DEVELOPMENT OF MOLECULAR MARKERS

FOR INTROGRESSION OF RESISTANCE TO

TURCICUM LEAF BLIGHT IN SORGHUM

Mayada Mamoun Beshir

B. Sc. *(Hons)* Khartoum University

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE IN PLANT BREEDING AND SEED SYSTEMS OF MAKERERE UNIVERSITY.

April 2011

DECLARATION

This thesis is a presentation of my original research work. Wherever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature, and acknowledgement of collaborative research and discussions.

This work was done under the guidance of Dr. Patrick Okori, at Department of Crop Science, Faculty of Agriculture, Makerere University, Kampala, Uganda and Prof. Abdelbagi M. Ali, at the Agricultural Research Corporation, Wad Medani, Sudan.

Signed …………………………….   Date:………………………………...
Mayada Mamoun Beshir

In my capacity as supervisor of the candidate's thesis, I certify that the above statements are true to the best of my knowledge.

Signed …………………………….   Date:………………………………...
Patrick Okori
(PhD)

Signed …………………………….   Date:………………………………...
Abdelbagi M. Ali
(PhD)

SUMMARY

Sorghum {*Sorghum bicolour* (L.) Moench (2n=2x=20)}, a C4 grass that diverged from maize about 15 million years ago, is the fifth major cereal crop in the world after wheat, rice, maize and barley. It has relatively small genome of 750 million base pairs. Sorghum production especially in the tropics is affected by several pests and diseases. *Turcicum* leaf blight (TLB) caused by the pathogen *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs (teliomorph: *Setosphaeria turcica* [Luttrell] Leonard and Suggs) is one of the threats to sorghum production. It is one of the most destructive foliar diseases of sorghum. Development of resistant varieties is the most economically viable solution for disease management for cereals in general. However, the design of the well targeted disease management strategies that involve deployment of resistant genotypes requires detailed characterisation of a pathogen's pathosystem. *E. turcicum* attacks both maize and sorghum. The maize *Exserohilum turcicum* pathosystem has been characterised, and host species specialisation occurs. However, a comprehensive review of the published literature shows that sorghum resistance to TLB has received limited research attention and the *E. turcicum* - pathosystem has limited studies. The objectives of this study were to (1) determine the mode of TLB inheritance in sorghum; (2) develop and validate SSR and RAPD markers linked to the TLB resistance loci and (3) use the polymorphic SSR markers to map QTL for resistance in sorghum to TLB.

The study was carried out in Uganda at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). Three populations derived from a cross of MUC007/009 (resistant) and Epuripuri (susceptible) an elite sorghum variety were used together with two parents and four checks GAO6/106 (Moderately resistant), Lulud (Susceptible), MUC007/010 (Resistant) and

GAO6/18 (Moderate Susceptible). A total of 304 F_2 segregating population, 278 $F_{2:3}$ and 246 $F_{2:4}$ segregating families were used. The experiments were set up following a completely randomised design with no replication to evaluate F_2 and $F_{2:3}$ and alpha lattice design to evaluate $F_{2:4}$ population. Generation mean analysis was used to determine the contribution of additive, dominant and epistatic genetic effects and also to confirm the genetic ratio analysis for the population distribution under a greenhouse and field conditions. Disease severity was assessed using percentage of leaf area affected on individual plant basis using a scale of 0 to 75, where 0 %= no disease and >75 % of leaf surface diseased. Assessment commenced at stage 4 (the growing point differentiation) 51 days after planting and continued on a weekly basis of disease severity and they were used to compute area under disease progress curves (AUPDC). To standardise area under disease progress curve the AUDPC, values were divided by the total period of epidemics. Data were subjected to analysis using GenStat Discovery Edition 12 to establish any association between AUDPC disease severity, lesion type and dates to flowering. Chi square (χ^2) analysis was used to test goodness of fit of the mode of TLB inheritance data to expected segregation ratios.

Disease severity of F_2 plants in the greenhouse condition indicated a normal distribution indicative of quantitative inheritance or minor gene effects. Under the field conditions, disease severities of $F_{2:3}$ and $F_{2:4}$ matched a normal distribution also suggesting quantitative inheritance. Though the performance of the resistant parent MUC007/009 and the susceptible parent Epuripuri was not different under the greenhouse environment, it was highly significant different (P<0.001) under the field conditions. There was transgressive segregation towards the resistance under both environments for F_2, $F_{2:3}$ and $F_{2:4}$ progenies. However all populations (F_2, $F_{2:3}$ and

$F_{2:4}$) from this cross, showed negative correlation between flowering dates and AUDPC. The early maturity lines had higher disease severity. In this study there was a clear difference between greenhouse and field environments. Similar reports have been made elsewhere. In this study the resistant parent MUC007/009 and the susceptible parent Epuripuri expressed distinctly different lesion types under both greenhouse and field environments. The resistant lesion type and the susceptible lesion type were use to screen the $F_{2:3}$ and $F_{2:4}$ families. The two distinct lesion types segregated according to the 1:2:1 ratio indicative of dominant gene inheritance. Partitioning of genetic effects into additive, epistatic and dominance components in this study shows that this type of resistance is attributed to additive and epistatic effects. These data are consistent with other studies in maize which also show that resistance to *E. turcicum* is quantitative in nature. The limited role of dominance effects under both greenhouse and field environments further demonstrates the bigger role of additive and epistatic effects.

This study involved screening eighty random amplified polymorphic DNA (RAPD) and ten simple sequence repeat (SSR) markers for polymorphism between the two distinct parents. Eight RAPD markers were polymorphic between the resistant parent MUC007/009 and the susceptible parent Epuripuri. These markers are recommended to be used for mapping of resistance to sorghum TLB. Out of ten SSR markers, three were polymorphic between the resistant parent MUC007/009 and the susceptible parent Epuripuri. The three polymorphic SSR markers are distributed along the sixth linkage group of sorghum consensus map. However the SSR marker Xtxp95 showed high significant association between the allelic groups and the resistant lesion type, while the SSR markers Xtxp57 and Xtxp247 did not show any significant association. The polymorphic information content (PIC) for SSRs was 0.490 (Xtxp57), 0.496 (Xtxp247) and

0.499 (Xtxp95). The SSR marker Xtxp95 was linked to the resistant lesion type and the analysis showed that 23.74% in $F_{2:3}$ and 14.09% in $F_{2:4}$ of the variability in the resistant lesion type is associated with the SSR marker Xtxp95 segregation. The results from this study show that the genomic region flanked by plant colour locus and Xtxp95 marker may harbour a locus for sorghum TLB lesion type.

DEDICATION

I would love to dedicate this thesis to the soul of my beloved mother who had raised me to be the person I am today. To my father, who taught me that the best kind of knowledge to have is that which is learned to be put into practice. To my aunt Sommaya who stood nearby in bad and good times. To my beloved sister Dhallia and beloved brothers Hani and Beshir. Thank you for all the unconditional love, guidance, and support that you have always given me and helping me to succeed. Finally, this thesis is dedicated to my mentors in Agricultural Research Corporation at Wad Medani, Sudan, and in the Department of Botany, Faculty of Science at University of Khartoum, Sudan. Thank you for giving the confidence that I am capable of doing anything I put my mind to.

ACKNOWLEDGEMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1   Biology and economic importance of sorghum

Sorghum {*Sorghum bicolour* (L.) Moench (2n=2x=20)} is a tropical C4 monocot plant belonging to the family Graminae and tribe Andropogonae. This genus has many species and subspecies. There are several types of sorghum, including grain sorghums, grass sorghums (for pasture and hay), sweet sorghums (for syrups), and broomcorn. It is indigenous to Africa and Asia and is believed to have been domesticated in Sub-Saharan Africa particularly in the Nile basin from where; it spreads to other parts of the world (Kimber, 2000). The genus bicolour has wild and domesticated races which are *bicolour*, *guinea*, *kafir*, *caudatum* and *durra* (Doggett, 1988). Sorghum is predominantly a self-pollinated crop with outcrossing rates between 3 to 15%, depending on genotype and environment. Genetically, *Sorghum* is a functional diploid although there is evidence of a tetraploid origin (Doggett, 1988).

Sorghum diverged from maize about 15 million years ago (Mullet *et al.*, 2001). It is the fifth major cereal crop in the world after wheat, rice, maize and barley (Doggett, 1988; FAOSTAT, 2003). Also it is a subject of plant genomics research based on its importance as one of the world's leading cereal crops, a bio-fuel crop of high and growing importance, a progenitor of one of the world's most noxious weeds, and as a botanical model for many tropical grasses with complex genomes (Paterson, 2008). Sorghum has a relatively small genome of about 750 million base pairs (Arumuganathan and Earle, 1991), incremental divergence from maize and rice (Doebly *et al.*, 1990), a small amount of repetitive DNA and co-linearity with other cereal

genomes (Bennetzen *et al*., 1998; Gale and Devos, 1998), and extensive diversity in agronomic traits (Dje *et al*., 2000; Kong *et al*., 2000). These qualities make it ideally suited for discovery and analysis of grass genes through comparative genomics (Mullet *et al*., 2001).

World sorghum production is about 60 million tons annually from a cultivated area of 46 million ha. The most important producers are the United State, Nigeria, India, Sudan, Ethiopia, Burkina Faso, China, Tanzania and Niger (FAOSTAT, 2007). More than 35% of sorghum is grown directly for human consumption and the rest is used primarily for animal feed and forage, alcohol production and industrial products (FAOSTAT, 1995; Awika and Rooney, 2004). It is of paramount importance that technological developments are used to increase productivity and sustainability of sorghum production and thereby provide a better quality of life for some of the poorest people on the continents of Africa, Asia and Latin America.

Sorghum performs relatively better than the other warm-season cereals in areas where the annual rainfall is in the range 500-700 mm per year. It is an important crop even in East Africa where the average annual rainfall is greater than 700 mm per year. This importance result from the rain in sub-tropical Africa being intermittent and characterised by brief periods of very high rainfall (Doggett, 1988).

In Sudan, sorghum is the main staple food, and is used in different forms (Grenier *et al*., 2004). The total cereal production in season 2008/09 was about 5.27 million tones of which 3.87 million tones was sorghum (FAOSTAT, 2008). Sorghum contributes about 65% of Sudan's consumption of grains, 70% of calories in the diet, and a considerable amount of protein. Grain sorghum is

grown mostly under rain-fed conditions in the Sudan, of which 75% in produced in the mechanised rain-fed sectors (Ibrahim and Abbas, 2006). In Uganda, sorghum ranked as the third most important cereal crop (Ebiyau and Oryokot, 2001; MAAIF, 2007; FAOSTAT, 2008). Uganda's total cereal production in 2008/09 was about 2.72 million tones of which 0.48 million tones was of sorghum from an area of 314,000 hectares (FAOSTAT, 2008).

1.2   Sorghum production constraints

Grain sorghum yields are especially low in Eastern Africa countries such as Uganda (15106Hg/Ha) as compared to yields in the United States (43548 Hg/Ha) and well below the genetic potential (FAOSTAT, 2009). These low yields of sorghum are attributed to a number of biotic stress{e.g. weeds such as e.g. *Striga sp*. (Ebiyau and Oryokot, 2001), Pests; such as *Stem borer*; includes *Chilo partellus Swinhoe*, *Busseola fusca Fuller*, *Diatraea sp*., *Eldana saccharina Walker*, *Ostrinia nubilalis Hubner*, and *Sesamia sp*. (Sharma *et al*., 2007) and diseases; such as *Turcicum* leaf blight (Adipala *et al*., 1993; Nkonya *et al*., 1998 and Tilahun *et al*., 2001)} and abiotic factors {e.g., drought (Kudadjie *et al*., 2004)}. The numerous biotic and abiotic constraints to yield result in a combined effect of much yield loss.

1.2.1   Abiotic constraints

Soil water deficits during crop establishment and early growth and during grain fill are the most important (Charles *et al*., 2006). Soil water deficit accounts for a total of approximately 1.8 million Mg of loss per year for Uganda and other eastern Africa (Charles *et al*., 2006). Also nitrogen deficiency accounts for about 1.2 million Mg yr $^{-1}$ loss. Salinity and the low pH complex are seen to be of relatively minor low importance in East Africa (Charles *et al*., 2006).

The weather during the growing seasons appears to have an influence on sorghum production. Moreover the lack of appropriate sorghum varieties that fit the current rainfall regime further contribute to reduced yield (Kudadjie *et al.*, 2004). These and other abiotic factors hamper sorghum production leading to low yields and overall remunerability.

1.2.2 Biotic constraints

Striga is a major constraint in sorghum production especially in eastern and northern Uganda (Ebiyau and Oryokot, 2001). At least two species of striga affect sorghum production *Striga hermonthica* (Del.) Benth. and *Striga asiatica* (L.) Kuntze (Scrophulariaceae). In districts of Tororo and Pallisa of eastern Uganda, it is estimated to be present in up to 80% of fields and causes an estimated 60-85% yield loss in infested fields (Ebiyau and Oryokot, 2001). Some striga-resistant sorghum varieties have been developed, but these generally offer lower yields than traditional cultivars and improved (but striga-susceptible) varieties (FAOSTAT, 2007), although recently developed varieties that are striga resistant are reported to be high yielding (Ejeta, 2006).

The most important arthropod pests of sorghum include sorghum midge {*Contarinia sorghicola* (Coquillet)}, sorghum shoot fly (*Atherigona soccata* Rond) which cause substantial losses in late and off-season sorghum in Uganda (Davies and Reddy, 1981) along with a number of stem borers include (*Chilo partellus* Swinhoe, *Buseola fusca* Fuller, *Diatraea sp.*, *Eldana saccharina* Walker, *Ostrinia nubilalis* Hubner, *Sesamia sp.* and *Buseola sorghuda sp.*) (Sharma *et al.*, 2007). *Chilo partellus* is found mainly in the semi-arid areas of East Africa while *Sesamia calamitis* and *Buseola sorghida* are distributed throughout sorghum growing areas of Africa (Kfir, 1997). Sorghum shoot fly and stem borers are endemic in most parts of Uganda (Gitau *et al.*, 2007).

Birds are one of the most important pests of sorghum worldwide. They are capable of inflicting heavy losses and causing real economic damage. In Uganda, the most notorious species is *Quelea quelea*. Earlier sorghums had higher tannin levels, which caused an offensive flavour and so was used advantageously to deter birds. These high-tannin sorghums are still grown where birds could cause significant losses.

The major diseases that affect sorghum include *Turcicum* leaf blight, (*Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs {teliomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs}; downy mildew {*Peronoscleropora sorghi* (Western & Uppal) Shaw}; anthracnose (*Colletrotrichium sublineolum* Henn.) (DeVries and Toeniessen, 2001) and sorghum smuts, including covered kernel smut (*Sporisorium sorghi* Ehrenberg (Link); loose smut (*Sphacelotheca cruenta* (Kuhn), Langdon and Fullerton) and long smuts (*Tolyposporium entrenbargii* (Kuhn) Pattouillard).

1.3   Justification of this study

Turcicum leaf blight, caused by *Exserohilum turcicum*, is reported to be widespread in the warm and humid growing regions of Ethiopia, Tanzania and Uganda (Tilahun *et al*., 2001). It is a foliar disease characterised by long, narrow, tapering lesions that are tan in the centre and bordered by defined reddish brown margins. The disease occurs whenever sorghum and maize are grown together (Ebiyau and Oryokot, 2001). In the United States, yield losses attributed to TLB can be up to 50% if the disease is established on susceptible varieties before panicle emergence (Narro *et al*., 1992; Mittal and Boora, 2005). Studies in Uganda have shown that disease epidemics are largely due to infested maize residues left in farm fields (Adipala *et al*.,

1993). On susceptible cultivars in Uganda, losses as high as 60% have been recorded on maize and as high as 70% elsewhere (Yeshitila, 2003).

Molecular markers associated with resistance to *Turcicum* leaf blight can be used for marker assisted breeding. Genetic study of the source of the resistance and the development of molecular markers is crucial for efficient breeding for resistant varieties. The limited success is due in part to an incomplete understanding of the genetics of sorghum *Turcicum* leaf blight resistance and the complex interaction of traits influencing the disease resistance. In this thesis effort is made to understand resistance to *Exserohilum turcicum*. The focus of this thesis is to confirm the usefulness of developing resistant varieties as main solution to the disease and this study is expected to contribute to the control of the disease. Development of a molecular marker breeding system will assist rapidly in developing a resistant sorghum variety to *Turcicum* leaf blight disease. A comprehensive and thorough review of the published work shows that sorghum resistance to *Turcicum* leaf blight has received limited research attention this in turn highlights the importance of this study.

1.4   Objectives

The overall objective of this research is to develop molecular markers to assist in breeding sorghum varieties resistant to *Turcicum* leaf blight.

The specific objectives are to:

1. Determine the mode of inheritance of resistance in sorghum to *Turcicum* leaf blight.

2. Discover and validate SSR and RAPD markers linked to the *Turcicum* leaf blight resistance in sorghum.

3. Use polymorphic SSR markers to map QTL for resistance to *Turcicum* leaf blight.

1.5 Hypotheses of the study

1. Available sorghum accessions display variable resistance against *Turcicum* leaf blight.

2. Polymorphic SSR and RAPD markers exit that can be used in marker assisted breeding to *Turcicum* leaf blight in sorghum.

CHAPTER TWO

LITERATURE REVIEW

2.1   Introduction

Turcicum leaf blight is highly destructive and drastically affects sorghum grain and fodder yield as well as fodder quality (Ogliaril *et al*., 2007). It is a fungal disease that thrives under humid conditions (Frederikson, 2000; Mohan *et al*., 2009). Estimated sorghum losses caused by TLB in Africa, Asia, and the Americas is up to or greater than 50% in most susceptible sorghum varieties (Mittal and Boora, 2005). In sorghum, a relationship between pigmented plant and resistance to foliar and panicle diseases was documented (Torres-Montalvo *et al*., 1992).

Development of resistant varieties is the most economically viable solution for disease management. The design of the well targeted disease management strategies requires detailed characterisation of a pathogen's pathosystem. Given that the maize *Exserohilum turcicum* pathosystem has been characterised, and the fact that host species specialisation is a possibility (Adipala *et al*., 1993). Moreover, a comprehensive review of the published literature shows that sorghum resistance to *Turcicum* leaf blight has received limited research attention. The objectives of this study were to determine the mode of *Turcicum* leaf blight inheritance using disease response, develop and validate SSR and RAPD markers linked to the *Turcicum* leaf blight resistance genes and use the polymorphic SSR markers to map QTL resistant for TLB.

Exerohilum turcicum and sorghum have co-evolved over a long period to generate a wide array of genotypes, both susceptible and resistant. Control of this disease through conventional

measures has been quite ineffective and difficult to sustain. The best way to control *Turcicum* leaf blight is by breeding sorghum genotypes resistant to *Turcicum* leaf blight with help of marker assisted breeding and also by deployment of multiple genes that confer either qualitative or quantitative resistance (Ogliaril *et al.*, 2007). Limited information is available for molecular markers that might be used in improving sorghum for TLB resistance.

2.2   The sorghum - *Exerohilum turcicum* pathosystem

2.2.1   Significance and epidemics of *Turcicum* leaf blight

Turcicum leaf blight, is caused by the ascomycete fungus *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs (teliomorph: *Setosphaeria turcica* [Luttrell] Leonard and Suggs., is one of the most destructive foliar diseases of sorghum (Carson, 1995; Ogliaril *et al.*, 2007). The nomenclature *Exserohilum turcicum* was suggested by Leonard *et al.* (1989) and it is widely used today. This pathogen has three major groups of hosts: maize (*Zea mays* L.) (Welz and Geiger, 2000), sorghum (*Sorghum bicolour*) (Ngugi, 2000) and wild relatives of sorghum or maize including Johnson grass (*Sorghum halapense*), teosinte and other grass species (Esele, 1995). *Turcicum* leaf blight causes yield losses of up to or greater than 50% in most susceptible sorghum varieties (Mittal and Boora, 2005) (Figure 1) and is favoured by mild temperatures and humid weather with heavy dews (Narro, 1992).

Pathogenic fitness and environmental conditions are important factors in determining disease development and epidemics of *Exserohilum turcicum* depend on its ability to infect, grow and sporulate (Levy, 1989). When wind disseminates the spores (Conidia) from   leaf lesions to

Figure 1. Distribution of *Turcicum* leaf blight in the world according to http://maizedoctor.cimmyt.org. Accessed 7, March, 2009.

susceptible leaves, infection occurs as a filament from the germinating spore penetrates the leaf surface. Leaf moisture is required for spore germination and infection. Within three to six days after infection, lesions appear and between six and 14 days, a new crop of spores are formed on the lesions. Spore production and lesion development occur between 10 - 34.4^{O}C (50 - 94°F) but optimum minimum daily temperatures for disease development are near or slightly below 16.1^{O}C (61°F) with average daily temperature near 22.2^{O}C (72°F). This fungus can also produce thick walled spores called chlamydospores, which lengthens the survival time of the organism in soil.

Exserohilum turcicum can survive from season to season as mycelia, sclerotia or chlamydospores on infected crop debris or in the soil (Casela *et al*., 1993). Alternate hosts and volunteer crops may also provide sources of primary inoculum, and transmission by seed has been reported (Nobel & Richardson, 1968). *Exserohilum turcicum* conidia are heavily melanised and can be

10

transmitted over long distances by wind (Bergquist, 1986). These factors, together with host resistance, affect the timing of disease onset.

Disease epidemics are favoured by high rainfall and relative humidity, moderate temperatures, and the presence of large amounts of inoculum (Hennessy *et al*., 1990). Previous studies on the epidemiology of the disease have indicated that leaf blight is often most severe on younger plants (Julian *et al*., 1994). However, there is little definitive information on the mechanisms underlying these observations (Ngugi, 2000). Studies in Uganda have shown that disease epidemics are due largely to infested maize residues in the farms fields (Adipala *et al*., 1993).

2.2.2 *Turcicum* leaf blight symptoms and etiology

The most commonly observed symptom of *Exerohilum turcicum* infection is long elliptical tan lesions that develop first on the lower leaves and progress upward. Symptoms can range from small cigar-shaped lesions to complete destruction of the foliage (Welz and Geiger, 2000). The earliest symptoms of infection are slightly oval, water-soaked, small spots on the leaves that grow into elongated, spindle-shaped necrotic lesions (Plate 1). They may appear first on lower leaves and increase in number as the plant develops and can lead to complete blighting of the foliage (Richards and Kucharek, 2006). Typical lesions are gray-green, elliptical or cigar-shaped and are typically 12 mm wide and 3-15 cm long with yellow to gray centres and red margins. Spore production causes the lesions to appear dark gray, olive or black (King and Mukuru, 1994). Sometimes lesions are multiple-pointed at the tips. A lesion may or may not be surrounded by a dark reddish-brown purple border or a narrow band of water soaking (Degefu, 1990; Kucharek, 2000).
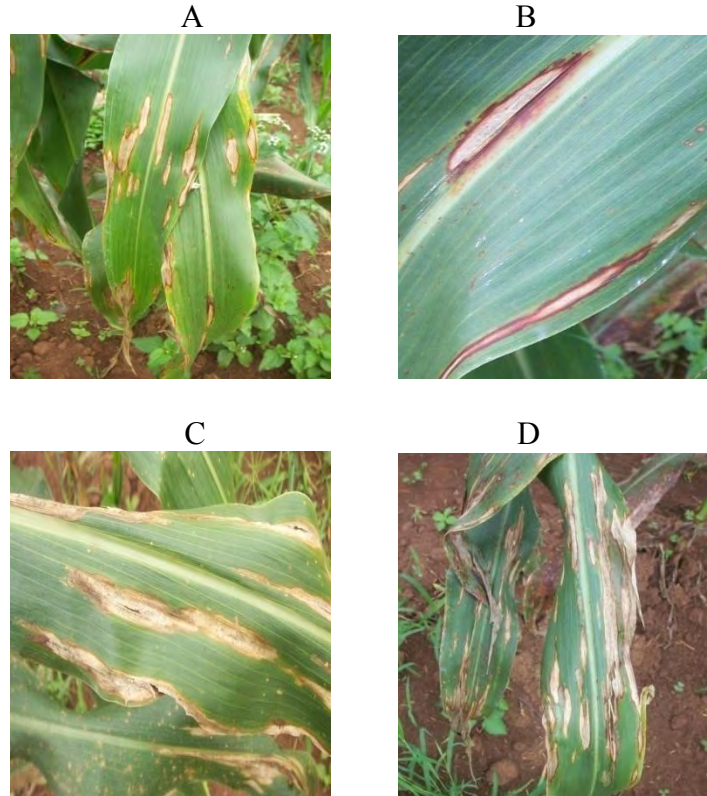
Plate 1. Early symptoms of *Exerohilum turcicum* infection comprising slightly oval lesions, water-soaked small spots on sorghum leaves (A and B) and necrotic wild-type elongated lesions (C and D) on sorghum plants.

2.2.3 *Exserohilum turcicum* physiological races

The classification of *Exserohilum turcicum* into races is based on differential reactions of isolates to specific *Ht* loci for resistance to *Helminthosporium turcicum* the name of the pathogen at the time the race classification system was developed (Berguist and Masias, 1974; Leonard *et al*., 1989). Five naturally occurring races of *Exserohilum turcicum* have been reported to overcome specific *Ht* resistance genes in the United States (Windes and Pedersen, 1991) and others have been reported from crosses of races in the laboratory (Fallah and Pataky, 1994). In Uganda, mating type analysis revealed the occurrence of *MAT 1, MAT 2* and *MAT 1, 2* on sorghum (Ramathani, 2010). Both mating types were found to occur in equal proportions in Soroti while *MAT* 2 was more common that *MAT 1* in other locations. The race differential study revealed

occurrence of races 0, 1, 2, and 3 in Uganda. Both the mating type and race differential study suggest a great potential of having more virulent races of *E. turcicum* in the future (Ramathani, 2010).

Moreover, some studies indicate that the pathogen exhibits host species specialisation (Robert, 1960). The vast majority of *Exserohilum turcicum* races have been isolated from maize even though the same pathogen has been isolated from species of grass crops. The early studies on this pathogen suggest that isolates from Johnson grass do not infect maize and conversely isolates from maize do not infect Johnson grass, suggesting host species specialisation (Robert, 1960; Tarumoto *et al*., 1977). Studies suggest the existence of races or isolates of differing pathotypes of *Exserohilum turcicum* on sorghum (Tarumoto *et al*., 1977).

2.2.4   Genetics of resistance to *Exserohilum turcicum* in cereals

2.2.4.1 Genetics of resistance to *Exserohilum turcicum* in maize

The earliest sources of resistance to *Turcicum* leaf blight were first found in ladyfinger popcorn in the 1940's (Hilu and Hooker, 1963). The *Ht1* gene identified from popcorn cv. Ladyfinger and field corn inbred GE440 was characterised by chlorotic lesions, reduced sporulation and smaller necrotic lesions (Hooker, 1963). It was further characterised by development of a green halo around the point of infection. Later, studies showed that this type of resistance reaction was conditioned by a single gene called *Ht* (*Helminthosporium turcicum*) (Hooker, 1963). A gene-for-gene relation was found and with the discovery of several new races, more *Ht* resistance loci have been reported (Carson, 1995) with five dominant genes controlling resistance (*Ht, Ht2, Ht3, HtM*, and *HtN)* (Simcox *et al*., 1993). Monogenic resistance is characterised by the formation of

chlorotic lesions, a delay in appearance of necrosis, and a marked reduction in sporulation (Raymundo and Hooker, 1982).

Partial resistance conditioned by relatively few genes is controlled by multiple genes, some with major effects (Jenkins and Robert, 1952). This is characterised by an increase in latent period, a reduction in lesion size and number, and infection efficiency (Adipala *et al*., 1993). The *Ht* genes seem to have unusually high environmental dependence, particularly with regard to light and temperature (Leath *et al*., 1990) and they tend to confer delayed lesion development or sporulation phenotypes rather than complete resistance (Balint-Kurti and Johal, 2009). Given that the maize *Exserohilum turcicum* pathosystem has been characterised (Adipala *et al*., 1993) and the fact that host species specialisation is a possibility, studying the pathogen-sorghum pathosystem becomes valuable. Aggressiveness of those races that infect corn or other grasses has not been determined in sorghum (Ngugi, 2000).

Turcicum leaf blight is unusual among necrotrophic diseases in that several dominant or partially dominant qualitative genes have been described that confer race-specific resistance to it, including *Ht1* (Hooker, 1963), *Ht* 2 (Hooker, 1977), *Ht* 3 (Hooker, 1981), *Htn* 1 (also known as *HtN* (Gevers, 1975) and *Ht* P (Ogliari *et al*., 2005). This anomaly might be explained by the fact that *Turcicum* leaf blight is arguably a hemibiotroph rather than a straightforward nectrotroph. It could be argued that the *Ht* genes are rather a typical plant major resistance genes and should be thought of as large-effect, race-specific QTL.

2.2.4.2 Genetics of resistance to *Exserohilum turcicum* in sorghum

In the *Turcicum* leaf blight pathosystem, resistance is controlled by mono- and poly- genes (Hooker and Kim, 1973; Lipps *et al*., 1997). Given that both sorghum and maize belong to the Poacea and the fact that both share large regions of colinearity (Bennetzen *et al*., 1998), resistance to *Turcicum* leaf blight in maize may then share common features. In sorghum resistance to infection or damage is often characterised by pigmentation (Torres-Montalvo *et al*., 1992). The accumulation of phytoalexins (Flavonoids) in sorghum affects the response to pathogen infection (Nicholson *et al*., 1987). It has been suggested that the type and quantity of anthocyanin flavonoids produced in response to pathogen attack in sorghum may vary (Klein *et al*., 2001). There may be a common biosynthetic pathway for the production of the type of flavonoids required for plant colour and for those involved in hypersensitivity. This observation would explain the linkage between the severity of symptoms for a set of pathogenically-unrelated diseases and the relationship between tan plant colour and the severity of diseases (Klein *et al*., 2001). The role of flavonoids in conferring disease resistance in plants was documented (Lamb *et al*., 1989), and in sorghum, the 3-deoxyanthocyanidins phytoalexins are the essential component in active defense mechanisms (Aguero *et al*., 2002).

Changes in environmental conditions from season to season and the probable occurrence of strains of *Exserohilum turcicum* are important external variables that affect resistance scoring (Tarumoto *et al*., 1977). Inheritance of resistance to leaf blight was found to be controlled by a single dominant gene (Boora *et al*., 1999; Mittal and Boora, 2005) with field inoculation being one of satisfactory methods for such studies (Tarumoto *et al*., 1977). Studies were initiated to identify cultivars with good combining abilities that could provide promising crosses for

breeding programs (Olujong *et al.*, 1996). High heritability values were obtained for both double-crosses and diallel crosses in Uganda, indicating that progenic resistance was highly heritable. Thus, breeding for resistance to *Turcicum* leaf blight in sorghum could be an alternative to cultural control practices in Uganda.

2.3 Molecular marker technologies available for breeding

2.3.1 Molecular breeding for sorghum-*Turcicum* leaf blight

The advent of new biotechnology techniques such as marker-assisted selection provides new opportunities to enhance sorghum disease resistance (Rooney and Klein, 2000). Resistance to *Turcicum* leaf blight in sorghum accession G-118 was found to segregate as a single dominant trait in a cross with susceptible cultivar HC-136. A molecular marker linked to the locus for resistance to *Turcicum* leaf blight was identified using simple sequence repeat (SSR) markers coupled with bulk segregant analysis (Mittal and Boora, 2005). In the same population, an SSR marker, Xtxp 309, produced amplification of a 450 bp band. This was found to be located at a distance of 3.12 cM away from the locus governing resistance to leaf blight which was considered to be closely linked and 7.95 cM away from the locus governing susceptibility to leaf blight (Mittal and Boora, 2005). By combining the random amplified polymorphic DNA (RAPD) technique with bulk-segregant analysis, it was possible to identify PCR amplification products that segregated with the response to *Turcicum* leaf blight (Boora *et al.*, 1999).

2.3.2 Molecular-marker assisted selection (MAS)

The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. The potential benefits

of using markers linked to genes of interest in breeding programmes, thus moving from phenotype-based towards genotype-based selection, have been obvious for many decades. However, realisation of this potential has been limited by the lack of markers. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers dispersed throughout the genome of any species of interest. These markers can be used to introgress loci of interest thus allowing marker assisted selection (MAS) in principle finally to become a reality (Bernardo, 2008). Use of MAS reduces the cost of field evaluation, increases breeding efficiency, and allows simultaneous selection for drought tolerance and other agronomic traits. Molecular markers have been used to assist backcrossing of resistance loci into elite cultivars (Babu *et al*., 2004). They have also been used to select alleles with major effects across multiple populations (Ejeta *et al*., 2000). However, their greatest potential appears to be in accelerating the rate of gain from selection for desirable genotypes and in the manipulation of quantitative trait loci (QTL) that condition complex economic traits. DNA markers also permit plant breeders to identify the chromosomal location of the various interacting genes that condition complex agronomic traits. Genetic mapping is essential for effective manipulation of important genes. Effective use of marker-based selection or marker assisted introgression should permit genetic recombination beyond the range possible in traditional breeding.

2.3.3   Types of markers used in plant breeding

The most common molecular markers are expressed sequence tagged (EST), simple sequence repeats (SSR) and random amplified polymorphism DNA (RAPD) (Trudy, 2009).

2.3.3.1 Simple sequence repeat (SSR) markers

Simple sequence repeats (SSR) are regions of DNA that consist of short, tandem repeated units (2-6 bp in length) found within the coding or noncoding regions of all eukaryotic organisms (Quellar *et al*., 1993). If nucleotide sequences in the flanking regions of the microsatellite are known, specific primers (generally 20–25 bp) can be designed to amplify the microsatellite by Polymerase Chain Reaction (PCR). Different alleles can be detected at a locus by PCR using conversed DNA sequences flanking the SSR as primers. SSR markers have been used initially to detect polymorphism between the parent cultivars (Klein *et al*., 2001). Although costly to develop relative to some other classes of genetic markers, once developed, analysis by SSR markers is both easy and inexpensive. The highly polymorphic nature (high information content) and other favourable characteristics make them excellent genetic markers for many types of investigations, including marker assisted selection and fingerprinting of germplasm collections (Kong *et al*., 2000). Different alleles can be detected at a locus by PCR using conserved DNA sequences flanking the SSR as primers. Combined, these maps include over 800 markers (Bennetzen *et al*., 2000).

2.3.3.2 Random amplified polymorphic DNA (RAPD) markers

RAPD markers are DNA fragments amplified by PCR using short synthetic primers (generally 10 bp) of random sequence (Trudy, 2009). These oligonucleotides serve as both forward and reverse primers, and each pair of primers is usually able to simultaneously amplify fragments from 1-10 genomic sites. Amplified fragments (usually within the 0.5 - 5 kb size range) are separated by agarose-gel electrophoresis and after ethidium bromide staining, polymorphisms are detected, as the presence or absence of bands of particular sizes. These polymorphisms are

considered to be due primarily to variation in the primer annealing sites, but can also be generated by genotype differences in the length of the sequence that amplified between the primer annealing sites. The main advantage of RAPD markers is that they are quick and easy to assay. Because PCR is involved, only low quantities of template DNA are required (usually 5-50 ng per reaction). Since random primers are commercially available, no sequence data for primer construction are needed. Moreover, RAPD markers have a very high genomic abundance and are randomly distributed throughout the genome. The main drawback of RAPD markers is their low reproducibility (Schierwater and Ender, 1993). Hence, experimental procedures need to be highly standardised because of their sensitivity to the precise conditions of the reaction. RAPD analyses generally require purified DNA of high molecular weight, and precautions are needed to avoid contamination of DNA samples because the short random primers that are used that are able to amplify DNA fragments in a variety of organisms. Altogether, the inherent problems of reproducibility make RAPDs unsuitable markers for MAS or for comparison of results among research teams working in a similar species and subject. As with most other multi - locus techniques, RAPD markers are not locus-specific, band profiles cannot be interpreted in terms of loci and alleles (dominance of markers), and similar sized fragments may not be homologous.

2.4   Quantitative trait loci (QTL) for *Turcicum* leaf blight resistance

2.4.1   Progress in sorghum genome characterisation

Sorghum offers novel learning opportunities relevant to biology and for the improvement of crops (Peterson, 2008). The small genome of sorghum has long been an attractive model for advancing understanding of the structure, function and evolution of cereal genomes. Sorghum represents tropical grasses in general, possessing a C4 carbon metabolism cycle, with its

complex biochemical and morphological specializations to improve carbon assimilation at high temperatures (Swigonova *et al.*, 2004). The relatively small size of sorghum genome (750 - 818 Mbp (Price *et al.*, 2005) suggests that sorghum will be highly amenable to structural genomics (Arumuganathan and Earle, 1991). Sorghum genome mapping based on DNA markers began in the early 1990s and has resulted in the publication of numerous genetic linkage maps in the last decade. These maps were based initially on RFLP markers, but more recent maps include AFLPs and SSRs (Mullet *et al.*, 2001) and even diversity array technology (DArT) markers (Mace *et al.*, 2009). Rapid progress has been made in the construction of an integrated *Sorghum bicolour* genome map (Peterson, 2008; Mace *et al.*, 2009). Combination of high throughput amplified fragment length polymorphism (AFLP) DNA marker technology (Klien *et al.*, 2000; Menz *et al.*, 2002), six-dimensional pooling of BAC clones (Klein *et al.*, 2001), cDNA capture technology (Childs *et al.*, 2001), sequence-based alignment of the genomes of sorghum and rice (Klein *et al.*, 2003), and BAC-based fluorescence in situ hybridization (FISH) have been used to construct an integrated *Sorghum bicolour* genetic map (Islam-Faridi *et al.*, 2002; Kim *et al.*, 2002).

The linkage maps of sorghum have been employed in the tagging (mapping) of genes for a large number of traits. Resistance genes have been tagged for numerous diseases (Klein *et al.*, 2005; Wang *et al.*, 2006), key insect pests (Tao *et al.*, 1998; Nagaraj *et al.*, 2001), and the parasitic weed, striga (Tao *et al.*, 2003; Klein *et al.*, 2005). Genes and QTLs have been identified that are related to abiotic stresses including post-reproductive stage drought tolerance (stay-green) (Crasta *et al.*, 1999; Haussmann *et al.*, 2004), preharvest sprouting (Carrari *et al.*, 2000; Haussmann *et al.*, 2002) and aluminium tolerance (Lijavetzky *et al.*, 2000). Additional

morphological characteristics have also been mapped in interspecific and/or intraspecific populations (Feltus *et al*., 2006).

2.4.2 Mapping quantitative trait loci for disease resistance in sorghum

Numerous studies have been published on the mapping of disease resistance quantitative trait loci (QTL) to particular genomic regions (Scheinost, 2001; Swigonova *et al*., 2004). For marker assisted breeding, use can be made of QTL where the position is only mapped within a 20 cM confidence window. However, such a wide window gives little clue as to the molecular identity of the genes involved. Scoring for any quantitative trait on an individual plant basis is difficult and is often inconsistent across environments making gene cloning techniques such as transposon tagging and map-based cloning challenging (Arumuganthan and Earle, 1991; Paterson *et al*., 1995). Most cases in which a quantitative gene has been successfully identified involved some combination of phenotypes that could be easily scored, defined environmental conditions, and the use of near-isogenic lines, facilitating accurate phenotypic scoring of the segregating population (Xu *et al*., 1994; Chittenden *et al*., 1994; Klein *et al*., 2000; Feltus *et al*., 2006). The genomic region flanked by a plant colour locus (*Pclor*) and a simple sequence repeat marker Xtxp95 on the sixth linkage group harbour disease response QTL for zonate leaf spot (ZLS), target leaf spot (TLS) and drechstera leaf blight (DLB) caused by fungal pathogens (Mohan *et al*., 2009). It is hypothesized that this region on the sixth linkage group could harbour a cluster of disease response loci to different pathogens as observed in the syntenic regions on rice chromosome 4 and maize chromosome 2 (Mohan *et al*., 2009).

2.4.3 Methods for mapping QTL

Genomic regions (loci) responsible for quantitative effects are known as quantitative trait loci (QTL). The QTL mapping approach has been proposed as means of increasing our understanding of the genetics underlying quantitative variation. The results from QTL mapping have provided information on the genetic architecture of complex traits, i.e., estimated number of QTL and magnitude of their estimated additive, dominance, and epistatic effects in multiple environments. Two parallel developments have allowed the aggressive use of molecular markers for studying quantitative traits. First, marker systems have increased the number and decreased the cost of markers in different crop species (Bernardo, 2008). For example, the development of single nucleotide polymorphism (SNP) genotyping has led to increase in the number of the data-points generated and decrease in the cost per data-point in a commercial maize breeding program (Bernardo, 2008). Second, statistical methods for detecting QTL and computer software for implementing these procedures have been developed. Methods for QTL mapping range from the simplest method of single-marker analysis (Sax, 1923) to more sophisticated methods such as interval mapping, joint mapping, multiple regression and composite interval mapping (Bernardo, 2008). Association mapping, which requires collections of germplasm instead of biparental populations, has also been developed as a method for finding genes underlying quantitative traits (Yu *et al.*, 2006). Software packages for mapping with F_2 or backcross populations or germplasm collections include MAPMAKER/QTL, JoinMap, QTL Cartographer, PLABQTL, QGene and TASSEL (Bernardo, 2008). Resistance QTL, once identified, can be transferred into adapted cultivars using marker-assisted selection (Haussmann *et al.*, 2004). The genetics of *Turcicum* leaf blight resistance in maize have been extensively studied (Welz and Geiger, 2000).

Combinations of qualitative and quantitative resistance genes are generally employed in breeding for resistance, with the emphasis now on quantitative genes, due to their higher phenotypic stability (Bernardo, 2008). For example, southern leaf blight (SLB), gray leaf spot (GLS) and northern leaf blight (NLB), which are all important foliar diseases impacting maize production, nine, eight, and six QTL were identified for SLB, GLS, and NLB resistance respectively (Zwonitzer *et al*., 2010). QTL for time to flowering were also identified at four of these six loci. Sorghum geneticists have long known that variation exists within sorghum for grain mold resistance but breeding to improve grain mold resistance in sorghum has had limited success (Esele *et al*., 1993). The limited success is due in part to an incomplete understanding of the genetics of sorghum grain mold resistance and the complex interaction of traits influencing grain mold resistance. Sorghum geneticists have long suspected that both qualitative and quantitative loci influence grain mold resistance. Several qualitatively inherited pericarp traits such as color and pigmented testa influence the level of grain mold resistance (Esele *et al*., 1993).

2.4.4   Approaches for studying modes of inheritance

Generally genetic diseases are either single-gene or multi-factorial, depending on the genetic cause. Some common diseases are caused by single-gene or chromosomal disorders, however, most common diseases are multifactorial, caused by a combination of genetic and non-genetic factors. The knowledge about different modes of inheritance contributes to accurate interpretation of family history and effective use of genetic testing.

Genetic resistance in plants is often divided into two major classes; Qualitative, or major-gene, resistance, is based on single major-effect resistance genes (R genes) and generally provides race-specific, high-level resistance. Quantitative resistance typically has a multi-genic basis and generally provides non-race-specific intermediate levels of resistance. Qualitative resistance is generally effective against biotrophic pathogens (pathogens that derive their nutrition from living host cells), while quantitative resistance is more often associated with resistance to necrotrophic pathogens (pathogens that derive nutrition from dead cells). The mechanisms of quantitative resistance have not been well characterised, but are likely to be variable depending on the specific interaction. In order to maintain agronomic performance, the breeder must minimize the amount of exotic germplasm that is introduced into adapted lines.

In respect to the similarity between sorghum and maize, *Exerohilum turcicum* races are defined according to their phenotypic reactions when inoculated onto a set of differential maize lines. Using this system, nine races of *Exerohilum turcicum* have been identified (Leonard *et al*., 1989). Five races of *Exerohilum turcicum* have been reported to overcome specific *Ht* resistance genes in the United States (Windes and Pedersen, 1991). Among cultivated crops, the vast majority of *Exerohilum turcicum* races have been isolated from maize. Yet the same pathogen has been isolated from several grass crop species (Esele, 1995). *Exerohilum turcicum* races have evolved in a gene-for-gene manner with five dominant genes controlling resistance (*Ht*1, *Ht*2, *Ht*3, *Ht*M, and *Ht*N) (Simox *et al*., 1993). Partial resistance conditioned by relatively few genes has also been reported (Jenkins and Robert, 1952). This type of resistance is characterised by an increase in the latent period, a reduction in the size and number of lesions, and of infection efficiency (Hooker, 1963; Pratt *et al*., 1997). A gene-for-gene relationship was recognised and

with the discovery of several new races, more *Ht* resistance loci have been reported (Carson, 1995). The classification of *Exerohilum turcicum* into races is based on the reactions of *Ht* resistance genes when plants are inoculated with different isolates (Berguist and Masias, 1974).

2.5   Sectional conclusion

Turcicum leaf blight is one of the most destructive foliage diseases of sorghum, resulting in yield losses of up to 50%. Caused by *Exerohilum turcicum*, its symptoms vary from small cigar - shaped lesions to complete destruction of the foliage. In Uganda, epidemics of this disease are due largely to infested maize residues left in farm fields. *Turcicum* leaf blight can be controlled through conventional measures, but this has not been effective and has been difficult to sustain. The most feasible way to control this disease is by breeding resistant varieties, using both qualitative genes and quantitative genes, incorporating marker-assisted selection when possible. However, resistance to *Turcicum* leaf blight in sorghum is poorly studied and the mechanism of resistance in sorghum to infection by *Exerohilum turcicum* is not confirmed yet. And also by deploying genes that confer either qualitative or quantitative resistance (Ogliaril *et al*., 2007). Genetic study of the source of the resistance and the development of molecular markers is crucial for efficient breeding for resistant varieties. The focus of this study is to investigate nature of resistance in sorghum to *Exerohilum turcicum* infection and nature of breeding programmes requires.

CHAPTER THREE

INHERITANCE OF RESISTANCE TO *TURCICUM* LEAF BLIGHT INHERITANCE IN SORGHUM

3.1 Introduction

Plant diseases result from an interaction between the host plant and the pathogen. The genetics of such host-pathogen interactions are of considerable biological interest and of the greatest importance in developing disease control strategies through resistance breeding effects. The variation in susceptibility to a pathogen among plant varieties is due to different kinds and numbers of genes for resistance that may be present in each variety. The effects of individual resistance genes vary from large to minute; depending on the importance of the functions they control (Agrios, 1997). *Turcicum* leaf blight caused by fungus *Exserohilum turcicum* is among the important foliar diseases that causes high yield losses in most susceptible sorghum varieties (Mittal and Boora, 2005). In the maize-*Exerohilum turcicum* pathosystem high heritability values were obtained under Ugandan conditions indicating the highly heritable nature of resistant to leaf blight in maize (Olujong *et al*., 1996). Breeding resistant varieties in sorghum is possible if high heritability is involved. This chapter describes the studies conducted to determine the mode of inheritance resistance to *Turcicum* leaf blight.

3.2 Materials and methods

3.2.1 Description of study site

This study was carried out in Uganda at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). MUARIK is at an elevation of 1200 m above sea level (O°28'N and

32°37'E). The populations used in the study were developed at the National Semi Arid Agricultural Research Institute (NaSAARI), Soroti. Laboratory analyses were done at the Biotechnology Laboratory of the Department of Crop Science, Makerere University.

3.2.2   Sorghum populations used in the study

Three populations derived from a cross of MUC007/009 and Epuripuri an elite sorghum variety were used along the two parents and four checks GAO6/106 (Moderately resistant), Lulud (Susceptible), MUC007/010 (Resistant) and GAO6/18 (Moderate susceptible). MUC007/009 is a sorghum variety resistant to *Turcicum* leaf blight. Epuripuri is susceptible parent. Epuripuri is a sorghum variety developed in 1972 by NaSARRI and released in 1995. It yields between 2,000 - 2,500 kg ha^{-1}, and has white kernels and takes approximately 110 days to flower (Mbeyagala, 2010). It is currently the most widely grown genotype in the country mainly for beer production. MUC007/009 has red seed and takes approximately 72 days to flower (Mbeyagala, 2010). A total of 304 F_2 segregating population, 278 $F_{2:3}$ and 246 $F_{2:4}$ segregating families were used. No selection was made for resistance to TLB or for any agronomic traits during the development of the three populations. It should be noted that the number of plants in the populations reduced in $F_{2:3}$ and $F_{2:4}$ because of germination failure and seedling death of some F_2 and $F_{2:3}$ individuals.

3.2.3   Layout of the experiments

3.2.3.1 Characterisation of mapping population

The three populations with their parents and some checks were evaluated for reaction to TLB at 51 days after planting (DAP). The experiment was set up following a completely randomised design (CRD) with no replication. The $F_{2:4}$ population was evaluated using an alpha lattice

design with three plots and five α-lattice blocks. MUC007/009 and Epuripuri were included in evaluation of all the populations. The F_2 population was evaluated in a greenhouse, while $F_{2:3}$ and $F_{2:4}$ populations were evaluated in the field. In the greenhouse the F_2 experiment was planted on 11th April, 2009 at MUARIK. The F_2 population, two parents and four checks (GAO6/106, Lulud, MUC007/010 and GAO6/18) were individually planted in separate pots filled with sterilized soil. The trial was harvested on September of the same year.

The $F_{2:3}$ and $F_{2:4}$ evaluations were planted on 9th October, 2009 and repeated in the first rains of 2010 (29th April, 2010) respectively at MUARIK. The experimental unit of $F_{2:3}$ measured 20 x 30 m with 44 sorghum rows planted at a spacing of 70 x 20 cm. The experimental unit of $F_{2:4}$ was the same as for $F_{2:3}$ and the spacing was 20 x 60 cm with 19 rows for each α-lattice block. Planting was done by hand with three to five seeds per hole to ensure germination. Seedlings were thinned to one per hill after one week of plantation. The two trials were weeded twice at after 3 and 7 weeks after planting.

3.2.3.2 Population development for generation mean analysis

A separate trial was set up to generate data for generation mean analysis under a greenhouse and field conditions in 28th March, 2010. Generation mean analysis was used to determine the contribution of additive (a), dominant (d) and epistatic (aa) genetic effects and also to confirm the ratio analysis for the population distribution. Due to insufficiency of F_1 seed, only the backcross BC_1F_1 to the susceptible parent (Epuripuri) was developed. Five out of six basic generations means were used plus $F_{2:3}$, $F_{2:4}$ and two checks namely GA06/106 (Moderate resistant) and GAO6/18 (Moderate susceptible). The populations included the average phenotype

(Mid parent value F_1) of the two parents and BC_1F_1 from a new cross of (MUC007/009 and Epuripuri) and Epuripuri, F_2, $F_{2:3}$ and $F_{2:4}$ from the previous crosses described in section 3.2.3.1. The experiment was repeated in the field and planted on 28th March, 2010, following completely randomized and alpha lattice designs in the greenhouse and the field respectively. The resistant parent MUC007/009 and the susceptible parent Epuripuri were selfed three times to insure that they are homozygous. Genetic ratios in table 1 were used to calculate the additive, dominance and epistatic genetics on the *Turcicum* leaf blight inheritance among the developed populations (Bernardo, 2002).

Table 1. Genetic ratios of additive (a) and dominance (d) effects and epistatic (aa) (Bernardo, 2002).

Population type	Mean	A	d	aa
BC_1F_1	1	0.5	0.5	0.25
F_1	1	0	1	0
F_2	1	0	0.5	0
$F_{2:3}$	1	0	0.25	0
$F_{2:4}$	1	0	0.125	0
MUC007/009	1	-1	0	1
Epuripuri	1	1	0	1

a = Additive effects.
d = Dominance effects.
aa = Epistatic.

3.2.4 Inoculum preparation and inoculation techniques

Sorghum infected leaves were collected from the field. Lesions were cut from these leaves and placed on moist paper towels in petri dishes for 48 hours to allow sporulation (Carson, 1995). Single spores were picked from the lesions with the aid of a compound microscope (LEICA MS5. Leica Microsystems Inc, Bannockburn, 60015 United States) and placed on potato dextrose agar (PDA) (Farm Eur. Laboratories Conda, SA. C/ La Forja, 9, Torrejon de Ardoz, Madrid 28850, Spain) plates and incubated at room temperature in a dark place. Individual spores of *Exserohilum turcicum* were subsequently sub-cultured to fresh PDA plates used to inoculate autoclaved sorghum kernels, and allowed to colonize the sorghum kernels for about 14 days (Carson, 1995). The colonised sorghum kernels were air-dried prior to field inoculation. Inoculation was done at the five leaf stage (Stage 2) (Vanderlip, 1993) by placing 20 to 30 colonized sorghum kernels into the leaf whorls. Inoculation was done in the evening when dew and ambient temperature are optimal to successful infection (Carson, 1995).

3.2.5 Disease assessment and evaluation

Disease severity was assessed using a scale of 0 to 75, where 0 %= no disease (no lesions identifiable on any of the leaves), 0.5 - 5 % of leaf surface diseased (a few restricted lesions on a few leaves); 5 - 10 % of leaf area diseased (several small or big lesions on many leaves); 10 - 20 % of leaf surface diseased (numerous small and large lesions on many leaves); 20 - 45 % of leaf surface diseased (many large and coalesced lesions on many leaves) and 45 - 75 % of leaf surface diseased; representing multitudes of coalesced lesions resulting in leaf wilting, tearing and blotching (Adipala *et al.,* 1993). Assessment commenced at stage 4 (the growing point

differentiation) (Vanderlip, 1993), 51 days after planting and continued on a weekly basis for eight weeks for F_2, six weeks for $F_{2:3}$ and four weeks for $F_{2:4}$.

3.2.6 Data collection and analysis

In the F_2 population, disease severity was assessed using percentage of leaf area affected on individual plant basis. In $F_{2:3}$ and $F_{2:4}$ populations disease severity was assessed as for F_2 population. Data was also taken on disease severity, lesion type and flowering dates. In the generation mean analysis experiment disease severity was assessed as described for F_2 populations. Means for all the developed populations were determined from data collected from the populations planted in the experimental plots.

Weekly assessments of disease severity were used to compute area under disease progress curves (AUPDC) as described by Campbell and Madden (1990) and Adipala *et al*. (1993). The formula for computing AUDPC was given as

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \right) \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{(1)}$$

Where:
t = The time in days of at reading.
y = The percentage of affected foliage at each reading.
n = The number of readings.

To standardise area under disease progress curve the AUDPC; values were divided by the total period of epidemics (Campell and Madden, 1990). All data were subjected to correlation and regression analysis using GenStat Discovery Edition 12 (Lawes Agricultural Trust, Rothamsted, UK) to establish any association between area under disease progress curve disease severity,

lesion type and flowering dates. Chi square (χ^2) analysis was used to test goodness of fit of the mode of TLB inheritance data to expected segregation ratios (Steel *et al*., 1997).

3.3   Results

3.3.1   Disease reactions to *Turcicum* leaf blight in F_2, $F_{2:3}$ and $F_{2:4}$ populations

The F_2 mean disease severity rating was 11.01% under greenhouse environment. It was much lower than the mid-parent value of the two parent's (27.96%) (Table 2) (Appendix 1). There was no significant difference between the two parents disease reaction in this environment. The F_2 disease severity ratings of TLB ranged from 0.63% to 51.88% and were skewed towards resistance (Figure 2). One hundred and ninety four (194) out of 304 segregating individuals scored less than 12% percent leaf area affected and showed transgressive segregation towards resistance (Figure 2). The resistant variety MUC007/010 had much more lower disease severity (5.82%) than the resistant parent MUC007/009 (26.54%) while the moderately resistant check GAO6/106 had slightly lower disease severity (23.65%) than MUC007/009 (Table 2). Two checks, the susceptible Lulud and the moderate susceptible GAO6/18 had higher disease severity (36.68% and 98.75% respectively) than the susceptible parent Epuripuri (29.39%) (Table 2). The distribution of AUDPC of F_2 segregating population under the greenhouse condition was normally distributed, consistent with a number of minor genes for resistance (Figure 2).

Table 2. Initial and final severity ratings and area under disease progress curves of TLB on F_2 families evaluated at MUARIK under greenhouse conditions during the first rains of 2009 (April - July).

	Disease Reaction	[a] AUDPC	[b] Initial Severity	[c] Final Severity
Parents				
MUC007/009	Resistance	26.54	0.80	23.0
Epuripuri	Susceptible	29.39	0.88	22.5
Mid-parent value		27.96	0.84	22.8
Checks				
GA06/106	Moderately resistant	23.65	0.75	21.7
Lulud	Susceptible	36.68	0.83	28.3
MUC007/010	Resistant	5.820	0.56	05.4
GAO6/18	Susceptible	98.75	2.00	75.0
F_2 Population				
Mean		11.01	1.21	14.1
Minimum		0.625	0	05.4
Maximum		51.88	130	28.2
LSD$\leq$ 0.05		1.22	0.25	0.67
CV%		0.02	0.13	0.34
SED		0.62	0.03	0.01

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
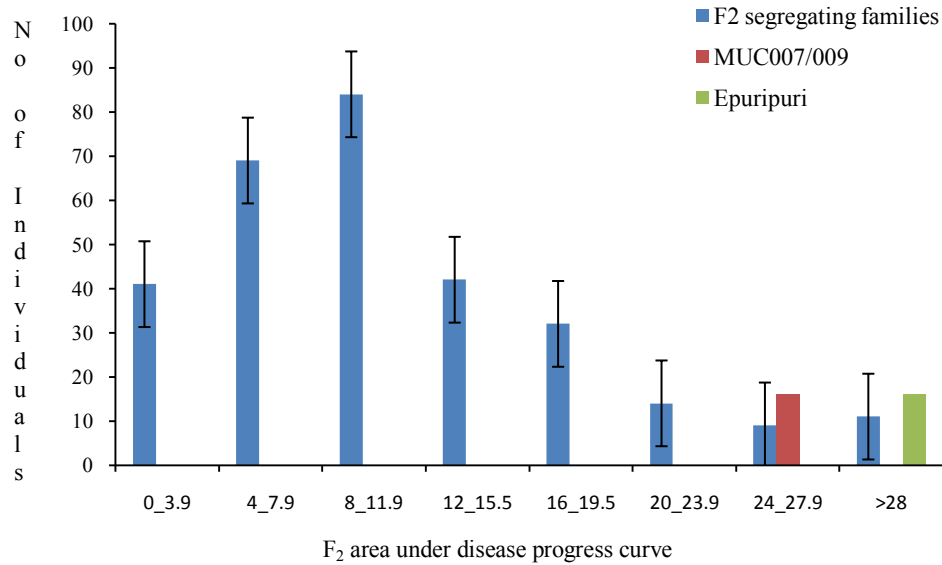
Figure 2. Segregation pattern of F_2 progeny from a resistant x susceptible cross of sorghum genotypes. The data was obtained from 304 segregating individuals.

Under the field environment, the mean disease severity of $F_{2:3}$ segregating families was 12.06%. The mean severity was slightly lower than the mid parent value of the two parents (12.84%). Disease severity ratings for the $F_{2:3}$ ranged from 5.98% to 37.88 % (Table 3) (Appendix 1). The $F_{2:3}$ plants showed highly significant differences between families. MUC007/009 had much lower disease severity (4.00%) than Epuripuri (21.70%). $F_{2:3}$ disease severity scores had normal distribution (Figure 3). The moderate resistant check variety GAO6/106 had disease severity ratings (3.40%) which were slightly lower disease severity than the resistant parent MUC007/009 (4.00%), while the resistant check MUC007/010 and the susceptible check GAO6/18 had slightly higher disease severity (4.46% and 7.13% respectively) than MUC007/009 (Table 3).

Table 3. Initial and final severity ratings and area under disease progress curves of TLB on $F_{2:3}$ families evaluated in the field at MUARIK during the second rains of 2009 (October - January).

	Disease Reaction	[a] AUDPC	[b] Initial Severity	[c] Final Severity
Parents				
MUC007/009	Resistance	04.0	0.10	6.80
Epuripuri	Susceptible	21.7	5.20	25.0
Mid-parent value		12.8	2.65	15.9
Checks				
GA06/106	Moderately resistant	03.4	0	5.40
Lulud	Susceptible	21.1	3.05	25.0
MUC007/010	Resistant	04.5	0	6.80
GAO6/18	Susceptible	07.1	0.35	8.40
$F_{2:3}$ Population				
Mean		12.1	1.01	11.2
Minimum		5.98	0	0.50
Maximum		37.9	4.00	100
LSD≤ 0.05		0.64	0.14	1.92
CV%		0.02	0.05	0.03
SED		0.33	0.07	0.98

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
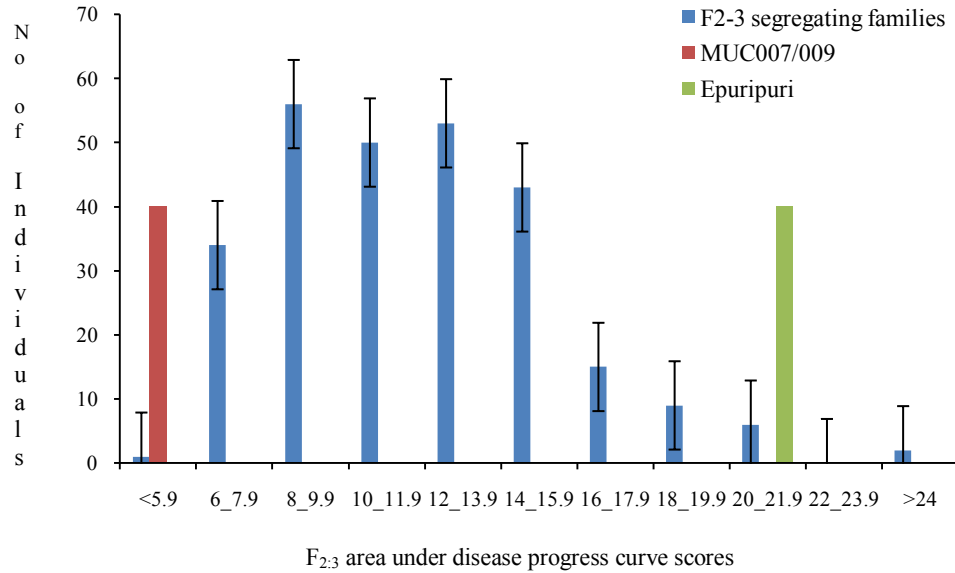
Figure 3. Segregation pattern of $F_{2:3}$ progeny from a resistant x susceptible cross of sorghum genotypes. The data was obtained from 278 segregating individuals.

Under the field environment the means of $F_{2:4}$ segregating families was 6.59% which was effectively equal to the mid parent value of the two parents (6.62%) (Table 4) (Appendix 1). *Turcicum* leaf blight scores for the $F_{2:4}$ ranged from 2.17% − 14.66% (Table 4). The mean resistant parent (MUC007/009) severity rating was 4.61% while the susceptible parent (Epuripuri) rating was 8.63%. Area under disease progress curve of $F_{2:4}$ segregating families was highly significantly different between families. $F_{2:4}$ segregating families severity scores had a normal distribution with transgressive segregation in both directions (Figure 4). The moderately resistant genotypes GAO6/106 and GA06/18 had similar severity ratings of 5.48% and 5.23% respectively (Table 4). However the disease severity scores for both materials were higher than the resistant parent MUC007/009 (4.61%) (Table 4).

Table 4. Initial and final severity ratings and area under disease progress curves of TLB on $F_{2:4}$ families evaluated at MUARIK during the first rains of 2010 (March - August).

	Disease Reaction	[a] AUDPC	[b] Initial Severity	[c] Final Severity
Parents				
MUC007/009	Resistance	4.61	0.65	4.33
Epuripuri	Susceptible	8.63	0.65	8.23
Mid-parent value		6.62	0.65	6.28
Checks				
GA06/106	Moderately resistant	5.48	0.53	5.56
GAO6/18	Moderately Resistant	5.23	0.64	5.40
$F_{2:4}$ Population				
Mean		6.59	0.47	6.69
Minimum		2.17	0	3.18
Maximum		14.6	3.69	15.5
LSD≤ 0.05		4.20	0.61	4.64
CV%		31.9	61.3	33.9
SED		3.06	0.31	2.36

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
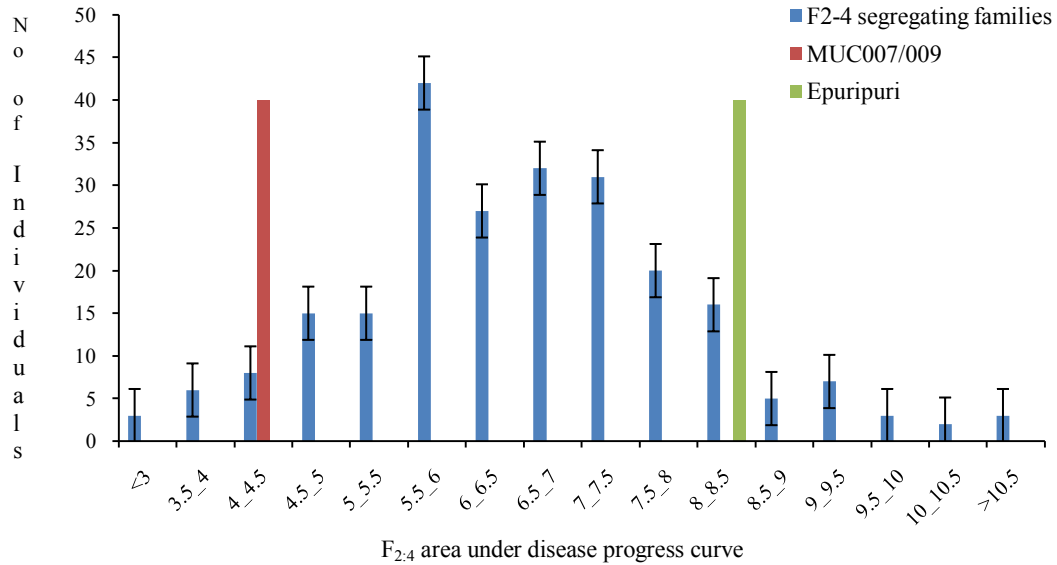
Figure 4. Segregation pattern of $F_{2:4}$ progeny from a resistant x susceptible cross of sorghum genotypes. The data was obtained from 246 segregating individuals.

.

3.3.2 Disease reaction in $F_{2:3}$ and $F_{2:4}$ progenies

MUC007/009 and Epuripuri had distinctly different lesion types (Plate 2). MUC007/009, the resistant parent, had narrow lesions with a red border; and Epuripuri, the susceptible parent, had wider lesions without a red border. The frequency of resistant lesion type and susceptible lesion type as assessed among the $F_{2:3}$ and $F_{2:4}$ families. The $F_{2:3}$ and $F_{2:4}$ families with a resistant lesion type were considered as homozygous resistant and families with the susceptible lesion type were considered homozygous for lesion type. A number of families had both susceptible and resistant lesion types and were considered as heterozygous. The two distinct lesion types segregated in a ratio of 1:2:1 ($\chi^2 = 0.57^{ns}$) (Figure 5). $F_{2:4}$ families similarly segregated with a ratio of 1:2:1 ($\chi^2 = 0.645^{ns}$) (Figure 5). The distribution of resistant lesion type among $F_{2:3}$ and $F_{2:4}$ ranged from 0 – 100%. The frequency of $F_{2:3}$ and $F_{2:4}$ populations segregating for resistant lesions type were 64% and 74% respectively which were higher than the mid parent value of the two parents for both

populations (50%). The resistant parent MUC007/009 had only the resistant lesion type, while the susceptible parent Epuripuri had no resistant lesion type.

(A)   (B)
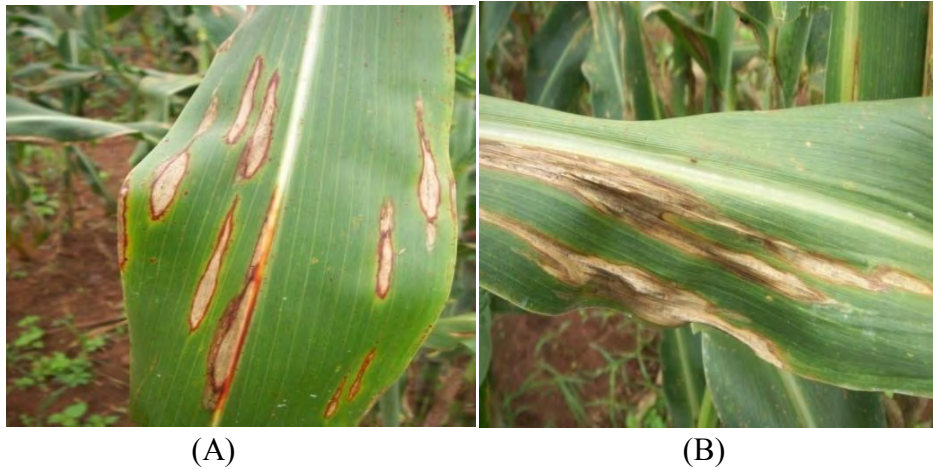
Plate 2. Reaction of two sorghum accessions to *Exserohilum turcicum* infection. Plate (A): Resistant lesion type characterised with narrow lesions with a distinctly red border and chloratic green halo (N). Plate (B): Susceptible lesion type characterised with wide greyish to tan lesions without a red border (W).
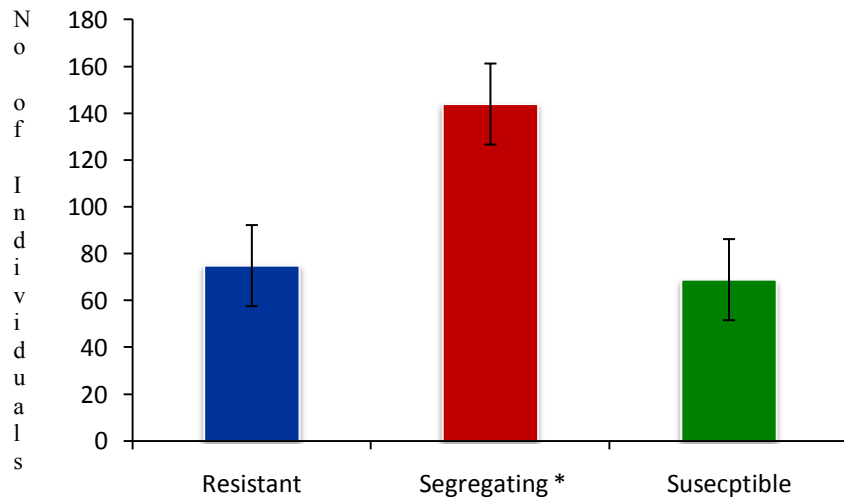
Figure 5. Segregation pattern for resistance to *Exserohilum turcicum* infection of the $F_{2:3}$ and $F_{2:4}$ segregating families.
* indicates the segregated families which had both susceptible and resistant lesion types that were considered as heterozygous.

3.3.3 Correlation analysis of AUDPC, days to flowering and lesion type

There was significant negative correlation between days to flowering among F_2, $F_{2:3}$ and $F_{2:4}$ progenies and the AUDPC under both environments (Greenhouse and field) (Table 5) and Table 6). In the $F_{2:3}$ segregating population under the field environment, there was significant but negative correlation between the days to 50% flowering and AUDPC ($r = -0.133^*$) (Table 5). There was significant, unexplained positive correlation between the percent resistant lesion type per family and AUDPC ($r = 0.107^+$) as well as between the resistant lesion type and the initial and final severity ratings (Table 5). In the $F_{2:4}$ population and under the field environment, there was a negative but significant correlation between days to 50% flowering and area under disease progress curve ($r = -0.221^{***}$), the initial and final weekly score of the $F_{2:4}$ segregating families (Table 6). There was no significant correlation between resistant lesion type and, the final severity ratings and the AUDPC, however, there was significant unexplained positive correlation between the resistant lesion type and the initial ratings (Table 6).

Table 5. Correlation of days to 50% flowering, lesion type and area under disease progress curves on $F_{2:3}$ families at MUARIK during the second rains of 2009.

	[a] AUDPC	[b] Initial Severity	[c] Final Severity
[a] AUDPC	—	0.794^{***}	0.689^{***}
Days to 50% Flowering	-0.133^*	-0.109^+	-0.088^{ns}
Resistant Lesion Type	0.107^+	0.145^*	0.1451^*

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
[ns] = Not significant; [+] =Significant at 0.1; [*] = Significant at 0.05; [***] = Significant at 0.001

Table 6. Correlation of days to flowering, lesion type and area under disease progress curves on *Turcicum* leaf blight of $F_{2:4}$ families during the first rains of 2010.

	[a] AUDPC	[b] Initial Severity	[c] Final Severity
[a] AUDPC	—	0.371^{***}	0.804^{**}
Days to 50% Flowering	-0.221^{***}	0.037^{ns}	-0.189^{**}
Resistant Lesion tType	0.007^{ns}	0.150^{*}	-0.050^{ns}

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al.*, 1993).
[ns] = Not significant; [*] = Significant at 0.05; [**] = Significant at 0.01; [***] = Significant at 0.001

3.3.4   Generation mean analysis

3.3.4.1 Area under disease progress curve (AUDPC), days to 50% flowering and percentage of resistant lesion type (N)

Under the greenhouse conditions, there were only slight and non significant differences between the generations including the two parents (MUC007/009 mean = 2.5%, and Epuripuri mean = 2.6%); while the F_1 (Mean = 2.4%) and F_2 (Mean = 2.2%) had lower AUDPC ratings than MUC007/009 the resistant parent (Table 7). The BC_1F_1 (Mean = 2.7%) and $F_{2:3}$ (Mean = 2.6%) had higher AUDPC ratings than Epuripuri the susceptible parent and $F_{2:4}$ progeny had the same AUDPC as MUC007/009 the resistant parent (Table 7). The mean of GA06/18 (Susceptible check) was greater than Epuripuri the susceptible parent, while the mean of GA06/106 (Moderatly resistant) was higher than the susceptible parent (Table 7).

Table 7. Generational means of area under disease progress curve, initial and final severity ratings, days to 50% flowering and resistant lesion type under the greenhouse condition during the first rains of 2010.

Population Type	Disease Reaction	No of Plants	[a]AUDPC	[b]Initial Severity	[c]Final Severity	Days to 50% Flowering	[d]Lesion Type%
Generation							
BC_1F_1		34	2.7	0.1	5.3	77	71
F_1		101	2.4	0.1	4.6	82	78
F_2		81	2.2	0.1	4.2	86	46
$F_{2:3}$		68	2.6	0.2	4.4	85	44
$F_{2:4}$		62	2.5	0.2	4.7	85	42
Parents							
MUC007/009	Resistance	38	2.5	0.1	4.7	81	100
Epuripuri	Susceptible	36	2.6	0.2	4.9	81	0
Checks							
GA06/106	Moderately resistant	16	3.1	0.5	5.6	86	88
GA06/18	Moderately susceptible	14	4.6	0.5	7.7	84	79
LSD≤ 0.05			0.64	0.41	0.98	6.42	
CV%			48.1	119.1	39.9	13.5	
SED			0.33	0.21	0.49	3.26	

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[d] = Percentage number of plants showing resistant lesion type.

Under the field condition, the two parents were significantly different, MUC007/009 mean AUDPC= 4.3%, and Epuripuri mean AUDPC = 9.8% (Table 8). Both F_1 and BC_1F_1 had the same mean AUDPC of 6.7%., which was higher than MUC007/009 the resistant parent (Table 8). F_2 and $F_{2:4}$ had the same mean AUDPC of 8.8% (Table 11). The $F_{2:3}$ progeny had lower

AUDPC of 7.2% than Epuripuri the susceptible parent. The mean AUDPC of GA06/18 the susceptible check was much lower (4.6%) than Epuripuri the susceptible parent, while the mean AUDPC of GA06/106 (Moderate resistant) was slightly higher than the resistant parent (Mean = 5.8%) (Table 8). Both parents had similar days to flowering under the greenhouse condition (MUC007/009 = 81 days and Epuripuri = 81 days), with one day difference under the field condition (Table 7) and (Table 8). F_1, BC_1F_1 and $F_{2:4}$ flowered earlier than the resistant parent while $F_{2:3}$ flowered at the same as the resistant parent.

3.3.4.2 Additive, dominance and epistatic model

The overall genetic effects on the AUDPC and dates to 50% flowering among different generations showed few significant effects in either the greenhouse and field conditions (Table 9). The non-dominance, Adititve and epistasis interaction, showed significance geneticseffects under the field conditions but it was significant under the greenhouse condition (Table 9). Under both environments, there was limited dominance effects (Table 9). Although there was significant epistatic effect under the greenhouse conditions and not under the field conditions (Table 9).

Table 8. Means of area under disease progress curve, initial and final severity ratings, days to 50% flowering and resistant lesion type under field condition during the first rains of 2010.

Population Type	Disease Reaction	[a] AUDPC	[b] Initial Severity	[c] Final Severity	Days to 50% Flowering	[d] Lesion Type %
Generation						
BC_1F_1		6.7	0.6	7.8	72	77
F_1		6.7	0.6	6.2	71	100
F_2		8.8	0.6	8.6	76	79
$F_{2:3}$		7.2	0.4	7.5	73	81
$F_{2:4}$		8.8	0.4	10.0	72	54
Parents						
MUC007/009	Resistant	4.3	0.3	5.3	73	100
Epuripuri	Susceptible	9.8	0.4	9.9	74	0
Checks						
GA06/106	Moderate resistant	5.8	0.7	6.2	73	98
GA06/18	Moderate susceptible	4.6	0.3	6.0	75	100
LSD≤ 0.05		2.38	0.40	2.86	4.45	18.8
CV%		32.1	89.1	36.1	6.12	25.40
SED		1.20	0.20	1.44	2.25	9.46

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Initial severity was taken 14 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[c] = Final severity was taken 40 days after inoculation based on scale 0 - 75%, (Adipala *et al*., 1993).
[d] = Percentage number of plants showing resistant lesion type.

Table 9. Additive, dominance and epistatic effects for area under disease progress curve and flowering dates under the greenhouse and field condition during the first rains of 2010.

	[a] AUDPC		Days to 50% flowering	
	Greenhouse	Field	Greenhouse	Field
Mean	2.513^{***}	8.59^{***}	86.46^{***}	73.47^{***}
[b] Additive effects	0.043^{ns}	4.14^{*}	-1^{ns}	-0.07^{ns}
[c] Dominance effects	-0.097^{ns}	-2.32^{ns}	-6.01^{ns}	-1.36^{ns}
[d] Epistatic	0.060^{ns}	-3.80^{*}	-5.69^{**}	0.09^{ns}

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b, c] and [d] = Computed as described by Bernardo (2002).
ns = Not significant; * = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001.

The dominance and epistatic effects computed on the basis of AUDPC among the different generations under both environments. The additive effect had significant effects under the field environment while it was not significant under the greenhouse condition. The lack of fit for different generation means was not significantly different from zero under greenhouse and field conditions. Additive and dominance effects were not significantly associated with days to 50% flowering in either field or greenhouse (Table 10). Epistatic effects significantly influenced days to 50% flowering under the greenhouse conditions (Table 10).

Table 10. Mean squares of additive, dominance and epistatic effects for area under disease progress curve and days to 50% flowering under greenhouse and field conditions during the first rains of 2010.

Source of Variation	df	[a] AUDPC		Days to 50% Flowering	
		Greenhouse	Field	Greenhouse	Field
Population type	6	0.022^{ns}	3.365^{ns}	9.784^{**}	2.773^{ns}
Additive effects	1	0.003^{ns}	11.927^{**}	2.790^{ns}	0.006^{ns}
Dominance effects	1	0.017^{ns}	0.509^{ns}	1.293^{ns}	1.563^{ns}
Epistatic	1	0.003^{ns}	2.349^{ns}	26.26^{**}	0.002^{ns}
Lack of fit	3	0.036^{ns}	1.801^{ns}	9.454^{*}	5.02^{ns}
Error		0.035	3.971	3.448	5.229

[a] = AUDPC computed as described by Campbell and Madden (1990).
ns = Not significant; * = Significant at 0.05; ** = Significant at 0.01; *** = Significant at 0.001.

3.4   Discussion

3.4.1 F_2, $F_{2:3}$ and $F_{2:4}$ sorghum progenies reaction to *Turcicum* leaf blight

Sorghum *Turcicum* leaf blight is one of the most destructive foliar diseases. The focus of this chapter is to confirm the usefulness of developing resistant varieties as main solution to the disease. The specific objective of this study was to determine the mode of inheritance of resistance to *Turcicum* leaf blight. Disease response of sorghum to *Turcicum* leaf blight assessed using area under disease progress curve, final and initial severity ratings varied among the three progenies under greenhouse and field environments. Disease severity of F_2 plants in the greenhouse condition showed a normal distribution indicative of quanitative inheritance or minor

gene effects. Under the field conditions, disease severities of $F_{2:3}$ and $F_{2:4}$ matched a normal distribution suggesting quantitative inheritance. Though the performance of the resistant parent MUC007/009 and the susceptible parent Epuripuri was not different under the greenhouse environment, it was highly significant different under the field conditions. There was transgressive segregation towards the resistance under both environments for F_2, $F_{2:3}$ and $F_{2:4}$ progenies. The resistant parent MUC007/009 and the susceptible parent Epuripuri expressed distinctly different lesion types. The resistant lesion type and the susceptible lesion type were screened among the $F_{2:3}$ and $F_{2:4}$ families. The two distinct lesion types segregated according to the 1:2:1 ratio indicative of dominant gene inheritance. There was a significant, unexplained positive correlation between resistant lesion type percentage and the area under disease progress curve under the greenhouse conditions. However all populations (F_2, $F_{2:3}$ and $F_{2:4}$) from this cross, showed negative correlation between flowering dates and AUDPC. The early maturity lines had higher disease severity.

In this study there was clearly difference between greenhouse and field environments. Similar reports have been made elsewhere (Tarumoto *et al*., 1977; Levy, 1989; Hennessy, 1990). Given that *E. turcicum* is a necrotoph that requires high humidity and warm temperature for infection; and such conditions are common under greenhouse conditions. This may explain the higher severities in the F_2 in the greenhouse, although the same was not seen in later seasons.

On maize - *E. turcicum* pathosystem, a gene–for-gene relation has been reported (Simox *et al*., 1993; Carson, 1995). Indeed with the discovery of several new races, more *Ht* resistance loci have been reported with five dominant genes controlling resistance (*Ht*, *Ht2*, *Ht3*, *HtM*, and *HtN*)

(Simcox *et al*., 1993; Carson, 1995). The *Ht* genes seem to have unusually high environmental dependence, particularly with regard to light and temperature (Leath *et al*., 1990) and they tend to confer delayed lesion development or sporulation phenotypes rather than complete resistance (Balint-Kurti and Johal, 2009). Several dominant or partially dominant qualitative genes have also been described that confer race-specific resistance, including *Ht1* (Hooker, 1963), *Ht* 2 (Hooker, 1977), *Ht* 3 (Hooker, 1981), *Htn* 1 (also known as *HtN*, Gevers, 1975) and *Ht* P (Ogliari *et al*., 2005). Resistance in sorghum to *E. turcicum* is poorly characterised. However, the close relatedness between sorghum and maize genome and the fact that both crops are attacked by the same pathogen suggests resistance mechanisms may be similar. Whereas the *Ht* based resistance is well characterised in maize, and shows dominance, in this study we did not find dominant gene action. Similarly in maize quantitative resistance has been reported (Welz and Geiger, 2000) just as the case of this study.

In this study the parents had distinctly different lesion types that were consistent under both greenhouse and field environments. The resistant parent had narrow lesions with a distinctly red border while the susceptible parent had wider lesions without a red border. In maize *Ht* reaction is also associated with characteristic green halo around lesions which limits both sporulation and lesion expansion (Hooker, 1963; 1977; 1981). It is therefore possible that in sorghum a similar *Ht* type of responce may be elicited. In the *Ht* reaction of maize phenolics have been implicated (Obi *et al*., 1979). And for many plants resistance to necrotrophs is often associated with production of phenolics (Lamb *et al*., 1989). In sorghum the 3-deoxyanthocyanidins phytoalexins are essential for resistance (Aguero *et al*., 2002). It is therefore plausible that the resistance

reaction observed general in this study may be conditioned by similar systems reported for *Ht* in maize.

3.4.2   The mode of inheritance of resistance in sorghum to *Turcicum* leaf blight

Generation mean analysis was used to investigate the contribution of additive (a), dominant (d) and epistatic (aa) effects on resistance in sorghum to *Turcicum* leaf blight. The generation means were observed on five basic generations; the resistant parent MUC007/009, the susceptible parent Epuripuri, F_1, F_2 and BC_1F_1 to the susceptible parent plus $F_{2:3}$, $F_{2:4}$ and two checks GA06/106 (Moderately resistant) and GAO6/18 (Moderately susceptible).

Partitioning of genetic effects into additive, epistatic and dominance components in this study shows that for most part resistance is attributed to additive and epistatic effects. These data are consistent with some studies in maize which also show that resistance to *E. turcicum* be quantitative in nature (Welz and Geiger, 2000; Ogliari *et al*., 2005). The limited role of dominance effects under both greenhouse and field environments further demonstrate the bigger role of additive x epistatic effects. In this study the segregation patterns of $F_{2:4}$ progeny was normally distributed (Figure 4) with a quantitatively inherited trait.

It is interesting however to note that lesion type segregated according to a typical qualitatively inherited pattern. The resistant lesion type had properties akin to the *Ht* chlorotic lesions; being smaller in size with a characteristic defining halo. In maize *Ht* resistance is qualitative in nature in some studies, and quantitative in others, with the latter characterised by smaller wild type lesions (Hakiza, 1993; Lipps *et al*., 1997). In this study we observed smaller lesions on the

resistant genotypes which had a characteristic purple halo. The two observations are therefore similar and mutually reinforcing. In this case however, production of the anthocianins commonly associated with sorghum under stress was observed (Lamb *et al.*, 1989; Klein *et al.*, 2001; Aguero *et al.*, 2002). Taken together, this study shows that resistance in sorghum to *E. turcicum* is regulated by additive and epistatic effects.

CHAPTER FOUR

IDENTIFICATION OF MOLECULAR MARKERS LINKED TO *TURCICUM* LEAF

BLIGHT RESISTANCE LOCI IN SORGHUM

4.1   Introduction

In recent years, progress has been made in mapping and tagging the genes of agronomically important traits which form the foundation of marker assisted selection (Mittal and Boora, 2005). Marker assisted selection is a process by which a marker (morphological or molecular marker) is used for indirect selection of a genetic determinant a trait of interest (Bernardo, 2008). Although morphological markers such as disease response can reveal genetic differences between resistant and susceptible genotypes they are associated with several general deficits that reduce their usefulness. In the case of plant diseases, where it is difficult to obtain right levels of disease innoculum screening and correct evaluations may be difficult. The use of methods that indirectly permit selection of a desired trait thus useful. The use of molecular markers that co-segregate with desired loci have been used successfully for the isolation of a number of important plant genes, including genes for resistance (Mohan *et al*., 2009). In the case of *Turcicum* leaf blight, molecular markers associated with resistance can be used for marker assisted breeding. Limited information is available for molecular markers used in improving sorghum for *Turcicum* leaf blight resistance. The aim of this chapter was to develop and validate random amplified polymorphic DNA (RAPD) for sorghum *Turcicum* leaf blight resistance. And also to identify simple sequence repeat (SSR) markers closely linked to the *Turcicum* leaf blight resistance gene (or genes) in sorghum.

4.2   Materials and methods

4.2.1   Mapping population and the study site description

Molecular characterisation was done in the biotechnology laboratory in the Department of Crop Science of Makerere University, Kampala, Uganda. A population developed from a cross between MUC007/009 (Resistant parent) and Epuripuri (Susceptible parent) was used in this study. Three hundred and four plants of F_2 segregating population were used to characterise the association between *Turcicum* leaf blight and resistance genes. Since the two parents are genetically and phenotypically contrasting, no selection was made for resistance to *Turcicum* leaf blight or for any agronomic traits during the development of this population. The phenotypic data of area under disease progress curve and resistant lesion type were used to identify significant phenotype-marker associations for *Turcicum* leaf blight resistance in MUC007/009 and Epuripuri, resistant and susceptible parents respectively.

 4.2.2   Molecular markers selection

Simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD) were used in this study. Ten SSR markers were selected from the sorghum consensus genetic map (Mace *et al*., 2009) (Table 11) (Appendix 2) (personal communication Dr. S. M. Mohan, National Research Centre for sorghum, Rajendranagar, Hyderabad 500 030, India, Appendix 3). The agronomic region flanked by plant colour locus (*Plcor*) and simple sequence repeat marker Xtxp95 on linkage group six which harbours disease-response QTL for some diseases caused by different fungal pathogens was selected (Mohan *et al*. 2009). Eighty RAPD markers were randomly selected to screen the whole genome in this specific sorghum population (Appendix 4).

Table 11. SSR markers selected from the sixth linkage group of sorghum consensus map used in the study.

No [*] Marker		Forward	Reverse
1	Xtxp17	CGGACCAACGACGATTATC	ACTCGTCTCACTGCAATACTG
2	Xtxp57	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC
3	Xtxp95	TCTCCGTTTGCCCGCCAG	CACCGTACCGCCTCCCGAATC
4	Xtxp97	CAAATAAACGGTGCACACTCA	GTATGATTGGAGACGAGACGG
5	Xtxp145	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC
6	Xtxp274	GAAATTACAATGCTACCCCTAAAAGT	ACTCTACTCCTTCCGTCCACAT
7	Xcup12	TGTTACAGAGACGCGCAGAG	GGCTGGTTGCTACCTTGTTC
8	Xcup17	CTGAGGAGTGGTTTCATCCC	CATCACCGTTCCCCTCTTTC
9	Xcup36	TGAGCTGATAATGGCTGCTG	GCGTCACGGAAGTTGGAC
10	Xcup37	CCCAGCCTTCCTCCTGATAC	GTACCGACTCCAATCCAACG

[*] = Markers are based on communication from Dr. S.M. Mohan, National Research Centre for sorghum, Rajendranagar, Hyderabad 500 030, India (Mace *et al*., 2009).

4.2.3 DNA extraction

DNA was isolated from all materials according to Edwards *et al*. (1991). Two week old leaves were collected from each of the 304 F_2 individuals and 278 $F_{2:3}$ segregating families as well as the two parents before they were inoculated. The leaf samples were stored at -80^0C until needed for use. Frozen leaves were ground to a very fine powder in liquid nitrogen using a mortar and pestle. One gram of leaf material was homogenised in a hot (60^oC) mixed 500 µl of super quick extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA pH 8.0 and 0.5% SDS) followed by incubation at 60^oC for 10 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, vortexed. This was followed by centrifugation at 9447 g for 10 minutes to resolve phases in a centrifuge (Eppendorf, model 5415C, Netheler-Hinz GmbH, 22331 Hamburg, Germany). The supernatant was transferred into a fresh tube. DNA was precipitated with 0.1 volume 3M sodium acetate (pH 5.2) and 0.7 volume cold and concentrated

ethanol and was pelleted by centrifuging at 9447 g for 10 min in a centrifuge (Eppendorf, model 5415C, Netheler-Hinz GmbH, 22331 Hamburg, Germany). The supernatant was removed and the pellet washed with 70% (v/v) ethanol by vortexing and centrifuged at 9447 g for 5 min. The pellet was resuspended in 100 µL of 1X TE buffer (10 mM Tris-HCl pH 7.5 and 1 mM EDTA pH 8.0).

4.2.4   DNA quantification and qualification

Six µl of genomic DNA extracted above was loaded on a 0.8% agarose gel and run in 1X TAE buffer in a horizontal gel system (Bio-Rad, model 96, Bio-Rad Laboratories, Inc. Life CA USA) at 100 volts for 120 min and stained in 1% ethidium bromide solution (Promega, Promega Corporation, Madison, WI USA) for 15 min, visualised under 100% UV light in a gel documentation system (Bio-Rad, model 1000, Bio-Rad Laboratories, Inc. CA USA) and then photographed. The DNA samples that produced sharp, single visible bands were taken to represent good amounts and quality of DNA. DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (NanoDrop technologies, Delaware, USA). The DNA was diluted to10 ng µL^{-1} and stored at -20$^{\text{o}}$C until use for later use in Polymerase Chain Reactions (PCR).

4.2.5   Random amplified polymorphic DNA (RAPD)

All RAPD markers were amplified in the thermocycler (GeneAmp PCR system 9700 thermocycler (Applied Biosystem, Cary California, USA). Eighty markers were selected and tested for polymorphism in MUC007/009 and Epuripuri. The primers were synthesised by the Molecular and Cell Biology laboratory of the University of Cape Town, South Africa.

Polymerase Chain Reaction (PCR) was performed for each RAPD primer on the parent genomic DNA. Each PCR reaction was carried out in a total volume of 10 μl, containing 1.0 μl of 10X Taq PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.6 μl of 50 mM MgCl$_2$, 0.08 μl of 25mM dNTP mix, 0.1 μl of 5 M RAPD primer, 1.0 μl of genomic DNA and 0.1 μl Taq DNA polymerase (5 U/μl) (Promega, Madison, USA). The thermal cycling program consisted of a pre-denaturation step at 95°C for 3 min and 35 cycles of 94°C for 0.45 min, 35°C for 1 min and 30 cycles and 72°C for 1 min followed by a final extension at 72°C for 7 min. The amplified products were subjected to electrophoresis on 1.0 % agarose gel in 1X TAE buffer at 130 V using a BIO-RAD electrophoresis system. The DNA was visualised by ethidium-bromide staining (Promega, Madison, USA), using Gel Doc 1000 documentation system (BIO-RAD Laboratories, California, USA).

4.2.6 Simple sequence repeats (SSR)

All SSR markers were amplified in the thermocycler (GeneAmp PCR system 9700) thermocycler (Applied Biosystem, Cary California, USA). Polymerase chain reaction (PCR) was performed for each simple sequence repeat (SSR) primer pair on the two parents genomic DNA. Each PCR reaction was carried out in a total volume of 10 μl, containing 1.0 μl of 10x Taq PCR buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.6 μl of 50 mM MgCl$_2$, 0.08 μl of 25mM dNTP mix, 0.1 μl of 5 M RAPD primer, 1.0 μl of genomic DNA and 0.1 μl Taq DNA polymerase (5 U/μl) (Promega, Madison, USA). The thermal cycling programme consisted of a pre-denaturation step at 95°C for 5 min and 35 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min followed by a final extension at 72°C for 7 min. The amplified products were subjected to electrophoresis on 3.0% w/v agarose metaphor gels {Seakam agarose (Cambrex,

Rockland, USA)} in 1X TBE buffer at 130 V using a BIO-RAD electrophoresis system (BIO-RAD Laboratories, California, USA). The DNA was visualised by ethidium-bromide staining (Promega, Madison, USA), using a Gel doc 1000 documentation system. All SSR markers were screened for polymorphism among a number of sorghum accessions. For each microsatellite locus, the polymorphic information content (PIC) values were used to calculate the genetic diversity according to the formula shown below:

$$PIC = 1 - \sum_{i=1}^{K} x_i^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where k is the total number of alleles detected for a microsatellite and *xi* is the frequency of the *i*th allele of the SSR loci (Abu Assar *et al.*, 2005). All microsatellites that were polymorphic among the selected sorghum including those used in developing the F_2 population were subsequently used in mapping studies.

4.2.7   Genotypic scoring of the populations

Three SSR markers (Xtxp274, Xtxp57 and Xtxp95) that were found to be polymorphic between the susceptible parent (Epuripuri) and resistant parent (MUC007/009) were used to genotype the mapping population. The three SSR markers were selected from the sixth linkage group of sorghum consensus map (Mace *et al.*, 2009) (Table 11) (Appendix 3). An equal quantity of fresh leaf tissue was harvested from each F_2 individual plants two weeks after planting and the total genomic DNA isolated from them. The F_2 individual lines carrying the allele from the susceptible parent at the polymorphic SSR loci were scored as 0, while those carrying the allele from the resistant parent were given a genotypic score of 2. The segregating individuals carrying alleles from both parents (heterozygote) were given a genotypic score of 1. The population was scored at the SSR marker loci that had significant association with *Turcicum* leaf blight

resistance. Although the area under disease progress curve and the resistant lesion type were not correlated, but there was correlation between the resistant lesion type and the fifth and sixth severity ratings and the initial and final severity ratings. The phenotypic data of the $F_{2:4}$ families were used to identify significant phenotype-marker associations.

4.2.8 Single-Marker analysis

The relationship between molecular markers and phenotypic scores were analysed by single marker analysis to identify SSR markers that had significant association with *Turcicum* leaf blight disease reactions like lesion type and severity scores. Chi square (χ^2) was used to detect the goodness of fit of Mendel's ratio 1:2:1 among F_2 genotypic data at significant phenotypic levels (α) 10%, 5%, 1% and 0.1%. ANOVA and regression analysis were used to detect the significance of the three allelic groups of each SSR marker. The Fisher's Protected Least Significant Differences (LSD) at P< 0.05 (Steel *et al.*, 1997) was used for the mean separation. The allelic groups are F_2 individual lines which are:

- carrying the allele from the susceptible parent at the polymorphic SSR loci were scored as 0,

- carrying the allele from the resistant parent were given a genotypic score as 2 and

- carrying alleles from both parents (Heterozygote) were given a genotypic score as 1.

 All data were analysed using GenStat Discovery Edition 12.

4.3 Results

4.3.1 Random amplified polymorphic DNA (RAPD) markers screening

Genomic DNA of the two parents, MUC007/009 (Resistant) and Epuripuri (Susceptible) was amplified with 80 RAPD primers. All primers that were polymorphic were screened twice to confirm reproducibility and to control for RAPD primer artifacts. Through this process, 26 RAPD polymorphic markers were found. Amplification occurred for one or both parents with all the RAPD markers producing a total of 81 discrete products and an average of 3 products per primer. The 26 RAPD markers were selected for the second screening. Out of 26 RAPDs, 8 RAPDs were consistently polymorphic between the two parents. 13 discrete products were polymorphic producing 19.2% of the total products.

4.3.2 Simple sequence repeat (SSR) markers screening

A total of 10 SSR markers were selected from the sixth linkage group of sorghum (Appendix 3) (Mace *et al.*, 2009). They were screened to amplify MUC007/009 (resistant parent) and Epuripuri (susceptible parent) genomic DNA. Three SSR markers, Xtxp95, Xtxp57 and Xtxp274, were polymorphic between the two parents, representing 30% of the tested SSR markers (Plate 3). They were selected to screen the genomic DNA of F_2 segregating population.
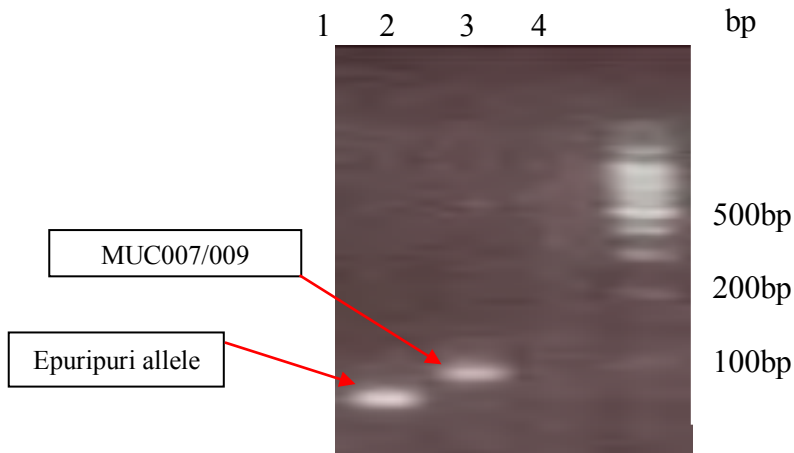
Plate 3. Simple sequence repeat profiles of L.C segregation pattern of SSR loci Xtxp95 between MUC007/009 and Epuripuri. Lane 1 is Epuripuri allele, 2 is MUC007/009 allele, 3 is Negative control while 4 represents the 100 bp DNA molecular size marker.

4.3.3 Segregation of SSR markers in F_2 segregating population

The SSR Xtxp274 had the highest number of individuals carrying the allele from the resistant parent followed by Xtxp57 and then Xtxp95. The heterozygous allele had 48% while the homozygous resistant allele received 27% and the homozygous susceptible allele received 18% of the total number of F_2 population (Figure 6). The polymorphic information content (PIC) for SSRs was 0.490 (Xtxp57), 0.496 (Xtxp247) and 0.499 (Xtxp95). In total 1353 alleles were detected among the 3 SSR loci among 203 F_2 segregated progeny. Chi square was done to test the goodness of fit of data to 1:2:1 ratio among the genotypic data of the three SSR markers in F_2 segregating progeny. The genotypic data for the SSR marker Xtxp95 showed that the genotypic data fits the 1:2:1 ratio (Table 12). In the markers Xtxp57 and Xtxp247, chi square was highly significant suggesting that the distribution of the genotypic data does not fit Mendel's segregation of ratio 1:2:1 (Table 12).

59
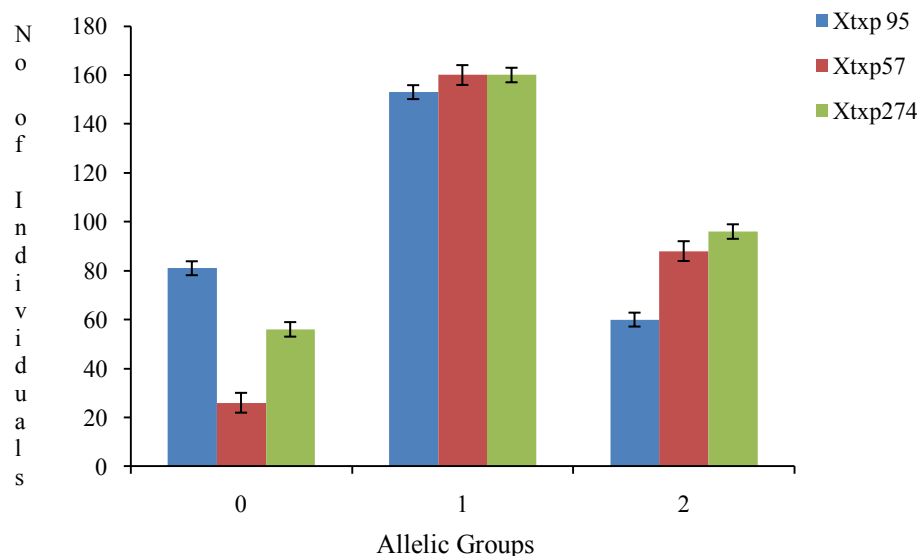
Figure 6. Segregation of Xtxp274, Xtxp57 and Xtxp95 as susceptible, heterozygous and resistant, respectively, among F_2 segregating population. Bars show the standard errors (±SE) for markers Xtxp95, Xtxp57 and Xtxp247 respectively.

Table 12. Segregation pattern, allele frequency and gene diversity of the three polymorphic SSR markers among F_2 progeny (MUC007/009 x Epuripuri).

Marker	[a] Linkage group	[b] Progeny Segregation [c] Res/Heter/Susc	[d] χ^2	Allele frequency Resistant/Susceptible	[e] Genetic diversity
Xtxp95	6	60/153/81	3.389[ns]	0.477/0.523	0.499
Xtxp57	6	88/160/26	35.78[***]	0.571/0.429	0.490
Xtxp274	6	96/125/56	14.18[***]	0.542/045	0.496

[a] = Linkage group according to Mace *et al*. (2009).
[b] = F_2 segregating population derived from MUC007/009 x Epuripuri. The ordered pairs of numbers represent the frequency of homozygous alleles from MUC007/009 allele, heterozygous and homozygous for Epuripuri allele, respectively.
[c] = Resistant/ Heterozygote /Susceptible.
[d] = Calculated Chi-square value as described by Steel *et al*. (1997) according to the expected Mendelian genotypic segregation ratio 1:2:1.
[e] = Calculted using the polymorphic information contents as described by Abu Assar *et al*. (2005).
[ns] = Not significant; [***] = Significant at 0.1%.

4.3.4 Single marker analysis

4.3.4.1 Single marker analysis of SSR Xtxp95

Single marker analysis for SSR marker Xtxp95 showed very highly significant (p≤0.001) association among the allelic groups and the resistant lesion type for $F_{2:3}$ and $F_{2:4}$ segregating population. The coefficient of determination (R^2) was 23.74% and 14.09% $F_{2:3}$ and $F_{2:4}$ segregating population respectively (Table 13). The three allelic groups 0, 1, 2 were significantly different from each other in $F_{2:4}$ while the first group 0 was significantly different from the other two in $F_{2:3}$ (Table 13). The association was not significant between the SSR marker Xtxp95 and the area under disease progress curve or flowering date for $F_{2:3}$ and $F_{2:4}$ (Table 13).

Table 13. Single marker analysis of variance for simple sequence repeat Xtxp95 marker.

Allelic group	[a] AUDPC		[b] Resistant lesion type		Dates to 50% flowering	
	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$
0	11.51	6.771	34.25	39.04	73.72	73.86
1	11.77	6.564	74.67	65.36	74.36	74.32
2	12.44	6.437	81.86	77.39	73.02	73.31
LSD (P≤0.05)	1.250	0.267	11.64	11.89	1.482	1.434
F_{Prob}	ns	ns	***	***	ns	ns
R^2	0.008	0.006	0.237	0.141	0.014	0.009
CV%	31.72	23.71	54.31	58.35	6.04	5.75

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Percentage number of plants showing resistant lesion type.
ns = Not significant; *** = Significant at 0.001.

4.3.4.2 Single marker analysis of SSR Xtxp57

The single marker analysis for SSR marker Xtxp57 showed highly significant ($p \leq 0.01$) association between the three allelic groups and the flowering dates for $F_{2:3}$ but was not significant in $F_{2:4}$ segregating population. The coefficient of determination (R^2) was 4.7% days for $F_{2:3}$ segregating population (Table 14). The three allelic groups 0, 1, 2 were significantly not different from each other (Table 14). The association was not significant between the SSR marker Xtxp57 and area under disease progress curve and resistant lesion type for $F_{2:3}$ and $F_{2:4}$ (Table 14).

Table 14. Single marker analysis of variance for simple sequence repeat Xtxp57 marker.

Allelic group	[a] AUDPC		[b] Resistant lesion type		Dates to 50% flowering	
	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$
0	11.6	7.16	64.4	59.2	76.0	74.6
1	11.8	6.61	64.9	58.8	74.2	74.1
2	12.1	6.52	63.7	63.9	72.7	73.2
LSD ($p \leq 0.01$)	1.59	0.65	16.7	15.8	1.818	0.538
F_{Prob}	ns	ns	ns	ns	**	ns
R^2	0.002	0.013	0.0002	0.004	0.047	0.011
CV%	32.24	23.43	62.06	62.68	5.9	5.76

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Percentage number of plants showing resistant lesion type.
ns = Not significant; ** = Significant at 0.01.

4.3.4.3 Single marker analysis of SSR Xtxp247

Single marker analysis for SSR marker Xtxp247 showed significant ($p \leq 0.1$) association between the three allelic groups and the area under disease progress curve for $F_{2:3}$ and was not significant in $F_{2:4}$ segregating population. The analysis showed that the coefficient of determination (R^2) was 2.2% in $F_{2:3}$ (Table 15). The three allelic groups 0, 1, 2 were significantly not different from each other (Table 15). The association was not significant between the SSR marker Xtxp247 the flowering dates and the resistant lesion type for $F_{2:3}$ and $F_{2:4}$ (Table 15).

Table 15. Single marker analysis of variance for simple sequence repeat Xtxp247 marker.

Allelic group	[a] AUDPC		[b] Resistant lesion type		Dates to 50% flowering	
	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$	$F_{2:3}$	$F_{2:4}$
0	12.9	6.33	64.3	61.9	73.8	73.5
1	11.5	6.75	68.6	62.3	74.1	73.9
2	11.5	6.65	58.3	55.4	73.6	74.0
LSD ($p \leq 0.01$)	1.28	0.54	13.7	13.2	1.53	1.47
F_{Prob}	+	ns	ns	ns	ns	ns
R^2	0.022	0.010	0.013	0.007	0.002	0.002
CV%	31.75	23.68	62.01	63.20	6.05	5.77

[a] = AUDPC computed as described by Campbell and Madden (1990).
[b] = Percentage number of plants showing resistant lesion type.
ns = Not significant; + = Significant at 0.1.

4.4   Discussion

The objective of this chapter was to develop and validate rapid amplified polymorphic DNA (RAPD) and simple sequence simple repeat (SSR) markers linked to the *Turcicum* leaf blight (TLB) resistance gene or (genes). This study involved screening eighty RAPD and ten SSR markers for polymorphism between the two distinct parents (MUC007/009, Resistant and Epuripuri, Susceptible). The validation of the RAPD markers result shows 8 RAPD markers (A4, A9, A16, A17, B6, B7, D1 and D18) can differentiate the two parents MUC007/009 (Resistant parent) and Epuripuri (Susceptible parent) and showed consistent polymorphic bands. Some of these polymorphic RAPDs amplified the resistant parent and others amplified the susceptible. These markers are recommended to be used for mapping resistance to sorghum *Turcicum* leaf blight.

Three polymorphic markers differentiated the two parents {MUC007/009 (Resistant parent) and Epuripuri (Susceptible parent)}. The three polymorphic SSR markers are distributed along the sixth linkage group of sorghum consensus map (Mace *et al.*, 2009). These markers can be used in mapping QTL for *Turcicum* leaf blight resistance in sorghum. The results from this chapter show that the genomic region flanked by plant colour locus (*Plcol*) and Xtxp95 marker to harbour the locus for sorghum *Turcicum* leaf blight lesion. The same genomic region flanked by sorghum colour locus and simple sequence repeat marker Xtxp95 harbour disease-response QTL for zonate leaf spot (ZLS), target leaf spot (TLS) and drechstera leaf blight (DLB) caused by fungal pathogens (Mohan *et al.*, 2009). This locus therefore appears to regulate resistance to many biotic stresses. The cluster of resistance loci especially for diseases has been reported in maize (Zwonitzer *et al.*, 2010) and rice (Wang *et al.*, 2009).

The F_2 individual lines carrying the allele from the susceptible parent at the polymorphic SSR loci were scored as 0, while those carrying the allele from the resistant parent were given a genotypic score of 2 and the segregating individuals carrying alleles from both parents (Heterozygote) were given a genotypic score of 1. The genotypic data for the SSR marker Xtxp95 fitted Mendel's 1:2:1 ratio, while the genotypic data for the SSR markers Xtxp57 and Xtxp247 did not fit significantly the 1:2:1 ratio. However marker Xtxp95 showed high significant association between the allelic groups and the resistant lesion type, while the SSR markers Xtxp57 and Xtxp247 did not show any significant association. The SSR marker Xtxp95 was linked to the resistant lesion type and the single marker analysis showed that 23.74% in $F_{2:3}$ and 14.09% in $F_{2:4}$ of the variability in the resistant lesion type is associated with the SSR marker Xtxp95 segregation.

The overall genomic region flanked by plant colour locus and simple sequence repeat marker Xtxp95 on the sixth linkage group harboured disease response QTL for some disease caused by fungal pathogen (Mohan *et al*., 2009). It is hypothesized that this region on the sixth linkage group could harbour a cluster of disease response loci to different pathogens as observed in the syntenic regions on rice chromosome 4 and maize chromosome 2 (Mohan *et al*., 2009). The information gained from this chapter can be used in deploying marker assisted selection for *Turcicum* leaf blight and map-based isolation of important disease resistant genes in sorghum.

CHAPTER FIVE

CONCLUSION, RECOMMENDATIONS AND FUTURE PROSPECTS

5.1   Conclusion

This study shows that the segregation of the *Turcicum* leaf blight is distributed normally suggested a quantitative inherited trait and the limited role of dominance effects under both greenhouse and field. This study shows that resistance in sorghum to *E. turcicum* is regulated by additive and epistatic effects. It also strengthens the role of environmental effects in the segregation and inheritance of *Turcicum* leaf blight. In this study the parents had distinctly different lesion types that were consistent under both greenhouse and field environments. It is noted that lesion type segregated according to a typical qualitatively inherited pattern. The SSR marker Xtxp95 was linked to the resistant lesion type and the single marker analysis showed that the segregation of this marker is associated with the resistant lesion type variability.

5.2   Recommendations

In this study, M007/009 was the source of *Turcicum* leaf blight resistance however it is possibly that the resistance source does exist within other varieties and land races. Therefore, there is need to evaluate and identify other sources of resistance under different environments. Also, there is need to investigate more on the linkage of *Turcicum* leaf blight resistance and the plant colour region on the sixth linkage group. Also in view of the potential benefits, it is recommended that further studies be conducted involving larger population size, additional markers near the plant colour region and a larger number of SSR markers well distributed throughout the genome from the synteny maps of maize and rice. The position of polymorphic SSR Xtxp95 marker can be used in

mapping the QTL for *Turcicum* leaf blight lesion type and possibly disease resistance in sorghum.

5.3   **Future prospective**

Introgression of targeted genomic regions with minimal linkage drag efficiently is the overall objective of any plant sorghum breeder. And to achieve this, there is need to characterise genomic regions beyond statistics such as QTL. The information gained from this study demonstrates the need for developing marker assisted selection for *Turcicum* leaf blight and map-based isolation of important disease resistant genes in sorghum. The study suggests the relationship between the resistant *Ht* genes in maize and the others in sorghum and also argued that the genes are rather a typical plant major resistance *Ht* genes and should be thought of as large-effect, race-specific QTL. This could be studied more by studying the gene expression of both sorghum and maize for these genes. And also as a transition from QTL analysis to gene discovery, there is need for focusing on identifying the candidate gene(s) underlying the mapped QTL. Advances in genomics, bioinformatics and proteomics offer opportunity to achieve this. For example the relationship between the plant colour and the *Turcicum* leaf blight resistance in sorghum needs more investigation using the functional genomics and gene expression.

REFERENCES

Abu Assar, A.H., Uptmoor, R., Abdelmula, A.A., Salih, M., Ordon, F. And Fried, W. 2005. Genetic variation inn sorghum germplasm from the Sudan, ICRISAT and USA assessed by simple sequence repeats. *Crop Science* **45:**1636-1644.

Adipala, E., Lipps, P.E. and Madden, L.V. 1993. Occurrence of *Exserohilum turcicum* on maize in Uganda. *Plant Disease* **77**:202-205.

Agrios, G.N. 1997. Plant pathology. Fourth Ed. New York: Academic Press.

Aguero, M.E., Gevens, A. and Nicholson, R.L. 2002. Interaction of *Cochliobolus heterostrophus* with phytoalexin inoclusions in *Sorghum bicolour*. *Physiological and Molecular Plant Pathology* **61**:267-271.

Arumuganathan, K. and Earle, E.D. 1991. Estimation of nuclear DNA content of plants by flow cytometry. *Plant Molecular Biology* **9:**229-233.

Awika, J.M. and Rooney, L.W. 2004. Sorghum phytochemicals and their potential aspects on human health. *Phytochemistry* **65**:1199-1221.

Babu, R., Nair, S.K., Prasanna, B.M. and Gupta, H.S. 2004. Integrating marker assisted selection in crop breeding - prospects and challenges. *Current Science* **87(5)**:607-619.

Balint Kurti, P.J. and Johal, G. 2009. Maize disease resistance. *Handbook of Maize* **1:**229-250.

Bennetzen, J.L., SanMiguel, P., Chen, M., Tikhonov, A., Francki M. and Avramova, Z. 1998. Grass genomes. *Natural Academic Science* **95**:1969-1970.

Bennetzen, J.L., Subramanian, V., Xu, J., Salimath, S.S., Subramanian, S., Bhattramakki, D. And Hart, G.E. 2000. A framework genetic map of sorghum containing RFLP, SSR and morphological markers. *In*: Philips, R.L. Vasil, I.L. (eds) DNA-based markers in plants, second edition. Klluwer cademic Puplishers, Boston.

Bergquist, R. 1986. Leaf blight. *In:* Compendium of Sorghum Diseases. Edited by Frederiksen. R.A.St. Paul, MN, USA: American phytopathological Society.

Bergquist, R.R. and Masias, O.R. 1974. Physiologic Specialisation in *Trichometasphaeria turcicum* f.sp. *zeae* and *T. turcicum* f. *Sp. sorghi* in Hawaii. *Phytopathology* **64**:645-649.

Bernardo, R. 2002. Breeding for quantitative traits in plants. First edition. United States of America. Stemma press.

Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: Learning from the last 20 years. *Crop Science* **48**:1649-1664.

Boora, K.S., Frederiksen, R.A. and Magill, C.W. 1999. A molecular marker that segregates with sorghum leaf blight resistance in one cross is maternally inherited in another. *Molecular and General Genetics* **261**:317-322.

Campbell, C.L. and Madden, L.V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.

Carrari, F., Frankel, N., Lijavetzky, D., Benech-Arnold, R., Sánchez, R. and Iusem, N.D. 2000. The TATA-less promoter of *VP1*, a plant gene controlling seed germination. *DNA Sequence* **12**:107-114.

Carson, M.L. 1995. A new gene in maize conferring the chlorotic halo reaction to infection by *Exserohilum turcicum*. *Plant Diseases* **79:**717-720.

Casela, C.R., Frederiksen, R.A. and Ferreira. A. S. 1993. Evidence for dilatory resistance to anthracnose in sorghum. *Plant Disease* **77:**908-911.

Charles, S., Girma, A., Christopher, M., Kiazzi, C., Elias, L. and Soares, X. 2006. Atlas of sorghum {*Sorghum bicolour* (L.) Moench}: Production in five countries in Africa. The

Board of Regents of the University of Nebraska on behalf of the University of Nebraska-Lincoln.

Childs, K.L., Klein, R.R., Klein, P.E., Morishige, D.T. and Mullet, J.E. 2001. Mapping genes on an integrated sorghum genetic and physical map using cDNA selection technology. *Plant Journal* **27:**243-255.

Chittenden, M.L., Schertz, K.F., Lin, Y.R., Wing, R.A. and Paterson, A.H. 1994. A detailed RFLP map of *Sorghum bicolour* and *S. Propinquum*, suitable for high density mapping, suggests ancestral duplication of Sorghum chromosomes or chromosomal segments. *Theoretical and Applied Genetics* **87:**925-933.

Crasta O.R., Xu, W.W., Nguyen, H.T., Rosenow, D.T. and Mullet, J. 1999. Mapping of post flowering drought resistance traits in grain sorghum: Association between QTLs influencing premature senescence and maturity. *Mol Gen. Genetics* **262**:579-588.

Davies, J.C. and Reddy, K. 1981. Shootfly species and their graminaceous hosts in Andhra Pradesh. *Insect Science and its Application* 33-37.

Degefu, Y. 1990. Evaluation of Ethiopian maize germplasms for resistance against leaf blight caused by *Helminthosporium turcicum*. Research Progress Report, Awassa College of Agriculture, *Addis Ababa University*.

DeVries, J. and Toeniessen, G. 2001. Securing the harvest Biotechnology, Breeding and seed system for Africa Crops. *CABI publishing*.

Dje, Y., Heuertz, M., Lefebvre, C. and Vekemans, X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theoretical and Applied Genetics* **100:**918-925.

70

Doebly, J., Durbin, M., Golenberg, E.M., Clegg M.T. and Ma., D.P. 1990. Evolutionary analysis of the large subunit of carboxylase (rbcl) nucleotide sequence among the grasses (Gramineae). *Evolution* **44**:1097-1108.

Doggett, H. 1988. Sorghum. Second edition. Longman Scientific and Technical, London.

Ebiyau, J. and Oryokot, O.E. 2001. Sorghum {*Sorghum bicolour* (L.) Moench}. Agriculture in Uganda. Volume II Crops: National Agricultural Research Organisation Fountain Publisher.

Edwards, K., Johnstone, C. and Thompson, C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acid Research* **19:**13-49.

Ejeta, G. 2006. The Striga Scourge in Africa: A growing pandemic? *In*: G. Ejeta and J. Gressel (eds.). Towards Ending the Witch-hunt: Proceedings of an International Symposium on Integrating New Technologies for Striga Control, 5-11 November, 2006, Addis Ababa, Ethiopia, World Scientifi c Publishing Company PTE LTD, Singapore .

Ejeta, G., Goldsbrough, P.B., Tuinstra, M.R., Grote, E.M., Menkir, A., Ibrahim, Y., Cisse, N., Weerasuriya, Y., Melake-Berhan, A. and Shaner, C.A. 2000. Molecular marker applications in sorghum. *In*: Haussmann, B.I.G., Geiger, H.H., Hash, C.T. and Bramel-Cox (eds). 2000. Application of molecula markers in plant breeding. Training manual for a seminar held in IITA, Ibadan, Nigeria, from 16-17 August 1999.

Esele, J.P. 1995. Foliar and head diseases of sorghum. *African Crop Science Journal* **3:**185-189.

Esele, J.P., Frederiksen, R.A. Miller, F.R. 1993. The association of genes controlling cayopsis traits with grain-mould resistance in sorghum. *Phytopathology* **83:**490-495.

Fallah, M.P. and Pataky, J.K. 1994. Reactions of isolates from matings of races 1 and 23N of *Exserohilum turcicum*. *Plant Disease* **78:**767-771.

FAOSTAT (Food and Agriculture Organization of the United Nations). 1995. Statistics Division 1995. Online available at http://faostat.fao.org.

----. 2003, FAOSTAT.

----. 2007, FAOSTAT.

----. 2008, FAOSTAT.

----. 2009, FAOSTAT.

Feltus, F.A., Hart, G.E., Schertz, K.F., Casa, A.M., Kresovich, S. and Abraham, S. 2006. Alignment of genetic maps and QTLs between inter-and intra- specific sorghum populations. *Theoretical and Applied Genetics* **112**:1295-1305.

Frederikson, R.A. 2000. Diseases and disease management in sorghum. *In*: Wayne Smith, C. and Frederikson, R.A. (eds), Sorghum: Origion, History, Technology and production, 497-534. John Wiley and Sons, New York.

Gale, M.D. and Devos, K.M. 1998. Plant comparative genetics after 10 years. *Science* **282:**656-659.

Gevers, H.O. 1975. A new major gene for resistance to *Helminthosporium turcicum* leaf blight in maize. *Plant Disease* **59:**296-299.

Gitau, C.W., Ngi-Song, A.J., Otieno, S.A. and Overholt, W.A. 2007. Host preference of *Xanthopimpla stemmator* (Hymenoptera: Ichneumonidae) and its reproductive performance on selected African lepidopteran stem borers. *Biocontrol Science and Technology* **17(5):** 499-511.

Grenier, C., Bramel, P.J., Dahlberg, J.A., El-Ahmadi, A., Mahmoud, M., Peterson, G.C., Rosenow, D.T. and Ejeta, G. 2004. Sorghums of the Sudan: analysis of regional diversity and distribution. *Genetic Resources and Crop Evolution* **51(5):**489-500.

Hakiza, J. J. 1993. Quantitative analysis and heritability of host resistance to *Exserohilum turcicum* in maize. (Dissertation). *The Ohio State University*.

Haussmann, B.I.G., Hess, D.E. and Omanya, G.O. 2004. Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. *Theoretical and Applied Genetics* **109(5):**1005-1016.

Haussmann, B.I.G., Mahalakshmi, V., Reddy, B.V.S., Seetharama, N., Hash, C.T. and Geiger, H.H. 2002. QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theoretical and Applied Genetics* **106:**133-142.

Hennessy, G.G., de Milliano W.A.J. and McLaren C.G. 1990. Influence of primary weather variables on sorghum leaf blight severity in Southern Africa. *Phytopathology* **80:**943-5.

Hilu, H.M. and Hooker, A.L. 1963. Monogenic chlorotic lesion resistance to *Helminthosporium turcicum* in corn seedlings. *Phytopathology* **53**:909-912.

Hooker, A.L. 1963. Mongenic resistance in *Zea mays* (L.) to *Helminthosporium turcicum*. *Crop Science* **3:**381-383.

Hooker, A.L. 1977. A second major gene locus in corn for chlorotic lesion resistance to *Helminthosporium turcicum*. *Crop Science* **17:**132-135.

Hooker, A.L. 1978. Additional sources of monogenic resistance in corn to *Helminthosporium turcicum*. *Crop Science* **18:**787-788.

Hooker, A.L. 1981. Resistance to *Helminthosporium turcicum* from *Tripsacum floridanum* incorporated into corn. *Maize Genetics Cooperation Newsletter* **55:**87-88

Hooker, A.L. and Kim, S.K. 1973. Monogenic and multigenic resistance to *Helminthosporium turcicum* in corn. *Plant Disease Reporter* **57:**586-589.

http://images.google.com/. Accessed on 2nd of March, 2009. http://maizedoctor.cimmyt.org/. Accessed 7th of March, 2009.

Hughes, G.R. and Hooker, A.L. 1971. Gene action conditioning resistance to northern leaf blight in Maize. *Crop Science* **11**:180-184.

Ibrahim, N.E. and Abbas, E. M. 2006. experience of sorghum and millet production in Africa. A paper presented in eastern and central Africa regional sorghum and millet network of ASARECA (ECARSAM), Machakos, Kenya, 24th - 28th July 2006.

Islam-Faridi, M.N., Childs, K.L., Klein, P.E., Hodnett, G. and Menz, M.A. 2002. A molecular cytogenetic map of sorghum chromosome: fluorescence in situ hybridization analysis with mapped bacterial artificial chromosomes. *Genetics* **161:**345-353.

Jenkins, M.T. and Robert, A.L. 1952. Inheritance of resistance to leaf blight of corn caused by *Helminthosporium turcicum*. *Agronomy* **44**:136-140.

Julian, A.M., Crowson, H.L. and de Milliano, W.J. 1994. Factors influencing epidemiology of *Exserohilum turcicum* on sorghum. *African Crop Science Conference proceedings* **1:**263-268.

Kfir, R. 1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America* **90**:619-624.

Kim, J.S., Childs, K.L., Islam-Faridi, M.N., Menz, M.A. and Klein. R.R. 2002. Integrated karyotyping of sorghum by *in situ* hybridization of landed BACs. *Genome* **45:**402-412.

Kimber, C.T. 2000. Origins of domesticated sorghum and its early diffusion to India and China. p. 3-98. *In*: Smith *et al.* (ed.) Sorghum: Origin, history, technology, and production. John Wiley & Sons, Inc., New York.

King, S.B. and Mukuru, S.Z. 1994. An overview of sorghum finger millet and pearl millet in Eastern Africa with special attention to diseases. Edited by D.D.L., Breeding for disease resistance with emphasis on durability Wageningen, Netherlands: *Wageningen Agricultural University.*

Klein, P.E., Klein, R.R., Cartinhour, S.W., Ulanch, P.E. and Dong, J. 2000 A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. *Genome Research* **10:**789–807.

Klein, P.E., Klein, R.R., Rebalov, J.V. and Mullet, J.E. 2003. Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 reveals extensive conservation of gene order and one major chromosomal rearrangement. *Plant Journal* **34:**605-621.

Klein, R.R., Klein, P.E., Mullet, J., Minx, P., Rooney, W.L. and Schertz, K.F. 2005. Fertility restorer locus Rf 1 of sorghum (*Sorghum bicolour* L.) encodes a pentatricopeptide repeat protein not present in the colinear region of rice chromosome 12. *Theoretical and Applied Genetics* **111**:994-1012.

Klein, R.R., Rodriguez-Herrera, R., Schlueter, J.A., Klein, P.E., Yu, Z.H. and Rooney, W.L. 2001. Identification of genomic regions that affect grain-mould incidence and other traits of agronomic importance in sorghum. *Theoretical and Applied Genetics* **102**:307-319.

Kong, L., Dong, J. and Hart, G.E. 2000. Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolour* (L.) Moench DNA simple-sequence repeats (SSRs). *Theoretical and Applied Genetics* **101**:438-448.

Kucharek, T. 2000**.** Foliar and Head Diseases of Sorghum in Florida. Volume 1073, Circular (Florida Cooperation Extension Service). Institute of Food and Agricultural Sciences, University of Florida, 1992.

Kudadjie, C.Y., Struik, P.C. Richards, P. and Offei, S.K. 2004. Assessing production constraints, management and use of sorghum diversity in north-east Ghana: a diagnostic study. *Wageningen Journal of Life Science* **3(4):**371-391.

Lamb. C.J., Lawton, M.A., Dron, M. and Dixon, R.A. 1989. Signal transduction mechanisms for activation of plant defenses against microbial attack. *Cell* **56**:215-224.

Leath, S., Thakur, R.P. and Leonard K.J. 1990. Variation in expression of monogenic resistance to *Exerohilum turcicum* race 3 under different temperature and light conditions. Phytopathology **80:**309-313.

Leonard, K.J., Levy, Y. and Smith, D.R. 1989. Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. *Plant disease* **73**:776-777.

Levy, Y. 1989. Analysis of epidemics of northern leaf blight on sweet corn in Israel. *The American Phytopathological Society* **79(11)**:1243-1245.

Lijavetzky, D., Muzzi, G., Wicker, T., Keller, B. and Wing R. 2000. Construction and characterisation of a bacterial artificial chromosome (BAC) library for a genome of wheat. *Genome* **42**:1176-1182.

Lipps, P.E., Pratt, R.C. and Hakiza, J.J. 1997. Interaction of *Ht* and partial resistance to *Exserohilum turcicum* in maize. *Plant Disease* **81**:277-282.

MAAIF. 2007. Basic facts on agricultural activities in Uganda: Sorghum production levels.

Mace, E.S., Rami, J-F., Bouchet, S., Klein, P.E., Klein, R.R., Kilian, A., Wenzl, P., Xia, L., Halloran, K. and Jordan, D.R. 2009. A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. *BMC Plant Biology* **9**:13.

Mbeyagala, K.E. 2010. Genetic diversity among sorghum {*Sorghum bicolour* (L.) Moench} landraces in Ugnada. (Dissertation). *Makerere University* P. 40-45.

Menz, M.A., Klein, R.R., Mullet, J.E., Obert, J.A., Unruth, N.C. and Klein, P.E. 2002. A high-density genetic map of *Sorghum bicolour* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. *Plant Molecular Biology* **48**:483-499.

Mittal, M. and Boora, K.S. 2005. Molecular tagging of gene conferring leaf blight resistance using microsatellites in sorghum {*Sorghum bicolour* (L.) Moench}. *Indian Journal of Experimental Biology* **43(5)**:462-466.

Mohan, S.M., Madhusudhana, R., Mathur, K., Howarth, C.J., Srinivas, G., Satish, K., Reddy, R.N. and Seetharama, N. 2009. Co-localization of quantitative trait loci for foliar disease resistance in sorghum. *Plant Breeding* **128**:532-535.

Mullet, J.E., Klein, R.R. and Klein P.E. 2001. *Sorghum bicolour* - An Important Species for Comparative Grass Genomics and a Source of Beneficial Genes for Agriculture. *Current Opinion in Plant Biology* **5**:118-121.

Nagaraj, N., Reese, J.C. and Tuinstra, M.R. 2001. Molecular mapping of sorghum genes expressing tolerance to damage by greenbug (Homoptera: Aphididae). *Journal of Economic Entomology* **98(2)**:595-602.

Narro, J., Betancourt, V.A. and Aguirre, J.L. 1992. Sorghum diseases in Mexico. *In*: de Milliano W.A.J., Frederiksen R.A., Bengston G.D. (eds) Sorghum and millet diseases: a second world review. ICRISAT, Pantancheru Andhra Pradash, India, p. 75-84.

Ngugi, H.K., Julian, A.M. and Peacocke B.J. 2000. Epidemiology of sorghum anthracnose (*Colletotrichum sublineolum*) and leaf blight (*Exserohilum turcicum*) in Kenya. *Plant Pathology* **49**:129-140.

Nicholson, R.L., Kollipara, S.S., Vincent, J.R., Lyons, P.C. and Cadena-Gomez, G. 1987. Phytoalexin synthesis by the sorghum mesocotyl in response to infection by pathogenic and nonpathogenic fungi. *National Academy of Science* **84**:5520-5524.

Nkonya, E., Xavery, E.P., Akonaay, H., Mwangi, W., Anandajasekeram, P., Verkuijl, H., Martella, D. and Moshi, A. 1998. Adoption of maize production technologies in Northern Tanzania. Mexico D.F.: International Maize and Wheat Improvement Center (CIMMYT). The United Republic of Tanzania and the Southern African Center for Cooperation in Agricultural Research (SACCAR).

Nobel, M. and Richardson, M.J. 1968. Annotated list of seed-borne diseases. Proceedings of International Seed Testing Association No. 33. Zurich, Switzerland: ISTA.

Obi, I.U. 1979. Additional phytoalexin-like compounds in *Ht-gene* resistance of corn to *Helminthosporium turcicum. Annals of Applied Biology* **92(3):**377-381.

Ogliaril, J.B., Guimarães, M.A. and Camargo, L.E.A. 2007. Chromosomal locations of the maize (*Zea mays* L.) *HtP* and *rt* genes that confer resistance to *Exserohilum turcicum. Genetics and Molecular Biology* **30(3)**:630-634

Olujong, H.F., Adipala, E. and Rubaihayo, P.R. 1996. Diallel analysis for reaction to *Exserohilum turcicum* of Maize cultivars and crosses. *African Crop Science* **4**:19-27.

Paterson, A.H. 2008. Genomics of sorghum. International Journal of Plant Genomics Volume 2008, Article ID 36245. Doi: 10.1155/2008/362451.

Peterson, T.C., Golubev, V.S. and Groisman, P. 1995. Evaporation losing its strength. *Nature* **377**: 687-688.

Pratt, **R.C., Lipps, E.P., Ssango, F., Hakiza, J.J. and Adipala, E.** 1997. Inheritance of race - nonspecific resistance to *Exerohilum turcicum* in maize synthetic population OhS10. *African Crop Science Journal* **5(1)**:55-63.

Price, H.J. Dillon, S.L. Hodnett, G., Rooney, W.L., Ross, L. and Johnston, J.S. 2005. Genome evolution in the genus *Sorghum* (Poaceae). *Annals of Botany* **95(1)**:219-227.

Quellar, D.C., Strassmann, J.E. and Hughes, C.R. 1993. Microsatellites and kinship. *Trends in Ecology and Evolution* **8**:285-288.

Ramathani, I. 2010. Characterisation of *Turcicum* leaf blight epidemics and pathogen populations in the *Exserohilum turcicum* - sorghum pathosystem of Uganda. (Dissertation). *University of Makerere* p. 88-92.

Raymundo, A.D. and Hooker, A.L. 1982. Single and combined effects of monogenic and polygenic resistance on certain components of northern leaf blight development. *Phytopathology* **72**:99-103.

Richards, R. and Kucharek, T. 2006. Florida plant disease management guide. *University of Florida.*

Robert, A.L. 1960. Physiologic specialisation in *Helminthosporium turcicum. Phytopathology* **50**:599-601.

Rooney W.L. and Klein R.R. 2000. Potential of marker assisted selection for improving grain mold resitance in sorghum. Pages 183-194. *In* Technical and institutional options for

sorghum grain mold management: proceedings of an international consultation, 18-19 May 2000, ICRISAT, Patancheru, India.

Sax, K. 1923. The association of size diff erences with seed-coat pattern and pigmentation in *Phaseolus vulgaris. Genetics* **8**:552-560.

Scheinost, P.L., Lammer, D.L., Cai, X., Murray, T.D. and Jones, S.S. 2001. Perennial wheat: the development of a sustainable cropping system for the U.S. Pacific Northwest. *American Journal of Alternative Agriculture* **16(4)**:147-151.

Schierwater, B. and Ender, A. 1993. Different thermostable DNA polymerases may amplify different amplification patterns. *Nucleic Acids Research* **21**:4647-4648.

Sharma, H.C., Dhillon, M., Pampapathy, G. and Reddy, B. 2007. Inheritance of resistance to spotted stem borer, *Chilo partellus*, in sorghum, *Sorghum bicolour. Euphytica* **156:**117-128.

Simcox, K.D., Pedersen, K.D. and Nickrent, D.L. 1993. Isozyme diversity in *Setosphaeria turcica. Canadian journal of plant pathology* **15**:91-96.

Steel, R.G.D and Torrie, J.H. 1997. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York.

Swigonova, Z., Lai, J., Ma., J., Ramakrishna, W., Llaca, V., Benne**tzen, J.L. and Messing, J.** 2004. Close split of sorghum and maize genome progenitors. *Genome Research* **14:**1916-1923

Tao, Y.Z., Hardy, A., Drenth, J., Henzell, R.G., Franzmann, B.A., Jordan, D.R., Butler, D.G. and McIntyre, C.L. 2003. Identification of two different mechanisms for sorghum midge resistance through QTL mapping. *Theoretical and Applied Genetics* **107:**116-122.

Tao, Y.Z., Jordan, D.R., Henzell, R.G. and McIntyre, C.L. 1998. Construction of a genetic map in a sorghum recombinant inbred line using probes from different sources and its

comparison with other sorghum maps. *Australian Journal of Agricultural Research* **49**:729-736.

Tarumoto, I., Isawa, K. And Watanabe, K. 1977. Inheritance of leaf blight resistance in sorghum-sudangrass and sorghum-sorghum hybrids. *Japan Journal of Breeding* **27(3)**:216-222.

Tilahun, T., Ayana, G., Abebe, F. and Wegary, D. 2001. Maize pathology research in Ethiopia. Edited by N. Mandefro, D. Tanner and S. Twumasi-Afriyie, Enhancing the contribution of maize to food security in Ethiopia. Proceedings of the second National workshop of Ethiopia. Addis Ababa, Ethiopia.

Torres-Montalvo, H., Mendoza-Onofre, L., Gonzalez-Hernandez, V. and Williams-Alanis, H. 1992. Reaction of tan and non-tan isogenic genotypes to head blight. *International Sorghum Millets Newsletter* **33**:36.

Trudy, F.C., Mackay, E.A.S. and Julien F.A. 2009. The genetics of quantitative traits: challenges and prospects. *Genetics* **10:**566-576.

Vanderlip, R.L. 1993. How a sorghum plant develops. Volume 1203, *Cooperative extension service*. Manhattan, Kansas: Kansas Agricultural Experiment Station.

Wang, M.L., Dean, R., Erpelding, J. and Pederson, G. 2006. Molecular genetic evaluation of sorghum germplasm differing in response to fungal diseases: rust (*Puccinia purpurea*) and anthracnose (*Collectotrichum graminicola*). *Euphytica* **148(3)**:319-330.

Wang, L., Xu, X., Lin, F. and Pan, Q. 2009. Characterisation of Rice Blast Resistance Genes in the *Pik* Cluster and Fine Mapping of the *Pik-p* Locus. *Genetics and Resistance* **99(8):**900-905.

Welz, H.G. and Geiger, H.H. 2000. Genes for resistance to Northern corn leaf blight in diverse maize populations. *Plant Breeding* **119**:1-14.

Windes, J.M. and Pedersen, W.L. 1991. An isolate of *Exserohilum turcicum* virulent on maize inbreeds with resistance gene *HtN*. *Plant disease* 75:430.

Xu, G.W., Magill, C.W., Schertz, K.F. and Hart. G.E. 1994. A RFLP linkage map of *Sorghum bicolour* (L) Moench. *Theoretical and Applied Genetics* **89**:139-145.

Yeshitila, D. 2003. Cloning and characterisation of Xylanase genes from phytopathogenic fungi with a special reference to *Helminthosporium turcicum* the cause of Northern Leaf Blight of maize. (Dissertation). *University of Helsinki* p. 37-38.

Yu, J., Pressoir, G., Briggs, W.H., Vroh Bi, I., Yamasaki, M., Doebley, J.F., McMullen, M.D., Gaut, B.S., Nielsen, D., Holland, J.B., Kresovich, S. and Buckler, E.S. 2006. A unified mixedmodel method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* **38**:203-208.

Zwonitzer, J.C., Coles, N.D., Krakowsky, M.D., Arellano, C., Holland, J.B., McMullen, M.D., Pratt, R.C., and Balint-Kurti, P.J. 2010. Mapping resistance quantitative trait loci for three foliar diseases in a maize recombinant inbred line population—evidence for multiple disease resistance*?*. *Phytopathology* **100:**72-79.

APPENDICES

Appendix 1. Means of area under disease progress curve of TLB, flowering dates and resistant lesion type on F_2, $F_{2:3}$ and $F_{2:4}$ evaluated at MUARIK under greenhouse and field conditions during the first and the second rains of 2009 and the first rains of 2010.

F_2 code	$F_{2:3}$ & $F_{2:4}$ code	F_2 Population		$F_{2:3}$ population			$F_{2:4}$ population		
		AUDPC	Flowering date	AUDPC	Flowering date	N lesion type	AUDPC	Flowering Dates	N Lesion Type
19	A1-1	4.3	81	13.8	76	75	10.3	74	14
29	A1-10	9.3	81	7.4	76	0	7.4	75	0
30	A1-11	21.9	67	19.1	76	43	4.8	71	23
20	A1-2	4.8	81	15.3	78	67	5.9	74	1
21	A1-3	8.9	71	6.1	71	100	8.3	75	89
22	A1-4	8.5	63	7.4	76	80	6.8	74	45
23	A1-5	27.6	69	8.7	76	100	4.5	70	99
25	A1-6	8.0	66	10.8	76	0	5.9	86	2
26	A1-7	5.5	71	7.3	74	0	6.5	71	0
27	A1-8	3.9	71	14.0	76	0	6.5	70	0
28	A1-9	2.0	69	14.9	91	60	8.4	82	92
104	A2-1	8.4	62	37.9	74	88	6.9	80	72
113	A2-10	2.6	68	8.2	69	100	8.4	70	90
115	A2-11	6.3	100	14.6	69	71	6.7	75	45
105	A2-2	12.8	60	16.6	71	78	5.6	73	70
106	A2-3	9.8	68	17.1	71	71	9.6	68	93
107	A2-4	7.5	75	11.2	71	0	4.7	74	0
108	A2-5	9.7	69	13.9	75	100	6.1	80	102
109	A2-6	2.1	62	6.7	87	73	5.7	79	50
110	A2-7	3.8	57	7.0	82	0	5.1	88	0
111	A2-8	4.8	66	7.2	71	88	3.9	79	100
202	A3-1	10.5	70	13.6	81	100	7.9	78	93
211	A3-10	3.4	62	16.1	67	71	3.6	78	67

83

213	A3-11	3.5	69	14.7	69	100	7.7	68	78
203	A3-2	11.3	74	13.1	71	0	5.7	77	0
204	A3-3	8.5	86	12.8	69	78	6.1	69	93
206	A3-5	9.4	67	15.3	64	0	6.1	72	0
207	A3-6	5.3	66	16.0	69	0	7.2	73	0
208	A3-7	8.8	69	11.6	68	75	3.5	81	63
210	A3-9	8.8	100	11.0	66	0	6.0	75	0
315	A4-1	8.5	75	17.7	71	100	5.8	78	91
316	A4-2	5.3	81	14.3	76	100	6.5	73	102
317	A4-3	8.5	66	13.2	72	100	7.3	73	82
318	A4-4	8.5	56	14.2	69	0	6.7	73	48
319	A4-5	12.1	56	10.7	69	100	5.5	73	97
320	A4-6	17.5	59	11.5	70	0	5.9	77	0
321	A4-7	17.1	68	12.7	71	80	6.0	73	43
31	B1-1	10.3	81	11.2	71	0	9.4	72	0
40	B1-10	9.8	63	12.2	80	70	5.9	75	96
41	B1-11	13.8	67	13.8	76	0	6.4	66	0
32	B1-2	27.9	76	8.6	71	75	4.0	80	66
34	B1-4	30.6	84	10.4	69	100	7.2	70	80
35	B1-5	17.5	69	8.2	71	0	5.9	65	1
36	B1-6	13.8	66	10.5	71	78	6.0	70	48
37	B1-7	18.8	85	7.6	74	100	10.8	76	100
38	B1-8	2.0	69	13.4	76	100	6.7	75	56
116	B2-1	6.9	66	9.0	71	0	6.5	72	41
128	B2-10	51.9	87	10.3	75	100	5.9	79	98
145	B2-11	0.6	62	12.6	69	100	6.5	77	92
118	B2-2	9.1	100	13.7	76	67	7.1	77	46
120	B2-3	6.8	61	18.5	74	86	6.4	69	104
123	B2-4	16.0	Died	11.1	78	40	7.3	74	69
124	B2-5	24.6	67	12.9	76	71	8.2	71	69
126	B2-6	17.5	56	9.3	71	83	6.2	72	52
119	B2-7	11.4	Died	16.6	76	75	6.5	76	80

122	B2-8	25.6	56	12.2	74	86	3.9	67	100
127	B2-9	28.1	68	9.8	74	0	7.0	74	48
214	B3-1	7.3	60	13.9	70	100	6.6	77	100
224	B3-11	12.2	56	10.7	66	86	8.1	77	65
215	B3-2	8.0	58	14.2	68	67	6.9	73	72
216	B3-3	7.0	56	15.8	76	0	5.0	79	70
217	B3-4	10.0	67	16.4	70	100	8.4	69	80
218	B3-5	12.8	81	18.9	65	100	6.9	74	79
219	B3-6	10.5	66	18.6	74	100	3.2	80	50
222	B3-9	10.6	56	13.4	69	100	7.1	75	50
322	B4-1	5.3	78	14.1	64	100	14.7	70	100
323	B4-2	8.8	68	12.6	71	75	7.4	77	98
324	B4-3	4.5	66	14.3	65	75	8.1	73	89
325	B4-4	2.0	65	11.6	75	80	8.3	70	85
326	B4-5	7.1	59	11.0	71	0	7.7	70	2
327	B4-6	12.0	100	14.5	69	0	Died	Died	Died
328	B4-7	4.1	74	24.3	69	100	5.4	75	64
43	C1-1	17.5	71	9.1	73	91	12.1	73	99
53	C1-10	14.8	66	10.8	87	71	7.6	75	66
54	C1-11	6.1	63	9.7	76	29	7.9	77	0
44	C1-2	4.0	73	7.1	73	73	7.3	74	56
45	C1-3	2.1	81	8.3	73	100	6.9	72	100
46	C1-4	4.9	73	8.2	76	89	9.0	71	84
47	C1-5	3.6	89	6.8	76	100	6.0	76	93
48	C1-6	5.3	71	9.7	73	0	7.1	70	2
50	C1-7	10.5	66	9.2	76	78	7.2	77	82
51	C1-8	5.4	66	9.1	76	100	6.9	78	98
52	C1-9	9.3	63	15.9	87	0	6.1	80	12
156	C2-10	11.4	59	Missing	Missing	Missing	5.4	73	63
148	C2-3	2.3	66	14.6	76	0	7.7	85	0
150	C2-4	4.0	56	18.4	75	80	5.8	78	64
151	C2-5	10.4	59	16.0	75	86	5.8	84	69

152	C2-6	12.3	73	11.8	75	0	10.2	69	0
153	C2-7	17.5	66	12.1	75	67	7.9	73	74
155	C2-9	11.5	66	Missing	Missing	Missing	5.1	73	75
225	C3-1	21.1	56	9.2	69	0	8.6	69	0
234	C3-10	5.1	80	11.8	79	86	6.6	68	96
235	C3-11	4.1	67	10.8	65	100	6.4	74	92
226	C3-2	12.3	67	13.0	75	0	4.6	74	25
227	C3-3	11.9	61	14.6	75	100	7.4	75	84
228	C3-4	12.3	62	13.6	75	100	6.1	73	43
229	C3-5	16.9	61	13.2	75	0	6.6	72	0
230	C3-6	12.0	62	11.1	66	100	6.1	75	81
231	C3-7	12.5	81	9.7	75	100	6.5	68	97
232	C3-8	7.8	59	9.6	71	100	7.6	75	99
233	C3-9	6.2	75	11.2	72	0	6.6	76	0
332	C4-4	8.2	65	14.4	69	0	5.9	82	100
333	C4-5	7.6	100	14.5	70	71	8.8	75	88
334	C4-6	0.8	78	17.0	71	100	4.9	76	43
335	C4-7	1.9	62	21.0	76	100	6.5	70	99
55	D1-1	5.8	62	9.3	76	86	7.2	78	62
64	D1-10	9.4	62	10.5	78	56	8.4	70	88
65	D1-11	22.5	62	12.0	78	100	5.5	75	100
56	D1-2	13.1	81	12.4	73	100	5.8	77	74
57	D1-3	12.8	86	8.5	76	67	6.4	69	100
58	D1-4	9.9	66	7.7	69	100	9.0	68	104
59	D1-5	17.5	69	7.0	76	0	5.9	76	0
60	D1-6	12.3	62	7.5	73	86	4.9	70	60
61	D1-7	12.3	66	9.6	76	88	6.1	82	24
62	D1-8	16.9	68	7.6	73	0	5.8	73	5
63	D1-9	12.3	65	8.5	76	100	7.2	68	101
158	D2-1	15.4	61	12.6	76	0	5.8	71	0
167	D2-10	4.0	56	12.9	69	100	9.2	75	100
168	D2-11	8.5	89	11.1	78	86	6.8	71	58

159	D2-2	31.3	56	14.9	76	88	7.3	75	92
160	D2-3	18.3	66	14.7	73	60	5.9	69	94
161	D2-4	33.1	62	12.3	73	100	5.0	71	100
162	D2-5	9.8	56	12.2	73	100	8.2	68	100
163	D2-6	12.8	56	12.1	73	80	Died	Died	Died
164	D2-7	20.4	56	10.3	78	100	7.1	73	95
165	D2-8	19.1	81	9.6	76	100	8.5	70	99
166	D2-9	14.5	73	11.1	69	100	7.5	71	70
246	D3-11	4.8	59	14.1	71	100	6.8	77	84
237	D3-2	7.8	68	13.5	69	0	5.2	67	2
238	D3-3	4.9	60	14.0	67	86	Died	Died	Died
240	D3-5	5.4	62	14.7	64	67	7.2	68	0
242	D3-7	13.4	100	21.7	71	100	5.4	69	4
243	D3-8	6.6	66	12.4	69	100	6.4	72	74
244	D3-9	2.9	61	13.2	73	100	6.5	78	100
353	D4-2	8.6	64	7.2	73	75	6.6	71	69
355	D4-3	12.5	62	11.1	73	0	5.6	75	0
356	D4-5	9.1	62	20.9	71	100	7.0	74	100
358	D4-6	21.0	69	17.5	73	100	5.4	87	72
359	D4-7	5.1	71	15.2	74	100	5.6	73	90
66	E1-1	11.4	66	8.5	73	70	6.9	70	33
76	E1-10	10.3	54	15.3	87	0	3.6	80	50
77	E1-11	11.5	57	9.2	87	100	5.9	77	99
67	E1-2	18.1	69	12.0	76	100	3.5	75	90
68	E1-3	11.4	100	12.9	73	0	8.1	72	0
69	E1-4	8.3	62	16.2	73	88	5.3	72	44
71	E1-5	9.4	65	15.0	73	40	6.6	75	75
72	E1-6	37.3	62	13.9	73	100	5.8	71	102
73	E1-7	13.5	62	10.8	76	78	5.5	81	47
74	E1-8	8.1	66	10.9	76	0	7.7	75	0
75	E1-9	3.5	67	12.1	87	88	6.4	76	100
169	E2-1	21.1	100	7.0	73	86	6.3	70	59

179	E2-11	1.9	56	8.0	78	100	6.6	74	94
170	E2-2	21.1	71	7.9	76	100	5.9	70	102
171	E2-3	8.8	78	8.6	78	80	4.9	79	54
173	E2-5	5.1	63	9.7	78	86	7.4	69	32
174	E2-6	4.5	67	8.1	78	100	4.0	78	102
175	E2-7	3.7	73	7.7	73	100	9.9	74	95
176	E2-8	1.9	61	13.4	73	100	4.5	73	50
247	E3-1	20.0	75	12.2	76	0	6.0	69	0
257	E3-10	19.6	59	14.6	73	100	Died	Died	Died
258	E3-11	13.3	62	11.8	69	0	Died	Died	Died
248	E3-2	21.3	68	12.0	78	86	7.5	76	34
250	E3-3	10.8	62	15.0	78	0	7.5	77	23
251	E3-4	18.8	Died	15.5	73	86	5.2	75	83
252	E3-5	16.8	71	Missing	69	83	5.5	72	80
254	E3-7	22.8	56	16.5	73	67	5.7	71	100
255	E3-8	42.3	56	13.7	73	100	4.4	77	100
256	E3-9	16.0	65	14.0	73	100	4.2	72	100
361	E4-2	12.5	68	13.0	73	0	5.6	71	3
363	E4-4	9.0	81	16.0	73	100	4.2	73	100
365	E4-6	8.8	76	18.9	78	60	6.0	79	56
367	E4-7	1.4	81	Missing	Missing	Missing	5.9	76	0
78	F1-1	7.8	69	7.9	76	30	8.1	72	43
88	F1-10	8.6	59	8.8	73	71	6.0	70	87
79	F1-2	2.6	55	6.2	76	100	4.0	79	75
80	F1-3	2.0	68	10.3	76	88	5.7	69	75
82	F1-4	3.0	67	7.2	69	78	7.6	70	45
83	F1-5	6.0	67	6.8	73	0	5.9	76	40
84	F1-6	2.9	62	8.9	73	0	6.0	73	57
85	F1-7	7.3	62	8.2	73	100	5.1	76	93
86	F1-8	10.3	65	9.4	76	71	5.6	71	87
87	F1-9	14.5	62	8.4	73	60	7.3	70	73
180	F2-1	12.9	74	15.7	76	100	7.9	72	99

190	F2-11	7.1	61	8.2	78	50	7.2	74	92
181	F2-2	6.6	69	11.1	73	0	5.7	74	0
183	F2-4	10.3	73	8.6	73	100	6.8	74	100
185	F2-6	8.4	56	13.1	87	71	8.8	73	99
187	F2-8	22.3	56	9.5	76	89	7.4	74	100
188	F2-9	29.8	66	8.3	73	100	5.1	68	99
259	F3-1	14.0	69	11.9	76	80	5.6	77	69
268	F3-10	16.6	100	12.8	69	0	7.6	71	0
270	F3-11	3.5	89	8.8	73	100	3.7	74	99
260	F3-2	16.9	81	10.1	87	100	2.2	84	100
261	F3-3	12.1	87	9.6	76	100	8.2	69	92
262	F3-4	4.4	69	9.7	81	0	9.1	79	50
263	F3-5	5.6	81	8.5	65	43	5.0	78	80
265	F3-7	5.8	68	16.7	73	0	6.6	82	0
266	F3-8	10.6	62	15.6	73	100	9.1	78	100
267	F3-9	9.6	68	13.2	69	0	7.0	71	0
374	F4-2	5.3	73	16.1	87	100	Died	Died	Died
375	F4-3	12.5	68	13.7	81	100	Died	Died	Died
376	F4-4	13.9	85	14.7	73	86	6.8	76	0
377	F4-5	18.9	66	12.3	81	100	7.3	75	64
378	F4-6	20.7	64	13.8	75	67	7.3	75	75
379	F4-7	19.1	100	14.8	73	100	4.5	75	100
90	g1-1	42.1	85	9.3	73	0	8.8	67	2
100	g1-10	12.5	60	12.0	76	0	8.5	74	0
102	g1-11	13.8	64	9.7	78	100	5.9	73	86
91	g1-2	16.9	89	8.9	73	63	7.9	72	60
92	g1-3	11.6	85	8.0	76	50	4.9	77	0
93	g1-4	5.3	89	10.4	73	100	6.3	78	100
94	g1-5	11.6	62	9.3	73	64	5.3	71	52
95	g1-6	17.5	59	9.5	73	0	6.4	75	6
96	g1-7	25.6	59	7.5	76	90	6.5	70	54
97	g1-8	26.4	100	7.6	73	0	7.3	74	0

98	g1-9	27.9	59	7.1	76	100	5.0	73	102
191	g2-1	3.5	56	7.6	76	100	8.2	70	100
200	g2-10	17.5	70	8.4	73	86	6.2	77	94
201	g2-11	11.6	75	6.7	69	0	7.9	75	8
192	g2-2	11.8	85	6.0	78	100	9.1	71	100
193	g2-3	14.8	56	7.0	69	0	7.4	75	0
194	g2-4	14.0	61	9.6	73	0	6.3	75	0
195	g2-5	18.4	62	9.5	76	100	6.2	77	100
196	g2-6	11.5	70	6.6	79	78	6.9	68	89
198	g2-8	28.9	67	8.9	78	75	6.9	66	79
199	g2-9	8.5	61	6.7	75	67	7.1	77	9
271	g3-1	9.4		8.8	76	100	5.0	78	100
313	g3-10	8.1	75	13.2	73	60	5.9	79	100
314	g3-11	16.9	81	14.0	70	100	7.1	76	100
305	g3-2	4.9	80	11.3	73	86	7.6	72	99
306	g3-3	4.5	80	11.7	73	25	6.4	70	25
307	g3-4	2.3	77	11.1	65	100	7.0	77	100
308	g3-5	4.8	66	9.6	76	100	8.0	79	81
309	g3-6	5.1	56	10.9	73	25	7.5	72	9
310	g3-7	3.4	66	10.2	73	0	4.4	74	1
311	g3-8	5.6	81	8.6	76	0	6.6	77	0
312	g3-9	7.3	56	14.4	73	20	5.2	76	50
380	g4-1	12.6	72	12.1	69	0	8.1	68	0
381	g4-2	9.6	71	12.1	75	67	5.7	79	50
382	g4-3	10.2	64	10.1	76	100	7.6	83	83
383	g4-4	11.3	100	10.1	73	71	9.7	90	61
384	g4-5	13.3	100	10.5	73	100	4.4	83	83

Appendix 2. Sorghum consensus map (Mace *et al*., 2009).
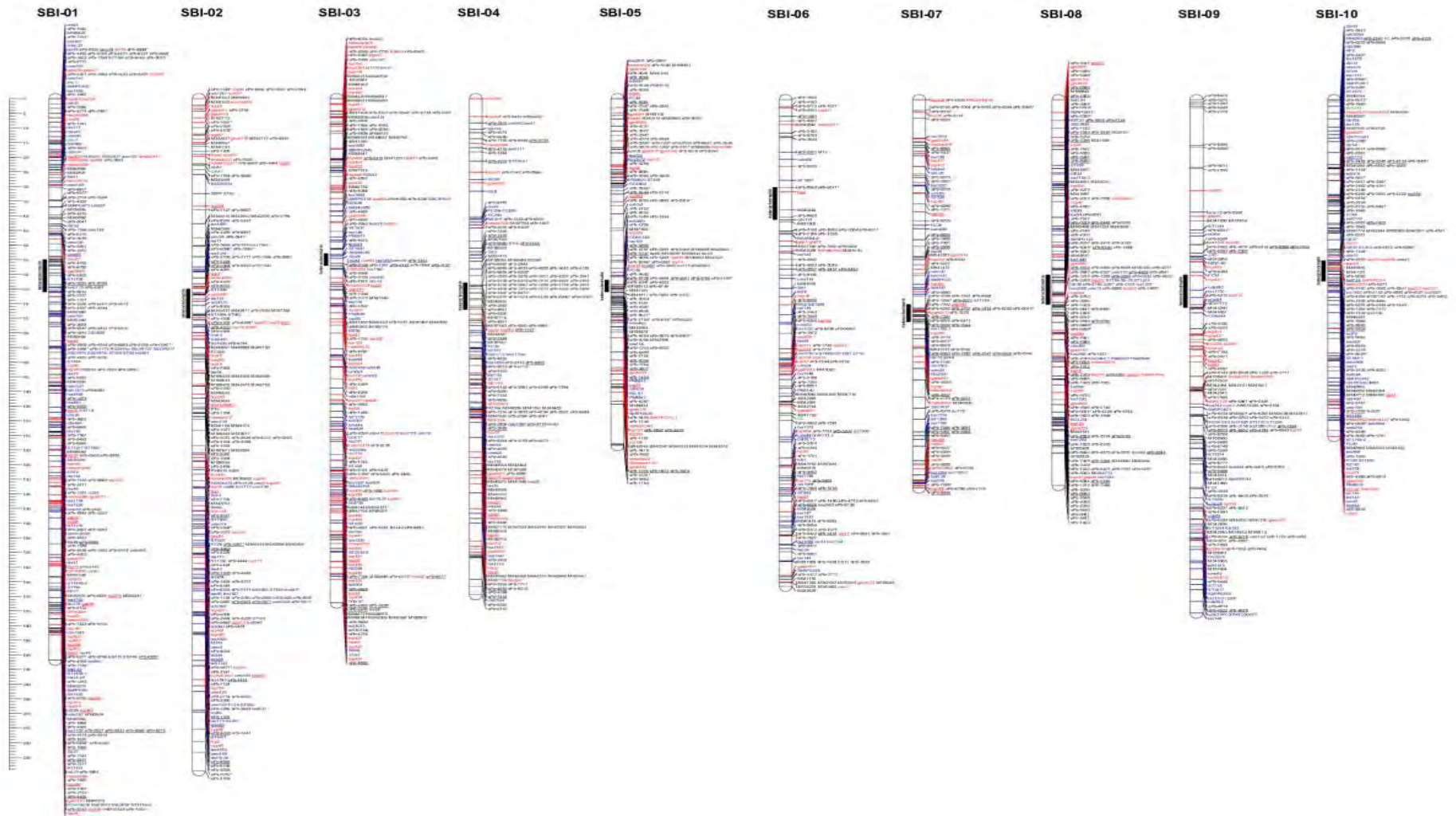
Appendix 3. Sixth linkage group of sorghum consensus map (Mace *et al*., 2009).
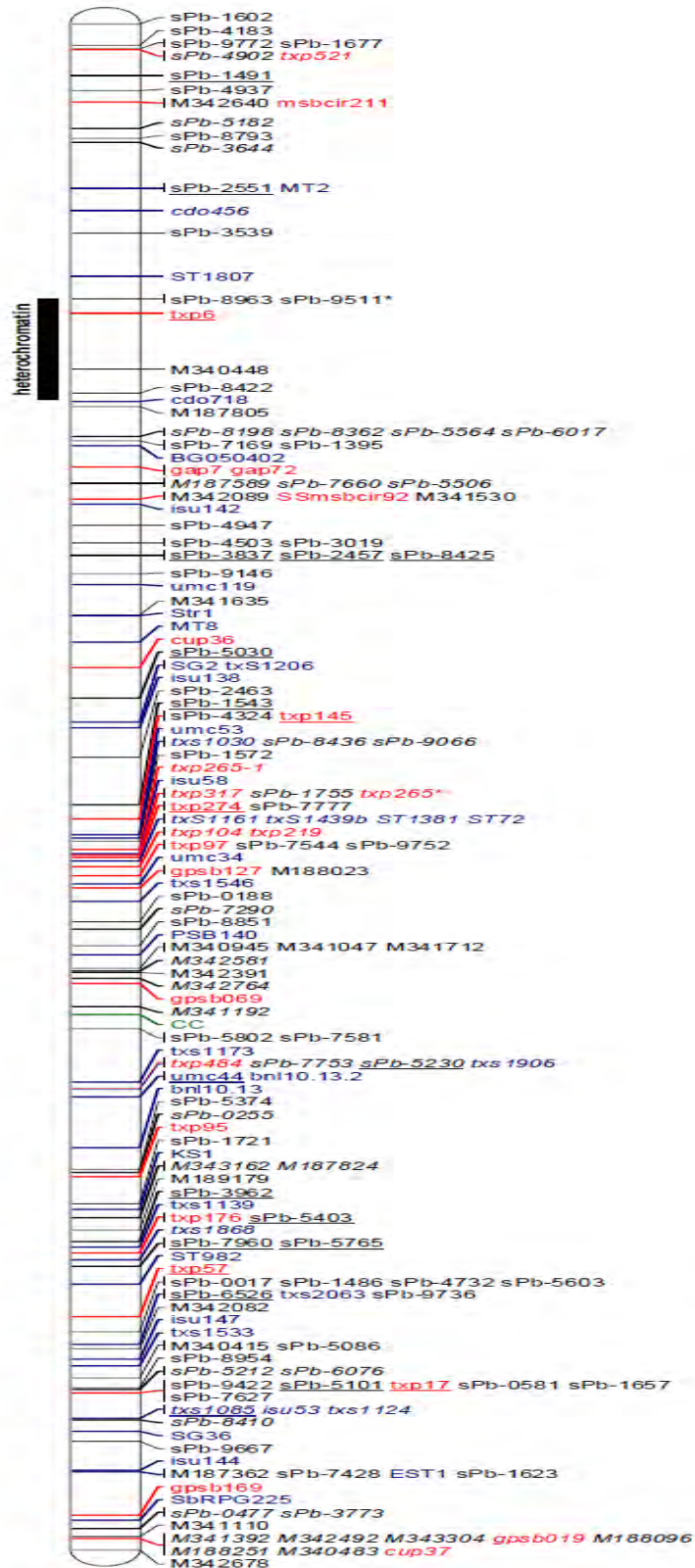
Appendix 4. Eighty Random Amplified Polymorphic DNA (RAPD) markers and their mT ºC.

No	Code	Primer	mT ºC
1	A1	CAGGCCCTTC	35
2	A2	TGCCGAGCTG	35
3	A3	AGTCAGCCAC	35
4	A4	AATCGGGCTG	35
5	A5	AGGGGTCTTG	35
6	A6	GGTCCCTGAC	35
7	A7	GAAACGGGTG	35
8	A8	GTGACGTAGG	35
9	A9	GGGTAACGCC	35
10	A10	GTGATCGCAG	35
11	A11	CAATCGCCGT	35
12	A12	TCGGCGATAG	35
13	A13	CAGCACCCAC	35
14	A14	TCTGTGCTGG	35
15	A15	TTCCGAACCC	35
16	A16	AGCCAGCGAA	35
17	A17	GACCGCTTGT	35
18	A18	AGGTGACCGT	35
19	A19	GTTGCGATCC	35
20	A20	CAAACGTCGG	35
21	B1	TGATCCCTGG	35
22	B2	GGACTGGAGT	35
23	B3	TGCTCTGCCC	35
24	B4	GTCCACACGG	35
25	B5	CTGCTGGGAC	35
26	B6	CCTTGACGCA	35
27	B7	TCCGCTCTGG	35
28	B8	TTTGCCCGGA	35
29	B9	CCACAGCAGT	35
30	B10	GGACCCTTAC	35
31	B11	GGAGGGTGTT	35
32	B12	AGGGAACGAG	35
33	B13	ACCCCCGAAG	35
34	B14	GTTTCGCTCC	35
35	B15	CATCCCCCTG	35
36	B16	TGCGCCCTTC	35
37	B17	GGTGACGCAG	35
38	B18	TGGGGGACTC	35

39	B19	GTAGACCCGT	35
40	B20	TTCCCCCGCT	35
41	C1	GTGAGGCGTC	35
42	C2	GGGGGTCTTT	35
43	C3	GATGACCGCC	35
44	C4	GTCCCGACGA	35
45	C5	CTCACCGTCC	35
46	C6	AAAGCTGCGG	35
47	C7	AAGCCTCGTC	35
48	C8	GACGGATCAG	35
49	C9	TTCCCCCCAG	35
50	C10	GTTGCCAGCC	35
51	C11	TTCGAGCCAG	35
52	C12	CCGCATCTAC	35
53	C13	GAACGGACTC	35
54	C14	TGGACCGGTG	35
55	C15	TGTCTGGGTG	35
56	C16	TGTCATCCCC	35
57	C17	TGCGTGCTTG	35
58	C18	CACACTCCAG	35
59	C19	TGAGTGGGTG	35
60	C20	ACTTCGCCAC	35
61	D1	ACCGCGAAGG	35
62	D2	GGACCCAACC	35
63	D3	TCTGGTGAGG	35
64	D4	ACCTGAACGG	35
65	D5	GTGTGCCCCA	35
66	D6	GGTCTACACC	35
67	D7	CACCGTATCC	35
68	D8	CTTCCCCAAG	35
69	D9	AGGGCGTAAG	35
70	D10	GAGAGCCAAC	35
71	D11	ACCCGGTCAC	35
72	D12	GTCGCCGTCA	35
73	D13	TGAGCGGACA	35
74	D14	TTGGCACGGG	35
75	D15	CTCTGGAGAC	35
76	D16	AGCGCCATTG	35
77	D17	GGGGTGACGA	35
78	D18	CATCCGTGCT	35
79	D19	TTTCCCACGG	35
80	D20	CTGGGGACTT	35