -by - sequencing was first developed for high resolution association studies in maize. However, since then it has been widely used on a number of species with complex genomes.

Genotyping -by-sequencing enhance plant breeders to implement genetic linkage analysis, genomic selection, GWAS and molecular marker discovery under breeding programs. It's simple, high reproducible, quick and reach to the important regions of the genome that sequence cannot reach, it also uses data directly from the population being genotyped thus limiting biasness toward a certain population. GBS is also cost effective making it more viable in plant breeding for providing a rapid tool to genotype. A work flow of GBS has been presented in (figure 3). The DNA samples, barcode and common adapters are placed and dried, the samples are then digested by a restriction enzyme APEK 1 and adapters ligated to the end of the genomic DNA fragment. Appropriate primers (primer 1 and primer 2) are then added by complementary sequence for amplifying restriction fragment with ligated adapters. PCR is then done to increase the fragment pools; later the PCR products are cleaned and the resulting libraries of fragment identified on the DNA analyzer.

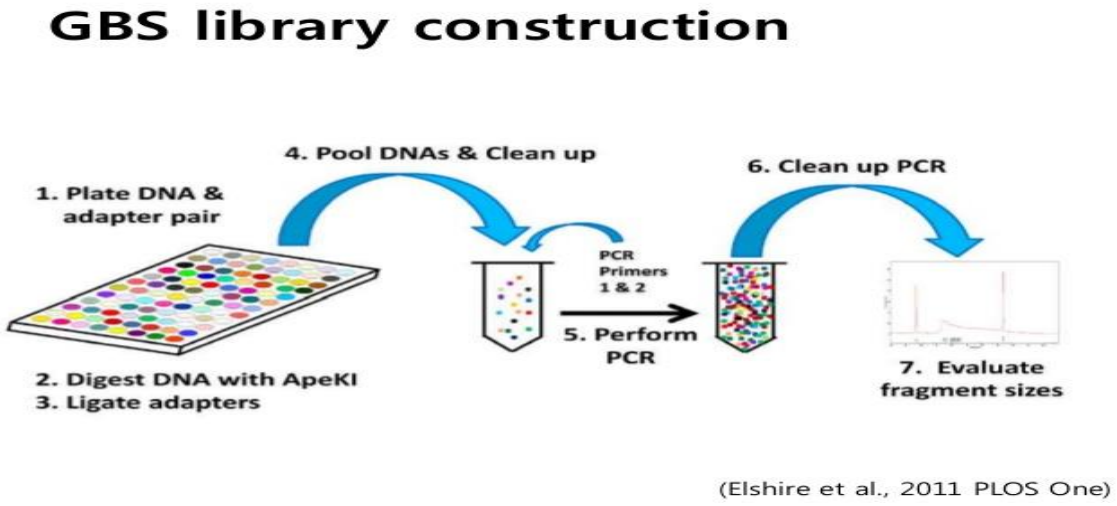

Figure 3: GBS libraries construction (picture taken from (Elshire, Glaubitz, Sun, Poland, Kawamoto, & Buckler, 2011) Elshire et al., 2011)

CHAPTER THREE

COMPARISON OF IMAGE ANALYSIS AND VISUAL SCORING FOR PHENOTYPING REACTION OF MAIZE TO NORTHERN LEAF BLIGHT

3.1 Abstract

Northern leaf blight (NLB) is a devastating fungal disease in Sub-Saharan Africa. The disease leads to a dramatic reduction in yields and the produce is unfit for consumption. Conventionally, it takes a couple of days to phenotype disease severity in a canopy of plants while the phenotyping data obtained is neither precise nor accurate. Modern phenotyping technologies which involve the use of digital imagery tools have gained rapid adoption in maize breeding programs. This study was designed to determine the effectiveness of visual scoring and image analyses in phenotyping reaction of maize to northern corn leaf blight. In this study, two parental lines obtained from the International Maize and Wheat Improvement Center (CIMMYT) gene pool were test-crossed to two single cross parents (CML312/LA Posta SeqC7 F64) and (LA Posta SeqC7 F64/CML539/) to generate 192 DH hybrids. The 192 hybrids and four commercial checks were grown on an alpha-lattice design, with two replications in two locations in Kenya under high NLB disease pressure conditions during 2016-2017 growing seasons. Scoring for NLB disease symptoms was achieved through visual scoring using the CIMMYT scoring scale of 1-5. Imagery analysis data was collected after flowering these was done by taking images of maize plants after flowering. Agronomic traits data for every population were as well collected, they included day to anthesis, plant aspects, AUDPC and NGRDI. Data obtained was analyzed using Meta-R software and some of the results were as follow, In population 1 that was from cross LPS64xCML312 the days to anthesis had a heritability of 0.8, CV f 2.5, and LSD of 2 respectively, plant aspect had a heritability of 0 and CV of 18, respectively, the

heritability estimates for (AUDPC) area under disease progressive curve was 0.43, a coefficient variance of 12.9 and LSD of 7.6 respectively. In population 2, cross LaPSF64 x CML 539, anthesis days had a heritability of 0.72, CV of 2.11 and LSD of 1.94, respectively; plant aspect had a heritability of 0.2, CV of 17.9 and LSD of 0.2 respectively, AUDPC had a heritability of 0.6, CV of 9.7 and LSD of 8.1 respectively. However, image and visual morphology were evaluated from the results obtained. Our results suggested that due to high accuracy on the AUDPC compared to the visual scoring, visual assessment remains subjective and prone to error unlike in image based phenotyping that provides more accurate scores, saves time are more efficient and repeatable. In visual scoring biased information was acquired. Therefore, imagery phenotyping is more preferred by plant breeders in their breeding programs because it enhances fast development of disease and pest resistant crop varieties.

3.2 Introduction

Plant phenotyping is not a new technology for recording quantitative and qualitative plant traits. It has been the backbone of most studies in ecology, agronomy, and eco-physiology to explore plant functional diversity, compare the performance of species, or study plant responses to different environment (Granier and Vile, 2014). Imagery tools have been developed to conduct acquisition of automated images, analysis and quantifying aspects in the field (Andrew, *et al*., 2015).

These methods are being used to study the development and growth of plants (Spalding and Miller, 2013). In numerous plant pathogens relationships less information is identified on the physiological mechanisms that shows symptoms of pathogens and diseases

produced on affected plants. Earlier, detecting of disease severity in plants depended on visual scoring. These visual based methods slowed early detection of plant diseases since small causing micro-organism are invisible to human eyes, the information obtained highly depended on the rater's experience, it took the rater lot of working hours in case of unfavorable weather condition and accessibility to the field was also a challenge.

However, introduction of Image-based phenotyping methods has led to a number of positive results in breeding programs due to their numerous benefits advantages; They are amenable to automation, making it easy to study large populations due to their high statistical power, they are non-destructive, meaning that phenotypic data can be collected from the same area over the course of a long experiment, can detect spatial patterns of heterogeneity and allow visualization of localized responses which may likely be a challenge to identify using other methods.

High-throughput techniques like thermal imaging and hyper spectral obtain data that cannot be identified with eyes. The image data generated image data from various phenotyping systems requires appropriate data management as well as an appropriate analytical framework for data interpretation (Florani and Schurr, 2013). Imaging techniques assist plant researchers' in identifying features and functionality of living plants through scanning temperature profiles, measuring photosynthetic rates, checking on growth rates, and getting into root physiology (Finkel, 2009).

3.3 Material and methods

3.3.1 Description of parental inbred lines used in the study

Three inbred lines CML 312, CML 539 and LA Posta sequia were used for evaluation of Northern leaf blight resistance in two regions, Kenya Agricultural and Livestock Research Organization (KALRO) - Embu and Kakamega. Double haploid populations obtained from CML312xLApostaseqF64B and CML539xLApostaSeqF64B were used in this study.

3.3.1.1 Inbred lines

CML 312

CIMMYT maize line (CML 312) is a tall intermediate maturing inbred line that has white, semi-flint kernels. Its source is from P500 with a pedigree of S89500F2-2-2-l-l-B*5 and developed by International Maize and Wheat Improvement Center (CIMMYT) Harare station. CML 312 matures late and is well adapted to subtropical regions. A genetic study also shows that the inbred line has good resistance to grey leaf spot and *Exserohilum turcicum* (Pswarayi and Vivek, 2004).

CML 539

CIMMYT maize line 539 inbred lines were developed by CIMMYT Harare station. It is tolerant to *Exerohilum* turcicum and early maturing.

LA Posta SeqF64B

Laposta Sequia has kernels that are dent and white in colour. It was obtained from CIMMYT population 43C through recurrent selection (CIMMYT, 1975). The maizeline is well adapted to lowland zones and it mature late.

Table 3. 1 Origin, genetics and agronomic character of plant materials

Inbred line	Germplasm resources	Country of origin	NCLB reaction
CML 312	CIMMYT	Zimbabwe	Resistance
CML 539	CIMMYT	Zimbabwe	Tolerant
LaPostaSeq (LPS64)	CIMMYT	Mexico	

3.3.2 Site description

3.3.2.1 KALRO- Embu

The experimental fields were situated at Kenya Agricultural and Livestock Research Organization (KALRO) - Embu which is in Eastern province, Embu County. The station lies at an altitude of 1060 m above the sea level, latitude 0°47'S and longitude 37°40'E. The mean annual rainfall ranges between 730 -1200 mm received during long rains (April to August) and short rains (September to December) respectively.

3.3.2.2 KALRO- Kakamega

The second experimental was conducted at KALRO- Kakamega which is in western region of Kenya, Kakamega County. The station lies an altitude of 1270 m above the sea level, latitude 0 °17 0 N and longitude 34° 44 58" E. The mean annual rainfall ranges between 1250- 1750 mm and an average temperature of 20. 5°c with two cropping seasons where long rains fall in March to July and short rains in August to November .The soils types are deep, friable, Basaltic loam, fertile and well drained (http:// www.kari.org, 2008). The soil at KALRO- Kakamega has conditions favorable for *Exserohilum turcicum* infection and thus this site was chosen as a NLB hot spot area.

3.3.3 Activities

3.3.3.1 Experimental Layout and design

The field experiments were conducted in KALRO Embu and Kakamega where double haploid lines obtained from the CIMMYT gene pool were test-crossed to two single cross parents (CML312/LA Posta SeqF64) and (LapostaSeq F64/CML539) in a North Carolina design to generate 192 DH hybrids. An alpha-lattice design with two replications was used to evaluate the 192 DH hybrids with other four commercial checks (CZH0616, WE1101, WH507 and CZH0616) across the two sites. The experiments were conducted under rain-fed condition, irrigation and under a high disease pressure conditions in 2016-2017

growing seasons. Each single-row plot measured 4m long spaced at 75cm between rows and 25cm between one hill to the other. Three seeds were sown per hill and later thinned to one seed per hill to achieve the targeted plant population. Crop husbandry practices for all experiments involved application of phosphate fertilizer (Di-ammonium Phosphate-DAP 18:46:0) of 3g per hill (80kg P2O5) at planting. Top- dressing was done six weeks after planting an amount of 2g in every hill (80kg N/ha) of nitrogenous fertilizer (Calcium ammonium Nitrate - CAN 26%N). Bull dock, 0.05 GR (Beta-cyfluhtrin) was used to control stalk borers and cutworms. Weeds were mostly controlled using round-up.

3.3.4 Data collection

3.3.4.1 Disease assessment

A score rating of Northern leaf blight (NLB) was done basing on a scale of five points as suggested by (CIMMYT, 1985). (Table 3.2)

Table 3. 2 NLB disease scoring

Score	Remarks
1	no infection, fully clean plants,
2	light infection with moderate number of lesions on lower leaves
3	moderate infection with abundant lesions on the lower leaves and a few lesions on the middle leaves,
4	heavy infection with lesions abundant on all leaves
5	Very heavy infection with all leaves fully covered by lesions.

NLB resistance ratings were done three times and were basing on visual evaluation of symptoms on each plant. Disease ratings were done from when the disease symptoms started showing till harvesting time. The Area under Disease Progress Curve (AUDPC) was determined according to the equation of (Campbell and Madden, 1991).

3.3.4.2 Agronomic data

i. Anthesis day (AD) refers to the number of days from planting to when 50% of plants had tassels

ii. Silking day (SD) refers to the number of days from planting to when 50% of plant had silk.

iii. Plant height (PH) was measured from the top of the soil to the node of flag leaf;

iv. Ear height (EH) was measured in centimeters as height between the base of a plant to the node bearing the upper ear respectively. Measurements were randomly obtained on 10 plants from every plot.

v. Ear aspect (EA) and Plant aspect (PA) was scored using a scale of 1 to 5. For the ear aspect, 1= indicated clean, uniform and large cobs with the preferred texture in the area whereas 5= indicated small non-uniform and diseased cobs with an undesirable texture. For the plant aspect, 1= indicated plants with uniform height, low and uniform ear placement and free of diseases whereas 5= indicated tall plants with high and irregular ear placement and are affected by diseases.

vi. Field weight (FW) was calculated using the ears weight per plot, it was done after the removal of husks.

vii. Grain yields (GY**)** was calculated as the weight of grains using shelled grains.

3.3.5 Statistical Analysis

Analysis of phenotypic data for estimating genetic components and heritability for quantitative traits is an important tool for breeders.

3.3.5.1 Analysis of variance

Analysis of variance (ANOVA) for NLB disease was based on the rating of individual plants. For the agronomic trait data such as plant height (PH), ear heights (EH), days to

silking, days to anthesis, ear and plant height and grain yield (GY) obtained across the environment were subjected to multi- environment trait analysis with R (META-R) software for analysis. The analysis generated the means, genetic variance, heritability, least significant difference and coefficient of variation. The environment and replication were treated as fixed effect while other components were treated as random effects. The ratio of genotypic variance to the phenotypic variance was used to estimate environment heritability (H^2). Best linear unbiased prediction (BLUP) of each line was estimated and the predicted means were used to generate histogram plots and boxplot to determine the distribution of the data, across and within populations.

3.3.5.2 Area under disease progressive curve (AUDPC)

Area under disease progressive curve was introduced and calculated according to the equation of (Campbell and Madden, 1991) using the following formula;

$$AUDPC = \sum_{t-1}^{n-1} [(xx_{i+\ 1^{+}x_i}) \ / \ 2] \ + t_{i+1^{+}}x_{i+1}$$

Where,

x_i Is the percentage expressing disease index at the 1th observation

$\boldsymbol{t_i}$ Represents the time at the 1th observation

n Represent the number of observation in total

3.3.5.3 Heritability

Heritability (h^2) in broad sense was calculated as the ratio of total phenotypic variance as expressed as percentage.

$$h^2 = \frac{\sigma^2 g}{\sigma^2 \rho} \ \times 100$$

Where;

$\sigma^2 g$ Is the genotypic variance

$\sigma^2 \rho$ Is the phenotypic variance

3.3.5.4 Correlation analysis

The correlation coefficient was carried out to determine the degree of association of a particular trait with yield and among other agronomic traits. Genotypic and phenotypic correlations were computed by using the formula given by (Weber and Moorthy, 1952).

$$r = \frac{cov(xy)p}{\sigma p_x \times \sigma p\gamma} \times 100$$

$$r = \frac{cov(xy)g}{\sigma g_x \times \sigma g_y} \times 100$$

Where;

r = Correlation coefficient
Cov$(x\gamma)$ =Covariance between the trait x and y
$\sigma\rho x$ *and* $\sigma\rho\gamma$ =phenotypic variance of the trait x and y
σgx *and* $\sigma g\gamma$ =Genotypic variance of the trait x and y

3.3.5.5 Image based Analysis

The images and annotation data collected were subjected to the integrated analysis platform (IAP) maize pipeline for analysis (Christian *et al*., 2014).

3.4 Results

3.4.1 Analysis of Variance

The analysis of variance showed significant difference ($P \leq 0.01$) among the parents for the disease severity and all other agronomic traits within the evaluated maize genotypes for Northern leaf blight resistance.

3.4.1.1 Cross LPS64xCML312

In the components of variance, anthesis day had a heritability of 0.78, LSD of 1.97, %CV of 2.5 respectively. Plant aspect had heritability of 0, LSD 0, and %CV of 18, respectively. For the AUDPC heritability was 0.41, LSD of 7.6 and %CV of 12.9 respectively. NGRDI-100 recorded heritability of 0.60, LSD of 1.60 and %CV of 228.4 respectively. Heritability

estimate was 0.75. AUDPC recorded a genotypic variance of 33.8, %CV of 9.7, heritability estimate of 0.55 and LSD of 8.05 respectively. Anthesis day and NGRDI-100 recorded high heritability values thus showing that the variability in the trait is due to genetic differences. Plant aspect recorded a heritability of zero this showed that all the variability in the trait is due to environment (Table 3.3).

3.4.1.2 Cross 1 CML539 x LPS64

On the variability the tasseling day had a heritability estimate of 0.74, LSD of 1.90, %CV of 2.048 respectively. Plant aspect had a heritability estimate of 0.17, LSD of 0.24, and %CV of 17.9 respectively. For the AUDPC, heritability estimate was 0.55, LSD of 8.05 and %CV of 9.7, respectively. Tasseling day and AUDPC recorded high values for heritability whereas plant aspect recorded a low value, this showing that almost all the variability in the trait are due to environment (Table 3.4).

Table 3. 3: Means, genotypic variance components (σ^2G), error variance (σ^2e), narrow sense heritability (h^2), least significant difference, and coefficient of variance, of CML312/LaPSF64 DH population evaluated for NCLB disease, agronomic and image traits across environments

Statistic	AD	PH	EH	PA	HC	NE	FW	EA	TURC1	TURC2	AUDPC	Necrosis Soil	Saturation	NGRDI_100
h^2	0.8	0.5	0.5	0	0.2	0.4	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.6
σ^2G	4.1**	67	22	0	0.4	1.9	0.1	0	0	0	23.6**	6.4	4.3	1.5
σ^2e	4.2**	201	72.3	0.1	3.7	8.1	0.2	0.2	0.1	0.2	92.2**	27.6	23.3*	3.5**
Mean	83.9	184	77.3	1.8	3	12.2	2	3.1	2.2	2.7	74.3	75.7	87.4	-0.8
LSD	2	11.9	6.9	0	1.1	2.1	0.5	0.3	0.3	0.3	7.6	4	3.4	1.6
CV	2.5	7.7	11	18	63.6	23.2	25.2	14.6	16.6	14.4	12.9	6.9	5.5	228.4*

**P≤0.001; **P≤0.01; *P≤0.05; AD= anthesis days; interval; PH=plant height; NE= number of ears; FW=Field weight; HC=husk cover EH=ear height; PA=Plant aspect; EA=ear Aspect; Turc= Turcicum; AUDPC== area under disease progress curve; NGRDI= normalized green- red difference index; σ^2g=genetic variance; LSD=least significant difference CV%=coefficient of variance; h^2=heritability

Table 3. 4: Means, genotypic variance components (σ^2G), error variance (σ^2e), narrow sense heritability (h^2), least significant difference, and coefficient of variance, of LaPSF64XCML 539 DH population evaluated for NCLB disease, agronomic and image traits across environments

Statistic	AD	SD	PH	EH	PA	FW	EA	Turc1	Turc2	Turc3	AUDPC	green veg	Greener veg
h^2	0.7	0.7	0.6	0.7	0.2	0.8	0.4	0.5	0.4	0.6	0.6	0.5	0.4
σ^2G	3.2**	3	67.6	39.6	0	0.4	0	0	0	0.1	33.8**	15.7	18.4
σ^2e	2.6**	2.8	118.6	58	0.2	0.4	0.2	0.1	0.1	0.1	36.1**	60.9	109.9
Mean	79.3	81.2	210.4	91.5	2.2	3	2.4	1.8	2.2	2.6	61.9	39.7	61.6
LSD	1.9	2.1	10.4	7.7	0.2	0.7	0.3	0.2	0.3	0.4	8.1	5.9	6.8
CV	2.1	2.1	5.2	8.3	17.9	19.9	16.6	13	13.9	11.4	9.7	19.6	17

**P≤0.001; **P≤0.01; *P≤0.05; AD= anthesis days; SD= silking days; interval; PH=plant height; EH=ear height; PA=Plant aspect; EA=ear Aspect; Turc= Turcicum; AUDPC= area under disease progress curve; σ^2g=genetic variance; LSD=least significant difference
CV% =coefficient of variance; h^2=heritability

3.4.2 Phenotypic correlations

The correlation study provides an idea about the traits whether or not they are associated with each other. Across the sites, grain yield had a negative correlation with flowering day (r= -0.46), silking Day (r = -0.48), root Lodging (r= -0.13), stem lodging (r= -0.04), ear Aspect (r =-0.72), ear rot (r = -0.103), AUDPC (r = -0.65), saturation (r= -0.12), brightness (r =-0.29). Grain yield also had significant and positive correlations with field weight (0.98) and number of ears (0.76). Flowering day, silking day, stem lodging, root lodging, ear rot and ear aspect had a positive correlation with turcicum. AUDPC-Turc showed a positive correlation with Flowering day, silking day, stem lodging, root lodging, ear rot and ear aspect but recorded a negative indirect effect for field weight with -0.788 respectively (Table 3.5).

Table 3. 5: Phenotypic correlation among different traits in a combined association measured for 192 DH lines

Traits	AD	SD	PA	NE	FW	EA	AUDPC	NGRDI-100
SD	0.83							
PA	-0.45	-0.39						
NE	-0.57	-0.61	0.31					
FW	-0.46	-0.48	0.07^{xx}	0.75				
EA	0.67	0.68	-0.31	-0.77	-0.73			
AUDPC-T	0.37^{xx}	0.39	-0.06	-0.55	-0.68	0.61		
NGRDI	-0.35	-0.4	0.09^{xx}	0.49	0.55	-0.59	-0.59	
GY	-0.46	-0.48	0.07^{xx}	0.76	0.98	-0.72	-0.67	0.54

Significance levels; **=P<0.01 and *=P<0.05, ns= non-significant; GY= grain yield; Turc =Northern leaf blight; SD= Silking day; PA= Plant aspect; EA=Ear aspect; NE=number of ears; FW= field weight; AUDPC= area under disease progress curve; NGRDI= normalized green- red difference index.

3.5 Discussion and conclusion

Maize is among important food crops benefitting both the industries, animals and man in the world. In the Sub-Saharan Africa, NLB caused by fungus *Exserohilum turcicum* (Pass) (Dunn and Namn, 1977) has become the major threatening foliar disease that is really affecting food security. Insufficient availability of adequate and diverse genetic source of resistance to NLB has been the main constraint. For plant breeders to achieve all these objectives in breeding resistant crop cultivars, phenotyping of the crops in the experimental fields has to be implemented. These phenotyping techniques help breeders to achieve in monitoring phenotypic traits of the crops. Phenotyping involves assessing of crops with diseases, pests and the severity caused by diseases.

The present study was conducted to identify the most effective phenotyping technology namely the visual assessment with the imagery technology. Considerable variation was found in visual scoring because most of the information on the quantitative traits obtained was biased due to human error while the digital imagery tools collected accurate and precise information. This study first confirmed that traditional ways of phenotyping quantitative traits was not efficient at all because it depended on the rater's experience, rater's ability to carry out effective assessment, weather condition during assessment period and accessibility of the fields. The differences observed between the digital imagery phenotyping tools and the visual methodology gives plant breeders basics for developing improved germplasm. The main objective is to lower the effects of diseases on agricultural production and also to identify the relationship between the disease symptoms and the effects on yields. Imaging could be help to identify many plant diseases that occur with

only internal symptoms for example the *Fusarium spp* fungi that cause maize ear rot (Mester *et al*., 2012). Previous studies have shown that these phenotyping technologies are neither precise nor sensitive compared to visual evaluation for certain host–pathogen. According to Olmstead *et al*. (2001), the use of image based technologies in estimating powdery mildew infection on sweet cherry leaves failed to produce correct estimates for the leaf area infected due to unclear colour between the infected and the uninfected leaf areas. An extensive morphological and genetic diversity for plant architecture traits in maize could aid to study complex traits (Flint, *et al*., 2005).

In the present study, the disease severity of one resistant parent CML312 and two susceptible parents CML539 and LA Posta seq were assessed at flowering, after flowering and at maturity. For the imagery technology, images of the maize crops were obtained at an interval of 10days from flowering time until maturity by collecting the data three times. Moreover, leaf scanners were used in scanning three maize leaves from every plot with the aim to observe the number of lesions on the leaves. Two DH populations from crosses LPS64x CML312and CML539x LPS64 were evaluated.

After a combined analysis across locations, CML539xLPS64 phenological and morphological traits, such as days to tasseling and silking, plant and ear height, showed high values for the mean, plant aspect, ear aspect, field weight and disease scores recorded low mean values. Heritability estimates for tasseling day, silking day, field weight and ear height was high at 0.74, 0.67, and 0.75 while the plant and ear aspect showed intermediate to low heritability. Genotypic variances analysis was highly significant. These findings

showed that the CML539 parental line had no resistance traits to Northern leaf blight with reference to its poor performance.

In cross LPS64xCML312, the morphological traits, the tasseling day, silking day, field weight and number of ears showed moderate to high heritability estimates, for plant and ear aspect, stem and root lodging they expressed a low heritability estimate. Mean values was high for tasseling day, silking day and area under disease progressive curve. The genotypic variance of the traits was significant.

This study revealed that the LPS64xCML312 DH hybrid was superior, indicating that the donor parent used in developing the DH lines had better resistance or favorable alleles to resist northern leaf blight in maize. Their parental components also showed presence of good genetic materials that should be further evaluated for commercial use.

CHAPTER FOUR

MAPPING GENOMIC REGION ASSOCIATED WITH RESISTANCE TO NORTHERN LEAF BLIGHT DISEASE IN TROPICAL GERMPLASM

4.1 Abstract

Northern leaf blight disease (NLB) is a devastating disease in the Sub-Saharan Africa (SSA) which causes significant yield loss in maize. The main purpose of breeding is to characterize diversity with an aim of developing germplasm that has durable genetic resistance to diseases and high yielding. In this study, the objective was to identify quantitative Trait Loci (QTL) related to northern leaf blight in tropical maize germplasm. An association mapping panel of 192 double haploid lines with different degrees of reaction to the disease derived from a CML312xLPS64 and CML 539xLPS64 were planted in Embu and Kakamega in 2016 and 2017. NLB score and other agronomic traits were investigated under high disease pressure areas. Significant phenotypic variation in NLB resistance was observed. Using a genetic map containing high density single-nucleotide polymorphisms with average genetic distance of 0.30Cm, qualitative trait loci for score and agronomic traits were analyzed. For NLB score, two stable QTLs were identified in 2016 respectively. For these *qNLB 7* located on chromosome 7 had the largest resistance effect, accounting for 33% of the phenotypic variation in 2016 respectively. The identification of quantitative trait loci conferring resistance to NLB may contribute to breeding programs seeking to protect the crop through improved genetic resistance.

4.2 Introduction

Northern leaf blight disease is one of the major biotic constraints in maize production system in Sub-Saharan Africa. It belongs to the division *Eumycota*, sub-division *Deuteromycotina*, order *Moniliales* and family *Dematiaceae*. NLB disease outbreaks often coincide with moderate temperatures and humidity (Balint and Johal, 2009). Maize yields are dramatically reduced by NLB for destroying the photosynthetic process at the period of grain-filling (Raymundo and Hooker, 1981). Therefore, it's considered to be first important disease of maize in the world then followed by gray leaf spot and maize streak virus (Pratt and Gordon, 2006). One of the most suitable ways of managing the NLB disease is through host plant resistance. Maize breeders from CIMMYT in collaboration with many national and international agricultural research institutions in SSA have been continually developing improved maize varieties with resistance that are routinely deployed in the region. Resistant lines possessing *Ht* gene have been reported and widely used in maize breeding programs (Welz and Geiger, 2000). Dominant genes (*Ht1, Ht2, Ht3* and *Htn1*) have been mapped with molecular markers. Gene *Ht1* has been mapped on bin 2.08 at chromosome 2 (Bentolila, *et al*., 1991). *Ht2* has been mapped *Tripsacum floridanum*on chromosome 8 on bin 8.06. Gene *Ht3* was introgressed from into maize (Inghelandt *et al*., 2012) and was mapped on chromosome 7 bin 7.04 (Zhang *et al*., 2014). Gene *Htn1* mapped on bin 8.05 is effective to most NCLB races however, its level of resistance depends on environment and genetic. Another major QTL on chromosome1 have been fine-mapped conferring resistance to NLB, *Stewart's wilt* and common rust (Jamann *et al*., 2016). In this study, evaluation of resistance to NLB was done performed in two different ecological zones, KALRO-Embu and Kakamega center where epidemic of

NLB has appeared. The main aim of this experiment was to identify the QTLs position associated with resistance to NLB in 192 DH lines. Inbred lines CML312, CML539 and La Posta seq were used with the molecular markers (SNPs). Studies aiming to know the genetic background of NLB resistance genes are important to guide maize breeders and plant pathologist for breeding programs.

4.3 Material and methods

4.3.1 Plant materials and field design

A mapping population of 192 double haploid lines was produced from a cross between CML312 (resistant), CML539 (susceptible) and LA Posta seq. The double haploid lines and their parents were planted at KALRO-Embu and Kakamega center in 2016 -2017. An alpha-lattice design with two replications was used in these studies. CML312x La Posta and CML539xLa Posta seq were planted respectively. Each single-row plot measured 4m long spaced at 75cm between rows and 25cm between hills. Three seeds were planted per hill and later thinned to one seed per hill to achieve the targeted plant population. Standard agricultural practices were followed throughout the growing season. Table 4.1 shows the origin and description of different lines.

Table 4. 1: Origin, genetics and agronomic characteristics of germplasm

Variety	Origin	Genetic constitution	NCLB reaction
CML 312	CIMMYT-Zimbabwe	Inbred line	Resistant
CML539	CIMMYT	Inbred line	Susceptible
LA Posta seq F64B	CIMMYT	Inbred line	

4.3.2 Evaluation of Resistance for Northern leaf blight

The 192 DH lines were grown at an experimental field of Kenya agricultural livestock research organization Embu and Kakamega in April to September 2016. Resistant levels assessment was performed on individual plants of each plot, mean score was also

calculated for every plot. NLB severity was scored using a five-point relative scale from (CIMMYT, 1985) where one indicated, highly resistant (no infection, fully clean plants) and five designated as highly susceptible (very heavy infection with lesions on all leaves).

4.3.3 Statistical data Analysis

Several range tests that includes genetic variance components, analysis of variance (ANOVA) and correlation coefficient of NLB rating in two locations and a combined analysis for NLB in DH lines were analyzed using Meta-R software program. Heritability of means was estimated from the variance components. In addition, best linear unbiased estimates (BLUEs) were estimated across locations assuming fixed genotype effects. For association analyses, best linear unbiased prediction (BLUP) of each line was calculated for across locations.

4.3.4 Genotypic data analyses

DNA was of extracted from the inbred lines that were grown in a greenhouse at 3–4 leaves stage in December 2016 at CIMMYT screening facility in the Kenya Agricultural and Livestock Research organization, KALRO- Naivasha. It lies at a Latitude 0 43’S, Longitude 36 26’E, 1896m above sea level. DNA was used for genotyping using GBS platform (Elshire *et al*., 2011) at Cornell University in the Genomic Diversity Institute, USA. Leaves were cut, folded and put in different perforated bags with ice-cubes and taken to laboratory where genotyping was done using high density markers through genotyping -by- sequencing (Elshire *et al*., 2011).

4.3.5 Linkage analysis and inclusive composite interval mapping

After SNPs number were minimized by choosing the homozygous and polymorphic markers, high quality SNP data were used to construct genetic linkage maps for two populations using QTL Ici-Mapping version 4.1.1 Software, MAP function (Meng *et al*., 2015). The genetic maps were constructed using the estimate map which uses Lander-Green algorithm. ICIM is a two-way method used to separate the cofactor selection from interval mapping process effectively hence controlling the background effects and improving mapping of QTL with additive effects (Meng *et al*., 2015). The highly significant markers were used to calculate the logarithms of odds (LOD) scores for each marker of more than 3.0 logarithms of odds (LOD) and a maximum distance of 30cM between the loci. In each population, BLUPs across environment and AUDPC value for NLB were incorporated to detect QTL based on inclusive composite interval mapping (ICIM). A threshold LOD score of 3 was used to determine a significant QTL and the phenotypic variation explained (PVE) across all QTL and in each trait (Tuberosa, *et al*., 2007).

4.3.6 Joint linkage association mapping (JLAM)

Two Double haploid populations genotyped with GBS were used for JLAM. For quality checking in both populations the SNPs that were either monomorphic, missing value, heterozygous, or with a minor allele frequency were eliminated from the analysis. 404SNPs of high quality were retained for further analysis in all populations. BLUPs estimated across population and regions were used in JLAM studies.

4.3.7 Genome –wide association analysis

BLUP of each line was used as phenotypes in association mapping. NCLB severity data were corrected for population structure using general linear model (GLM), as well as population structure and kinship (Q + K) using mixed linear model (MLM) algorithm (Flint, *et al*., 2005). GWAS and principal component (PC) analysis was performed using TASSEL version 5.2 (Bradbury *et al*., 2007). The first three PCs were used to correct the population structure.

4.4 Results

4.4.1 Phenotypic variation and heritability

Descriptive variation was observed for Northern leaf blight AUDPC values in two double haploid populations (Table 3.3 and 3.4). Between the two CMLs used as parents in DH population, CML312 was resistant with a mean of 74.3 whereas CML539 was susceptible with a mean of 61.9 for AUDPC value, respectively. The combined analyses of the two populations revealed an average mean of 67.8 for AUDPC. The ANOVA across the environment revealed significant genotypic variance for AUDPC value in each and across the DH populations. Heritability h^2 estimates ranged from moderate to high with 0.42 in DH pop2 for AUDPC to 0.55 in DH pop1 for AUDPC value, respectively (Table 4.2).

Table 4. 2: Combined Means, components of variance for disease severity, agronomic traits, image traits and area under disease progress curve (AUDPC) measured for both pop 1 and pop 2 DH populations in Embu and Kakamega.

Statistic	AD	SD	PH	EH	PA	FW	EA	AUDPC_T	Necrosis_ soil	Satur-ation	NGRDI_100	GY
h^2	0.7	0.4	2.71	0	0	0.2	0.4	0.4	0.5	0.6	0.3	0.3
σ^2G	6.7	6.8	1.07	0	0	0.2	0.1	51.9	12.9	13.2	1.1	0.3
σ^2e	4.9	25.1	111.2	40.9	0.2	0.3	0.3	83.4	51.7	30.9	6.7	0.2
Mean	81.7	84.3	199.2	85.8	2	2.5	2.8	67.8	77.7	85.9	0.3	2.5
LSD	3.1	4	2.35	0	0	0.7	0.5	10.9	5.2	4.8	1.8	0.8
CV	2.7	5.9	5.39	7.5	20.9	21	17.9	13.5	9.2	6.5	803.2	18.3

**P≤0.001; **P≤0.01; *P≤0.05; AD= anthesis days; SD= silking days; interval; PH=plant height; EH=ear height; PA=Plant aspect; EA=ear Aspect; FW= field weight; AUDPC= area under disease progress curve; Turc= Turcicum; GY=grain yield; σ^2g=genetic variance; LSD=least significant difference; CV%=coefficient of variance; h^2=heritability

4.4.2 QTL mapping for NLB Resistance

An Inclusive Composite Interval Mapping carried out on cross CML312xLaPS64 with 192DH lines identified AD, SD, Green veg, saturation and Greenerveg QTLs on chromosome 6, with a LOD score ranging between 2.55 and 3.71, greener veg had the highest phenotypic variance of 43.76. EH was identified on chromosome 3 with a LOD score value of 3.48, phenotypic variance of 22.58 and a R^2 of 21.58.

Two more QTLs conferring resistance to Northern leaf blight were detected on chromosome 5 and 7. A major QTL which explained 32.9% of the phenotypic variance and a LOD score value of 3.48 was detected on chromosome 7 between markers PZA00424-1 and S7128895684. Another QTL was identified on chromosomes 5 between markers S5170023977 and PZA014101 which explained a LOD score value of 3.15, a phenotypic variance of 26%, after a cross validation was carried out R^2 values explained 23.7%. Both QTL detected were contributed by the resistant parent, CML312 where one QTL detected on chromosome 7 which explained 32.9% of the total phenotypic variation was found to have the largest effect and the favorable alleles (Table 4.3).

On cross CML539xLaPS64, five QTLs were identified on chromosome 1, 8, 6 and 10 respectively, two QTLs were detected on chromosome 1 and 8 in AD with a LOD score values of 2.66 -3.12 and a R^2 value of 14.95. Other QTLs were identified on chromosome 6 and 10 on Ear aspect and grain yields. It was concluded that the cross CML539xLaPS64 was susceptible to Northern leaf blight.

Table 4. 3: Detection of QTL associated with resistance to NCLB, their physical positions and genetic effects of the QTL in two DH populations CML539xLaPOSTA F64 and LAPOSTAF64xCML 312

Trait name	Chromosome	Position	Left marker	Right marker	LOD score	PVE %	Add	R^2
CML312 X LPSF64								
AD	6	145	PZA00440_15	PZA02673_1	3.49	23.39	1.55	15.32
SD	6	29	PZA00910_1	PHM4662_153	2.73	9.02	0.43	
	8	44	S8_102533570	PZB02155_1	4.17	13.86	-0.49	39.51
EH	3	38	S3_144088367	PHM15475_27	3.48	22.58	-2.73	21.58
Turc3	5	199	S5_170023977	PZA01410_1	3.15	26.00	0.28	
	7	163	PZA00424_1	S7_128895684	3.48	32.97	0.09	23.72
Greenveg	6	119	PHM2658_129	PHM7922_8	2.55	18.52	-1.05	12.01
Greenerveg	4	124	PZA01905_12	S4_6209167	2.71	43.76	1.64	
	6	143	PZA01884_1	PZA00942_2	3.71	42.14	-1.83	
	8	200	PZA00739_1	PHM14104_23	3.33	43.21	-1.53	
	9	58	PZB00221_3	S9_145906361	3.50	41.99	-1.77	12.54
Saturation	1	177	PHM1438_34	PZA00175_2	2.65	11.06	0.27	
	6	119	PHM2658_129	PHM7922_8	3.67	25.45	-0.47	21.22
CML539 X LPSF64								
AD	1	91	S1_173654738	PHM4053_15	3.12	16.95	-0.70	
	8	139	PZA02746_2	PZA02019_1	2.66	19.82	1.71	14.95
GY_FW	10	123	PHM4066_11	PZA02961_6	2.89	15.03	-0.21	14.25
EA	6	26	S6_93211949	PZB01009_1	2.89	27.30	0.05	
	10	112	PZA01456_2	S10_120670943	2.90	15.40	0.04	14.54

LOD=logarithm of odds; PVE= phenotypic variance explained; Add= additive effect; AD=anthesis day; SD= silking day; EH=ear height; Turc=turcicum; Saturation= intensity of colour present; Green veg= green vegetation; Greener veg= greener vegetation; EA=ear aspect; GY-FW=grain weight

4.4.3 Joint linkage association mapping (JLAM) analysis

Combined analysis of DH populations through JLAM revealed four QTLs from model A, three QTLs from model B, and two QTLs from model C on Anthesis date (AD) explaining a phenotypic variance of between 0.3 and 0.4; a P value of zero on model A and B respectively. On *Turcicum,* four QTLs from model A, ten QTLs from model B and two QTLs from model C were identified. The QTLs were distributed across 7 chromosomes namely 1, 3, 4,5,7,8 and 10. These QTLs individually explained 3.4-11.1% phenotypic variance in model A, 1.9-9.7% for model B, and 6.8-9% for model C. One QTL *qNLB8-23* was the common QTL to the three models while the others were specific. QTL *qNLB5-147* detected on chromosome 5 has the largest effect QTL and it explained 11.1% of phenotypic variance followed by *qNLB8-23* on chromosome 8 which explained 9.2% phenotypic variance. QTL *qNLB8-23* was consistently detected for the three models. All the detected QTLs together explained 46%, 81% and 53% of total phenotypic variance for model A, B and C respectively. On NGRDI, six QTLs were identified on model A that had a total phenotypic variance of 0.4, two QTLs from model B with a phenotypic variance of 0.2, and three QTLs from model C with a phenotypic variance of 0.6. It was noted that *turcicum, had* the highest phenotypic variance on model A, B and C compared to other traits (Table 4.4).

Table 4. 4: Analysis of traits associated markers, allele substitution (α), effects and total phenotypic variance (R^2) of the joint linkage association mapping in Double haploid population based on three different biometric models

Trait	Chromosome	Position (Mbp)	Model A			Model B			Model C		
			α-effect	P value	PVE (%)	α-effect	P value	PVE (%)	α-effect	P value	PVE (%)
Anthesis Date											
PHM1956_90	3	0.0	3.0	0.0	14.4	3.0	0.0	19.7	2.3	4.0	22.4
S3_82056859	8	123.3	-0.8	0.0	12.6	-0.8	0.0	9.2	-	-	-
PZA01796_1	10	8.8	0.4	0.0	3.6	0.4	0.0	3.6	-	-	-
PZA03713_1	7	137.3	-	-	-	-	-	-	0.5	0.4	6.1
Total PVE (%)					0.3			0.3			0.4
Silking Date											
PHM15278_6	1	176.7	-0.6	0.0	8.2	-0.5	0.0	6.1	0.3	-0.5	6.0
PZA03226_3	1	208.1	-0.4	0.0	1.8	-	-	-	-	-	-
PHM1956_90	3	0.0	1.2	0.0	12.7	-	-	-	2.8	3.4	17.1
PZA02792_26	3	33.0	-0.2	0.0	1.9	-	-	-	-	-	-
PHM13440_13	6	86.5	2.0	0.0	5.9	-	-	-	-	-	-
PHM662_27	6	155.4	0.4	0.0	3.4	-	-	-	0.1	0.0	4.9
PZA02681_8	1	298.9	-	-	-	0.2	0.0	2.1	-	-	-
S6_21007530	5	42.3	-	-	-	2.8	0.0	17.8	-	-	-
S4_19430220	8	123.3	-	-	-	-0.6	0.0	8.3	-	-	-
PZA02564_2	9	142.3	-	-	-	0.3	0.0	3.7	-	-	-
PZA00240_6	9	109.6	-	-	-	-	-	-	0.3	0.2	2.6
Total PVE (%)					0.4			0.4			0.5
Plant Height											
PHM1576_25	1	60.2	2.6	0.0	3.3	2.3	0.0	2.3	-	-	-
PHM499_19	3	48.5	-1.8	0.1	1.7	-	-	-	-	-	-
PZB01358_1	7	137.8	0.4	0.5	0.4	1.9	0.0	4.7	-	-	-

Table 4. 4: Analysis of traits associated markers, allele substitution (α), effects and total phenotypic variance (R^2) of the joint linkage association mapping in Double haploid population based on three different biometric models

Trait	Chromosome	Position (Mbp)	Model A			Model B			Model C		
			α-effect	P value	PVE (%)	α-effect	P value	PVE (%)	α-effect	P value	PVE (%)
PHM635_23	2	186.2	-	-	-	-	-	-	22.4	-3.7	7.5
S1_18838432	7	162.0	-	-	-	-2.3	0.0	7.1	3.1	-1.4	7.5
S10_91685820	9	106.8	-	-	-	2.5	0.0	7.2	2.1	2.2	7.3
S4_149896839	4	150.3	-	-	-	2.2	0.0	2.3	-	-	-
S9_108521912	5	160.3	-	-	-	-3.0	0.0	7.6	-	-	-
PHM2130_29	6	0.0	-	-	-	15.7	0.0	7.4	-	-	-
Total (PVE %)					0.1			0.4			0.3
Ear Aspect											
PZA03742_1	5	211.9	0.05	0.0	7.0	-0.18	0.0	7.0	-	-	-
PZA01905_12	6	129.9	0.032	0.0	5.9	-	-	-	-	-	-
PHM1505_31	2	170.3	-	-	-	-	-	-	0.1	0.0	7.1
Total (PVE %)					0.2			0.1			0.1
Turcicum											
S4_6601124	4	226.9	0.04	0.0	3.4	0.71	0.0	5.1	-	-	-
S10_147595373	5	191.1	0.07	0.0	11.1	0.06	0.0	5.5	-	-	-
S7_127970539	5	196.1	0.21	0.0	6.4	-0.06	0.0	9.7	-	-	-
S4_237313660	8	155.5	0.08	0.0	10.4	0.06	0.0	4.4	0.06	0.1	9.2
PZA03733_1	1	44.5	-	-	-	-	-	-	0.02	0.1	6.8
S5_217019076	8	163.6	-	-	-	-0.1	0.0	2.1	-	-	-
PZA00726_8	10	124.2	-	-	-	0.06	0.0	5.7	-	-	-
PZA01254_2	7	128.9	-	-	-	0.08	0.0	3.0	-	-	-
bt2_7	7	157.5	-	-	-	0.05	0.0	4.7	-	-	-

Table 4. 4: Analysis of traits associated markers, allele substitution (α), effects and total phenotypic variance (R^2) of the joint linkage association mapping in Double haploid population based on three different biometric models

Trait	Chromosome	Position (Mbp)	Model A			Model B			Model C		
			α-effect	P value	PVE (%)	α-effect	P value	PVE (%)	α-effect	P value	PVE (%)
S3_106337279	8	63.5	-	-	-	0.05	0.0	5.1	-	-	-
S1_173421054	3	180.5	-	-	-	-0.04	0.0	1.9	-	-	-
Total (PVE %)					0.46			0.81			0.53
Green vegetation											
PZA03733_1	1	44.5	1.2s	0.0	8.5	1.2	0.0	8.4	0.4	1.3	10.4
PZA02164_16	1	143.8	-8.2	0.0	5.5	0.5	0.0	6.4	0.0	-8.9	7.1
PHM5185_13	2	169.3	-0.5	0.0	1.0	-	-	-	-	-	-
S4_6544767	4	215.3	0.8	0.0	5.6	-2.5	0.0	4.9	-	-	-
PZA00240_6	9	109.5	0.6	0.0	5.1	0.6	0.0	3.6	-	-	-
PZA02746_2	10	105.9			-	-4.7	0.0	4.0	-	-	-
Total (PVE %)					0.5			0.5			0.3
Greener vegetation											
PZA00273_5	10	0.0	-	-	-	-0.7	0.0	8.5	-	-	-
PZA03733_1	1	44.5	0.8	0.0	7.0	-	-	-	-	-	-
S1_46411896	3	170.4	-	-	-	-	-	-	1.6	-1.4	10.9
Total (PVE %)					0.1			0.2			0.3
Saturation											
PHM5484_22	1	53.4	-0.3	0.0	3.5	-	-	-	-	-	-
S9_146012201	5	112.2	0.2	0.0	2.3	-	-	-	-	-	-
S10_147595373	5	191.1	0.5	0.0	7.5	-	0.0	10.9	0.4	0.5	12.1
PHM563_9	6	158.5	-0.5	0.0	6.1	-	0.0	5.7	0.4	-0.4	6.3
PZA02746_2	10	105.9	-2.5	0.0	8.1	-	0.0	8.1	-	-	-

Table 4. 4: Analysis of traits associated markers, allele substitution (α), effects and total phenotypic variance (R^2) of the joint linkage association mapping in Double haploid population based on three different biometric models

Trait	Chromosome	Position (Mbp)	Model A			Model B			Model C		
			α-effect	P value	PVE (%)	α-effect	P value	PVE (%)	α-effect	P value	PVE (%)
PHM14475_7	9	113.1	-	-	-	-	-	-	O	-3.2	6.1
Total (PVE %)					0.4			0.3			0.3
NGRDI											
PZA01290_1	1	68.9	-0.4	0.0	3.7	-	-	-	-	-	-
PZA02164_16	1	143.8	2.1	0.0	4.7	-	-	-	-	-	-
PHM5306_16	3	92.6	-0.4	0.0	3.7	-	-	-	-	-	-
PHM563_9	6	158.5	0.4	0.0	6.7	0.4	0.0	6.7	0.7	0.5	11.1
S7_167230991	9	16.3	-0.2	0.1	1.6	-	-	-	-	-	-
PZA02746_2	10	105.8	1.8	0.1	6.7	1.7	0.0	6.2	2.2	2.3	11.2
S3_173290199	8	105.8	-	-	-	-	-	-	-5.0	0.9	3.8
Total (PVE %)					0.4			0.2			0.6

PVE- phenotypic variance; NGRDI – normalized green- red difference index

4.5 Discussion and Conclusion

Developing improved maize varieties with genetic resistance to NLB is an important component of sustainable crop management strategy in SSA. International institutions such as CIMMYT and IITA have collaborated with several regional and national institutions to develop and introduce a number of maize hybrids and OPVs with enhanced levels of NLB resistance in SSA, especially through conventional breeding. Improved tropical and sub-tropical maize germplasm developed at CIMMYT in Mexico, are routinely deployed in SSA. Adoption of maize varieties in SSA is mostly conditional upon reasonable levels of NLB resistance along with high grain yield. Molecular markers that are associated with NLB resistance could highly enhance pre-selection of genomic regions in the tropical germplasm developed within and outside SSA leading to accelerated genetic gains.

4.5.1 QTL mapping of NLB resistance

Initial mapping for reaction to NLB based on 192 progenies of CML312 (resistant)/La Posta seq (susceptible) identified a QTL on chromosome 7 between PZA00424-1 and S7128895684 markers had a large effect that explained a phenotypic variance of 32.9%. This QTL co-localized with *qNLB7;* a large effect QTL identified in earlier studies involving multiple NLB -resistant lines and evaluated for reaction to NLB in various environments in Africa. In this study, we have not attempted to validate these using testcrosses. In addition to *qNLB7*, one more minor QTL was identified in the current study on chromosomes 5. The source of resistance was from CML 312, a maize line from CIMMYT. These results corroborate with those reported in an earlier research by Chen *et al*. (2015) that showed two stable QTLs namely *qNLB*5.04 located on chromosome 5 (bin

5.04) that had the largest resistance effect accounting for 19 and 20% of the phenotypic variation and *qNLB*7.05 accounting for 11% phenotypic variance respectively.

Several types of resistance to *Exerohilium turcicum* exist in maize (Hooker, 1975). A major QTL for northern corn leaf blight resistance on chromosome 7 has been reported in several studies on NLB resistance with the proportion of phenotypic variance ranging from 24 to 92 % with the locus being a major gene (Welz *et al*., 1998). Similar studies using CIMMYT maize line 202 as the source of resistance identified 12 QTLs significantly conferring resistance across three agro-ecological zones. Three QTLs on chromosome 5, 8 and 9 were affective in the entire season (Schechert *et al*., 1999). In another study, 13 QTLs were identified for NLB resistance in a population obtained from a resistant European line D32 where five QTLs were identified on chromosomal bin 1.06, 3.07, 4.03, 5.04 and 8.06 that were significantly associated to northern leaf blight (Welz *et al*., 1998). In general, three QTLs on chromosome 3, 5 and 8 were repeatedly identified to be significant in different populations increasing the probability that these QTLs regions are shared in different population. Due to this consistency, they make suitable candidates for marker assisted selection (MAS) in resistance breeding.

Understanding and exploiting quantitative resistance of maize to foliar pathogens is of great interest to plant breeders. This is due the emergence of new pathogen races due to major alleles being introduced followed by their suppression (Leonard, *et al*., 1985). The current has identified genetic resources for NLB resistance. The causal gene identified on

chromosome 5 and 7 through joint linkage association mapping contributes the understanding of the molecular basis of the NLB resistance.

CHAPTER FIVE

5.1 GENERAL DISCUSSION

Northern Leaf Blight (NLB), also known as *Turcicum* leaf blight caused by *Exserohilum turcicum* Leonard & Suggs. NLB is a major foliar disease threatening production of maize across Sub-Saharan Africa region. Several maize lines resistant to NLB have been identified. These elite materials with resistance to NLB have been subjected to different high-throughput platforms such as hyper spectral, multispectral or thermal imaging for further screening. In screening of these elite materials for their NLB disease severity, the study aimed to a) to compare between the digital imagery tools and phenotypic evaluation for foliar diseases in maize and b) To identify genomic region associated with resistance to northern leaf blight disease in tropical germplasm.

The study identified that the digital imagery tools proposed to phenotype disease severity in the maize fields can offer more specific methods of quantifying, identifying, monitoring plant diseases and could save time as well as expenses. The use of visual crop evaluation in assessing disease resistance in the field is faced by a myriad of challenges namely; the trials were large, multiple locations of trials, harsh weather conditions, biased data due to fatigue, trials locations leading to poor accessibility, mishandling of collected data, consumes lot time causing delay in data analysis.

The study also established the genomic regions associated with the NLB resistance. The 192 maize inbred lines were screened in two agro-ecological zones namely KALRO-Embu and Kakamega**.** Genotypic and phenotypic data was used to identify the QTL with the help

of QTL Ici-Mapping Version Software, MAP function (Meng *et al*., 2015). The study reported that a cross with promising resistance to the NLB namely CML312xLaposta seq showed low NLB severity and statistically low AUDPC values. Genotyping was done with only 404 polymorphic SNPs markers which were identified during the parental polymorphism survey of developing a mapping population. Two QTLs (QTL 5 and QTL 7) were identified, which were flanked by the markers *S5170023977-PZA014101* and *PZA004241- S7128895684* and with LOD score 3.15 and 3.48 respectively.

5.2 CONCLUSION AND RECOMMENDATION

In Conventional phenotyping, the relationship between plants' genotype have slowed the ability to understand the interaction between a plant's genotype and its surrounding environment. The choice of any phenotyping methodology play a critical role in breeding since adoption of visual analysis is intensive, time-consuming, costly, and can easily lack consistency across workers. There is need for adoption of more advanced imagery tools by plant breeders in various research centers to enhance phenotyping of numerous phenological traits ensuring efficient breeding of resistant plant materials.

Recommendations from this study are:

1. There is need for the adoption of digital imagery tools to facilitate accurate high-throughput phenotyping for resistance to foliar diseases in maize, helping reduce the cost and time required to develop improved maize germplasm.
2. Continued research is recommended to come up with more NLB resistant varieties.

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