Studies of Sorghum Dual Resistance to Turcicum Leaf Blight and Anthracnose

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Declaration

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

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

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Dedication

To my Mum Liela.

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Arrangement of the thesis

This thesis is based on published articles from the work conducted in this study and is arranged under the following result chapters.

Chapter 3 is based on objective one published already in Wudpecker Journal of Agricultural Research.

Beshir, M.M., Ahmed, N.E., Ali, A.M., Babiker, I.H., Rubaihayo, P. and Okori, P. 2015. Prevalence and severity of sorghum leaf blight in the sorghum growing areas of central Sudan. Wudpecker Journal of Agricultural Research 4(6):54-60. ISSN 2315-7259.

Chapter 4 is based on objective one and is submitted for review in Plant Disease Journal for publication.

Beshir, M.M., Ahmed, N.E., Ali, A.M., Rubaihayo, P. and Okori, P. Reaction of sorghum genotypes to dual infection by *Colletotrichum sublineolum* and *Exserohilum turcicum* in Uganda and Sudan agro-ecologies.

Chapter 5 is based on objective two and is submitted in Plant Breeding Journal and is accepted for publication.

Beshir, M.M., Ahmed, N.E., Ali, A.M., Rubaihayo, P. and Okori, P. Effects of genes conditioning resistance to anthracnose and turcicum leaf blight in dual infection of sorghum.

Chapter 6 is paper on objective three is submitted in African Crop Science Journal and is accepted for publication.

Beshir, M.M., Ahmed, N.E., Ali, A.M., Rubaihayo, P. and Okori, P. Simple sequence repeat markers associated with turcicum leaf blight resistance loci in sorghum. African Crop Science Journal.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Sorghum (*Sorghum bicolour* L. Moench (2n=20)) and fungal pathogens have continuously co-evolved in a battle for growth and survival. In this rivalry sorghum evolved a stunning array of structural and gene-based defences designed to combat diverse pathogens and so did pathogens by developing new races. Anthracnose, caused by C*olletotrichum sublineolum*, and turcicum leaf blight, caused by *Exserohilum turcicum*, are two major foliar diseases of sorghum that limit its productivity in Sub Saharan Africa. Both diseases are endemic in Africa and thus presumably have co-evolved with sorghum. Deployment of resistant varieties is the most cost effective way to manage both diseases especially when integrated with appropriate agronomy, practices. Unfortunately, most commercial varieties are mostly bred for either of the two diseases and rarely of any to both pathogens. Development of sorghum varieties with multiple resistance to anthracnose and turcicum leaf blight could improve productivity and underpin studies of hots pathogen co-evolution. There are very limited studies of such phenomena in sorghum, a crop with unusually large number of diseases and pests.

This thesis is one of the few that has investigated three related objectives of (1) establishing the reaction of sorghum lines to dual infection by both pathogenes in Sudan and Uganda; (2) identifying gene action conditioning resistance to both pathogenes in sorghum; and (3) identifying simple sequence repeats that co-segregate with anthracnose and turcicum leaf blight resistance loci.

Four field and two greenhouse experiments were conducted in sorghum growing regions of Sudan and Uganda that are centers of diversity (two seasons/ four environments). The main investigation locations were Wad Medani in Sudan and MUARIK in Uganda that are ideal sites for both leaf anthracnose and turcicum leaf blight epidemics especially concomitant

infection by both fungi. The results showed a varied response of test genotypes under field and greenhouse conditions across locations in both Sudan and Uganda. Cultivars, Jesu 91-104DL and Butana (farmer preferred varieties in either country) were tolerant to both diseases. The advanced breeding line MUTLB1003 exhibited high levels of resistance to anthracnose and moderate reaction to turcicum leaf blight. Genotype main effect (G) plus genotype-by-environment (GE) interaction (GGE) analysis revealed that breeding for leaf anthracnose resistant genotypes was equally effective in all environments while it was not the same for turcicum leaf blight. Some of the genotypes in the study were resistant to dual infection and are thus suitable for multiple trait breeding.

Segregating progeny generated through half diallel mating of six parents indigenous to East and Central Africa i.e. HD1, Epuripuri, Sekedo, GA06/106, GA06/18 and MUC007/009, with varied reaction to leaf blight and anthracnose was used to study the mode of inheritance of resistance to foliar anthracnose and turcicum leaf blight. The results showed a negative but significant correlation between anthracnose and turcicum leaf blight disease intensity (incidence and severity). Thus suggested that that loci conditioning resiatnce to anthracnose were different from those that affect turcicum leaf blight. Dominant and additive variance components were almost equal indicating the significant role of both additive and non – additive genetic variance towards anthracnose resistance as supported by the Baker's ratio of 0.4. The contribution of additive gene effects towards turcicum leaf blight resistance was greater than non – additive gene effects as supported by the Baker's ratio of 0.6. The results of this study thus showed that sorghum genotypes studied such as GA06/18 had resistant alleles to both diseases. Two crosses GA06/106 x Epuripuri and MUK007/009 x Epuripuri clearly demonstrated that they were good starting populations for TLB and anthracnose resistance breeding programmes.

Mapping of resistance to anthracnose and turcicum leaf blight was undertaken in 126 $F_{8:9}$ sorghum recombinant inbred lines (RILs) derived from a cross between MUC007/009 and Epuripuri under Uganda and Sudan field conditions. Transgressive segregation was observed in RILs indicating that both parents carried minor alleles for resistance. High polymorphic information content, gene diversity and allele frequency were observed suggesting that all of polymorphic SSR markers evaluated could contribute substantive information to breeding research and construction of genetic map of sorghum. The information gained from this study can be used in deploying marker assisted selection for dual diseases infection of anthracnose and turcicum leaf blight and map-based isolation of important resistant genes in sorghum.

Chapter One

General introduction

1.1 Significance of sorghum

1.1.1 A hardy crop for drylands

Sorghum (*Sorghum bicolour* L. Moench (2n=20)) is a cereal grain that originated in Africa and is now grown throughout the semi-arid tropical and temperate regions of the world (Perumal *et al*., 2009). The cultivated races of *Sorghum bicolor* are *bicolor*, *guinea*, *kafir*, *caudatum* and *durra* (Doggett, 1988). Sorghum accounts for over 65% of the carbohydrate requirements and 39% of the daily calorie intake for millions of people in developing countries (FAOSTAT, 2012). While sorghum is a staple food for millions of people in India and Africa, livestock feeding accounts for most of the sorghum use in the developed world (Perumal *et al*., 2009). The hardy nature of sorghum, especially its resiliance to drought and low input agriculture, make it an ideal crop for the majority of resource poor farmers in Africa. It is the grain of 21st century in Africa and the success and continuity of its production makes it a key player in global food security, especially in Sub Saharan Africa (Perumal *et al*., 2009). There is also an increased demand for sorghum as most countries work towards attaining the sustainable development number one (SDG 1) on ending hunger, achieving food security and improved nutrition and promotion of sustainable agriculture (FAOSTAT, 2015). The demand for sorghum products implies that the national sorghum improvement programmes ought to increase and sustain high sorghum production levels especially in Africa that has many vulnerable populations.

1.1.2 Significance to economies in East and Central Africa

Today, sorghum is the dietary staple of 500 million people in 30 countries in the world (FAOSTAT, 2013). The biggest sorghum crop the world produced in the last 40 years was in 1985, with 77.6 million tons harvested (FAOSTAT, 2006). World sorghum grain production

was about 63 million metric tons (MT) during 2010 – 2011. In 2012 about 50 million Ha of land produced 70 million MT of grain (FAOSTAT, 2012). More than 90% of the production was in developing countries and most of this was in the semi-arid areas of Africa and Asia (FAS, 2012). Expansion of acreage in Africa increased at about 3.6%/year, although yields declined at 1.0%/year (FAOSTAT, 2012). In Sudan, sorghum is the major food crop and the majority of the people consider it as the national bread (Elbashir and Ali, 2014). In Uganda, sorghum is ranked the third most important cereal crop (FAOSTAT, 2015) and several bottled beer brewery industries use sorghum as a substitute to imported barley (MAAIF, 2007). Recent statistics show that Sudan and Uganda are leading sorghum producers (FAOSTAT, 2015), Sudan accounting for 4.524 million MT from 7.2 million Ha planted whilst Uganda accounted for 3 million MT from 3.5 million Ha (FAOSTAT, 2015). A major factor driving low productivity is the large number of foliar and grain diseases of the crop especially in the tropics.

1.2 Diseases as major production constrains of sorghum

Sorghum yields in East and Central Africa (ECA) are especially low; productivity in East Africa is 1183 Kg/Ha compared to yields in the United States of America (4354 Kg/Ha) (FAOSTAT, 2011). The low productivity is in part caused by several biotic stresses especially striga (Yasir and Mohamed, 2013), diseases (Esele, 1995) and pests (Muturi *et al*., 2014) being the major challenges. However, the diseases are exacerbated in the tropics of Africa by high rainfall and high relative humidity, moderate temperatures, and large amounts of inoculums (Ngugi *et al*., 2000).

Sorghum and fungal pathogens have been continuously confronting each other during evolution in a battle for growth and survival (Esele, 1995). In this rivalry, sorghum has evolved a stunning array of structural, chemical, and gene-based defences, designed to combat pathogens of different nature (Taylor *et al*., 2006) and so did pathogens by developing new

races (Tesso *et al*., 2012). Two key pathogenic fungi namely *Colletotrichum sublineolum* Ces. Munt.-Cvetk. (anamorph *Colletotrichum graminicola*), causes anthracnose, and *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs (teliomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs), causes turcicum leaf blight, continue to have a huge impact on sorghum production in temperate and tropical of the wrold regions (Ngugi *et al*., 2000).

The symptoms of TLB on sorghum are large, elongated, spindle-shaped spots and grey to tan lesions while anthracnose symptoms appear on all above ground parts of the sorghum plant, essentially as leaf spots (Dube *al al*., 2010; Reddy and Prasad, 2013). Anthracnose exhibits a high degree of variability that allows it to easily adapt to prevailing resistant genotypes thus breaking their resistance mechanism quickly (Costa, 2011). The symptoms of both diseases at later stages of infection appear only on above ground plant parts and develop in both living and dead tissues (Reddy and Prasad, 2013). Both pathogens cause grain abortion of up to 70% and significant reduction in grain yield through reduced kernel weight (Reddy and Prasad, 2013). Several control measures have been attempted but host-plant resistance is the most economical approach for successful management of these diseases (Hess *et al*., 2002). Mohan *et al*. (2010) have suggested that the availability of sources of resistance is a prerequisite for breeding adapted resistant and high yielding sorghum genotypes, but there is a need to determine the nature of inheritance of resistance to both diseases in genotypes.

1.3 Diseases management strategies

1.3.1 Agronomic management options

Worldwide, farmers have been developing new practices for managing plant diseases. The discovery of the causes of plant diseases in the early nineteenth century offered opportunities for understanding of the interactions of pathogen and host (http://www.apsnet.org/ accessed 1st February, 2015). Plant disease epidemics can be classified into two basic types, monocyclic

and polycyclic, depending on the number of infection cycles per crop cycle. The early stages of a monocyclic epidemic can be described quite well by a linear model, while the early stages of a polycyclic epidemic can be described with an exponential model (http://www.apsnet.org/ accessed 1st February, 2015). There is concern with keeping the disease levels well below 100%, there is no need to adjust the models for approaching the upper limit, and then there is need to use the simple linear and exponential models to plan strategies. Clearly developing a disease management strategy requires enough knowledge of the biology of the pathogen and host to select the appropriate epidemiological model (Ramathani, 2009). It also requires at least "ball-park" estimates of the model parameters and the magnitude of the impact of each specific tactic on the initial inoculum or the apparent infection rate (Ngugi *et al*., 2000). Failure to adopt such a quantitative approach can lead to very costly errors.

1.3.2 Disease resistance breeding strategies

Genetic resistance is the most effective strategy for breeding foliar diseases of sorghum especially among resource-contributed farmer's (Silva *et al*., 2015). However, resistance is hampered by high genetic variability of pathogen populations (Costa *et al*., 2009). Information on management practices for sorghum disease is very scarce (Silva *et al*., 2015). Host resistance strategies available for sorghum disease management include crop and cultivar rotation cultivation that involve varieties pyramided with different resistence genes and or differ in the types of resistance genes (gene rotation). However, genetic resistance to some foliar diseases has not been stable in certain situations due to the high variability present in the pathogen population (Cesale *et al*., 2001). Therefore, the instability of race specific resistance, often associated with vertical resistance has promoted the search for more stable forms of resistance (Ramathani, 2009). Other alternative strategies such as the use of sorghum hybrids with no virulence association in pathogen population and the use of cultivar mixtures have been explored (Casela, 1998). A continuous process of identification of sources of resistance to

fungal pathogens is needed not only to be incorporated in breeding programs, but also to be explored in management strategies to increase the stability of this resistance (Ramathani, 2009). On the other side, molecular marker technology greatly facilitates the study of multiple diseases resistance and has made it possible to dissect the polygenes controlling such traits into individual Mendelian factors (Paterson *et al*., 2008).

1.4 Factors affecting anthracnose and turcicum leaf blight epidemics

There are four major interacting factors affecting anthracnose and turcicum leaf blight pathosystem namely the host (sorghum, maize and wild relatives), the pathogens, the environment and the human influence (crop and farming systems) (Ngugi *et al*., 2000 and Ramathani, 2009). These factors contribute to the development of turcicum leaf blight epidemics in central and eastern Africa (Ngugi *et al*., 2001). The differential response of genotypes across environments (GE) limits the response to selection and subsequently progress in breeding programme (Bernardo, 2008). In the maize- *E. turcicum* pathosystems, characterized host species specialization has been reported (Adipala *et al*., 1993). Efforts have been made to characterise turcicum leaf blight epidemics as means to provide required data for designing and deploy disease management strategies. Temporal and spatial studies attribute of epidemics elucidate modes of inoculum spread over time and are the basis of cultural control methods of sorghum turcicum leaf blight (Ramathani, 2009). The best way to control anthracnose and turcicum leaf blight is by breeding resistant sorghum genotypes with help of marker-assisted breeding and also by deployment of multiple genes that confer either qualitative or quantitative resistance (Ogliaril *et al*., 2007).

1.5 Statement of the problem

Successful and continuous production of sorghum is key to global food security especially in the semi – arid tropics. However, the gap between achievable and actual yields in tropical

farming systems is quite large because of various foliar diseases damage among others constraints (Mohan *et al*., 2010). Foliar diseases are reported to be widespread in the warm and humid sorghum growing regions of Sudan (Beshir *et al*., 2015) Ethiopia (Fetene *et al*., 2011), Tanzania (Tilahun *et al*., 2001), Kenya (Ngugi *et al*., 2000) and Uganda (Ramathani *et al*., 2011). These diseases are caused by fungal, viruses and bacteria and include zonate leaf spot is caused by *Gloeocercospora sorghi*, rough spot caused by *Ascochyta sorghina* and *A. sorghi*, turcicum leaf blight caused by *E. turcicum* (*Helminthosporium turcicum*), anthracnose caused by *C. sublineolum*, sorghum rust caused by the fungus *Puccinia purpurea*, downy mildew caused by *Peronosclerospora sorghi* and head mold caused by several athogens include *Fusarium* spp., *Curvularia* spp., *Colletotrichum* spp., *Alternaria* spp., *Helminthosporium* spp. And among other fungi. The bacterial diseases include bacterial leaf stripe (bacterial stripe) caused by *Pseudomonas andropogonis* (Adipala *et al*., 1993; Casela *et al*., 1993; Esele, 1995).

Colletotrichum sublineolum and *E. turcicum* survive across cropping seasons on infected crop debris or in the soil (Casela *et al*., 1993). Fungal pathogenes have alternative hosts and volunteer crops as sources of primary inoculum (Sserumaga *et al*., 2013). Seed transmission has also been reported for *C. sublineolum* (Cardwell *et al*., 1989) and *E. turcicum* (Nobel and Richardson, 1968). *Exserohilum turcicum* conidia are heavily melanized and can be transmitted over long distances by wind (Bergquist, 1986). However, limited information is available for molecular markers that might be used in improving sorghum for foliar diseases resistance.

Anthracnose (caused by *Colletotrichum sublineolum*) and turcicum leaf blight (caused by *Exserohilum turcicum* (Leonard and Suggs, 1974) are considered the most important constraints to sorghum production in East and Central Africa (Ngugi *et al*., 2000). Anthracnose and turcicum leaf blight reduce the amount of green leaf area available for photosynthesis and

affect the quality of fodder by reducing protein *in vitro* dry matter digestibility (Rana *et al*., 1999). On susceptible sorghum cultivars, estimated yield losses as high as 70% have been recorded in Africa (Ngugi *et al*., 2000). In the United States, the largest sorghum producer worldwide, yield losses can be up to 50% if on susceptible varieties before panicle emergence (Narro *et al*., 1992). Previous studies on the epidemiology of these diseases have indicated that turcicum leaf blight is often more severe on younger plants (Julian *et al*., 1994), while anthracnose is associated with mature plants (Ashok-Mishra *et al*., 1992).

1.6 Justification of this study

Today, sorghum is the dietary staple for over 500 million people in 30 countries in the world (FAOSTAT, 2013). Recent statistics show that Sudan and Uganda are leading producers, Sudan accounting for 4.524 million MT from 7.2 million hectares while Uganda accounted for 3 million MT from 3.5 million hectares (FAOSTAT, 2015). The yield gap, however, still remains large with grain yield declining by 1.0% annually (FAOSTAT, 2012). The fungal diseases, anthracnose and turcium leaf blight are perhaps the most widespread causing major threats to sorghum productivity (Reddy and Prasad, 2013). Deployment of varieties with dual resistance is the most cost effective way to manage both diseases which when integrated with appropriate agronomy, provide suitable protection levels. Unfortunately, most commercial varieties are mostly bred for either anthracnose (Tesso *et al*., 2012) or turcicum leaf blight (Reddy and Prasad 2013) resistance.

Resistance in sorghum to anthracnose and TLB is quantitative and qualitative (Dube *et al*., 2010). A new plant resistant gene, which designated as *St* referring to *S. turcica*, is located on chromosome 5 in sorghum (Martin *et al*., 2011). Also genes for anthracnose resistance in sorghum have been mapped to chromosomes SBI-05 and SBI-08 (Perumal *et al*., 2009) and chromosome SBI-09 (Biruma *et al*., 2012). Breeding for such complex traits is often

compounded by genotype x environment interactions (Ngugi *et al*., 2000). Host plant resistance is part of an integrated foliar diseases management approach that can be used for controlling the fungi pathogenes (Mohan *et al*., 2010). Development of sorghum resistant varieties to dual diseases infection with *C. sublineolum* and *E. turcicum* would help to increase the production to small scall farmers in the region. There is need to identify new sources of resistance among sorghum breeding lines in East and Central Africa (Ngugi *et al*., 2000). Therefore, it is also essential to screen more locally adapted germplasm for possible identification of more sources of resistance with dual foliar diseases.

Understanding the mode of resistance to anthracnose and TLB in sorghum is essential because significant yield loss in sorghum growing regions of Africa is attributed to these stresses (Ngugi *et al*., 2000). However, information on the level of resistant dual diseases on farmer's preferred varities in Uganda and Sudan is limited. Therefore, urgent research is needed to understand the genetics of the inheritance of these resistance. Similarly, there is limited knowledge on the combining ability effects of the elite inbred lines, though such information is essential for the selection of lines with dual resistance (Reddy and Prasad, 2013). Determination of general combining abilities (GCA) and specific combining ability (SCA) are also needed for development resistant lines.

Deployment of multiple genes carrying cultivars that confer either qualitative or quantitative resistance Marker assisted breeding systems have helped (Mohan *et al*., 2010). DNA-based molecular markers delimiting disease resistance loci in sorghum have been reported for turcicum leaf blight (Mittal and Boora, 2005). In principle, the genetic analysis of QTLs underlying the complex traits that partly contribute for the complex phenomenon of foliar disease resistance should provide an understanding of their complementary nature and applicability in breeding programs. The limited success is due in part to an incomplete

understanding of the relationship between the genetics of sorghum foliar disease resistance and the complex interaction of traits influencing the disease resistance (Ramathani, 2009).

1.7 Objectives

1.7.1 Main objective

This study was carried out to contribute to the knowledge of dual resistance to anthracnose and turcicum leaf blight in sorghum in East and Central Africa.

1.7.2 Specific objectives

1. Establish the reaction of sorghum lines to dual infection by both pathogenes in Sudan and Uganda.
2. Identify gene action conditioning resistance to both pathogens in sorghum.
3. Identify simple sequence repeats that co-segregate with anthracnose and turcicum leaf blight resitance loci.

1.8 Hypotheses

1. Dual infection by both pathogenes exists among sorghum genotypes in Sudan and Uganda.
2. Resistance to anthracnose and turcicum leaf blight is controlled by additive gene action.
3. Simple sequence repeat markers co-segregate with resistant genes for anthracnose and turcicum leaf blight diseases in sorghum.

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Chapter Two

Literature review

2.1 Reaction of sorghum lines to dual infection by *Colletotrichum sublineolum* and *Exserohilum turcicum*

2.1.1 Etiology and host range of both pathogens

The fungal diseases cause severe reduction in sorghum grain and fodder yield to the tune of 70% and more (Reddy and Brasad, 2013). TLB has particularly been noticed to cause significant maize (Rajeshwar *et al*., 2014) and sorghum (Reddy and Prasad, 2013) yield reduction in many production regions. It is caused by a fungal pathogen *E. turcicum* (Pass.) (Leonard and Suggs, 1974) (synonyms: *Helminthosprium turcicum* (Pass.) (perfect stage: *Setosphaeria turcica* (Luttrell) Leonard and Suggs and *Trichometasphaeria turcica* (Luttrell). Hosts of *E. turcicum* include sorghum, maize, Sudan grass, Johnson grass, teosinte and other grass species (Esele, 1995). Ngugi *et al*. (2000) reported that TLB is sporadic in occurrence, depending on the environmental conditions and the level of disease resistance in the variety. Also Gregory (2004) listed the moderate temperature (18-27^{o}C), relative humidity from 90 to 100%, low luminosity, the presence of large amount of inoculums and long dew periods as the main factors influencing TLB epiphytotics.

Anthracnose, caused by *C. sublineolum* P. Henn., Kabát and Bubák, is one of the most destructive foliar diseases and, presently, it is found in most sorghum growing regions (Prom *et al*., 2012). Anthracnose was first reported in Togo, West Africa (Sutton, 1980) and has since been observed in most of the regions where sorghum is grown. Diagnostic symptoms of anthracnose include acervuli in the center of circular or elliptical lesions (Dube *et al*., 2010). Symptoms on leaves will depend on the cultivar type and environmental conditions. Symptoms can range from small, circular or elliptical spots to elongated necrotic lesions with abundant

acervuli formation (Thakur and Mather, 2000). Under severe conditions, *C. sublineolum* causes premature defoliation thereby delaying plant development (Ngugi *et al*., 2000). Infection of panicles affects the quality and quantity of the grain (Thakur and Mather, 2000). The earliest symptoms of anthracnose and TLB infections are slightly oval small spots on leaves as lesions. These lesions may appear first on lower leaves and increase in number as the plant develops and this can lead to complete blighting of the foliage (Richards and Kucharek, 2006). Typical TLB lesions are grey-green, elliptical or cigar-shaped and are typically 12 mm wide and 3-15 cm long and have yellow to gray centres and red margins. Spore production causes the lesions to appear dark gray, olive or black (King and Mukuru, 1994). Further spread of the disease within and between fields occurs by conidia produced abundantly on leaf lesions (Ngugi *et al*., 2000; Ramathani, 2009). Since the fungus survives between seasons on crop residues (Adipala *et al*., 1993). Crop rotation with legumes like soy bean or other non host crops can reduce inoculum build-up (Nyvall, 1989).

2.1.2 Epidemics under dual infection and single infection

Colletotrichum sublineolum and *E. turcicum* infect all above ground parts of plants with infection of leaves being more common (Reddy and Prasad, 2013; Prom *et al*., 2012). Anthracnose can occur during plant development, but symptoms are generally observed after flowering (Thakur and Mather, 2000). Yield losses of up to 50% may occur under severe foliar infection of susceptible cultivars; whereas panicle infection can cause losses of 30 - 50% (Ngugi *et al*., 2002). Disease management in sorghum relies heavily on using disease resistant hybrids and employing sound agronomic practices. Currently, the only practical management strategy for the pathogen involves deployment of resistant germplasm. However, fungal pathogens are known to display high levels of pathogenic variability and under optimal conditions for epidemics, host plant resistance breaks down rapidly (Ngugi *et al*., 2002). On susceptible cultivars, anthracnose may defoliate the plant and in severe cases, the plant will die

before it reaches maturity (Dube *et al*., 2010). Under optimal conditions for epidemics, the host plant resistance breaks down rapidly (Dube *et al*., 2010). Disease resistance, which is the most efficient way to control the disease, has been transitory due to variability in the population of this pathogen (Casela *et al*., 2001). Dilatory resistance, which is characterized by a slow rate of disease development of genetic mixtures, can be a way to diversify the host population and to manage resistance genes (Wilson *et al*., 2001). Alternative hosts and volunteer crops may also provide sources of primary inoculum, and seed transmission has been reported for *C. sublineolum* (Prom *et al*., 2012) and *E. turcicum* (Adipala *et al*., 1993). *Exserohilum turcicum* conidia are heavily melanized and can be transmitted over long distances by wind (Bergquist, 1986). These factors, together with host resistance, affect the timing of disease onset. Previous studies on the epidemiology of these diseases have indicated that leaf blight is often more severe on younger plants (Julian *et al*., 1994), while severe anthracnose is associated with mature plants (Ashok-Mishra *et al*., 1992).

2.1.3 Host defence under dual infection

Thakur *et al*. (2007) used correlation and cluster analysis to test sorghum lines at different locations and found some lines with sources of resistance to anthracnose. Ngugi *et al*. (2000) found that leaf blight epidemics always started earlier than those of anthracnose, but exhibited lower disease severity at crop maturity using nonlinear logistic model in Kenya. Also Ngugi *et al*. (2000) reported that planting date of sorghum is a critical issue in screening for resistance to anthracnose and TLB and proposed that test entries should be planted at least 15 days later than the normal planting time, usually defined by the onset of seasonal rains in eastern Africa. Narusak *et al*. (2009) provided a dual resistance-gene system against fungal and bacterial pathogens in sorghum. Sorghum varieties with multiple resistance to anthracnose and TLB would contribute to productivity increase as well as insights to the evolution of resistance to multiple infections, which is a fairly common phenomenon in nature. Previous studies have

characterized incidence of *C. sublineolum* (Sserumaga *et al*., 2013) and *E. turcicum* (Ramathani *et al*., 2011) on sorghum and resistance to either disease in Uganda.

Given that both sorghum and maize belong to the Poacea and share large regions of co-linearity (Bennetzen *et al*., 1998), resistance to TLB in sorghum may then share common features. Resistance to TLB in sorghum is controlled by mono- and polygenes similar to resistance in maize (Hooker and Kim, 1973; Lipps *et al*., 1997). It should be noted that resistance in sorghum to foliar infection is often characterised by pigmentation including flavenoids (Nicholson *et al*., 1987; Torres-Montalvo *et al*., 1992). Flavonoids have been implicated in plant disease resistance (Lamb *et al*., 1989); and in sorghum, the 3-deoxyanthocyanidins phytoalexins are the essential component in active defense mechanisms (Aguero *et al*., 2002). The type and quantity of flavonoids produced during pathogenesis, however may varied and may be related to pathogen species or their host pathogen intercations (Klein *et al*., 2001).

2.2 Gene action conditioning resistance to *Colletotrichum sublineolum* and *Exserohilum turcicum*

2.2.1 Race specific resistance

Six races of *E. turcicum* have been identified agroecological areas (Hooker and Kim, 1973; Leonard *et al*., 1989; Lipps *et al*., 1997; Ramathani *et al*., 2011). Virulence formulae have been designated demonstrating gene for gene relationships for *E. urcicum accordinglg to* Leonard *et al*. (1989). Race 0 has virulence formula *Ht1, Ht2, Ht3, HtN* and its distribution is commonly in Africa (Leonard *et al*., 1989; Ramathani *et al*., 2011). Race 1 has virulent formula *Ht2, Ht3, HtN/Ht1* (Bergquiest and Masias, 1974; Leonard *et al*., 1989) while race 23 has formula *Ht1, HtN/Ht2* and *Ht3* (Leonard *et al*., 1989; Leath *et al*., 1990). The development of new races shortens durability of the *Ht* based resistance (Ceballos *et al.*, 1991). Boora *et al.*

(1999) reported that the inheritance of resistance to sorghum leaf blight was controlled by a single dominant gene. Interestingly, host resistance in sorghum to *C. sublineolum* a highly variable pathogen is controlled by dominant genes (Singh *et al*., 2006).

2.2.2 Non race specific resistance to *E. turcicum*

Partial resistance in cereals to TLB ranges from a high level with few, small lesions to a low level with many, large sporulating lesions (Raymundo and Hooker, 1982). Introgression of these genes into a background with partial resistance confers the most effective resistance to *E. turcicum*, as displayed by reduced sporulation and number and size of lesions (Jiansheng and Jilin, 1984). Thus polygenic or partial resistance to be more durable (Lipps, 1982). Durable resistance is characterized by reduced number of lesions and decrease in lesion size and amount of sporulation, which is typical of polygenic resistance (Ullstrup, 1970). Whereas several quantitative genes have been found, resistance break down is quite common (Mohan *et al*., 2010). Therefore, a combination of monogenic resistance with partial resistance permits additive or complementary inter-allelic interactions that may enhance the overall level of resistance (Rajeshwar *et al*., 2014).

2.2.3 Resistance to dual infection

The multiple foliar disease resistance in sorghum is poorly understood and highly affected by environment x genotype interactions (Mohan *et al*., 2010). Genetic correlations between resistance to different diseases in plant can be used to determine the mode of selection and the success of plant breeding to multiple diseases (Leimu and Koricheva, 2006). Quantitative genetic analysis can be used simultaneously to address resistance against multiple pathogens (Zwonitzer *et al*., 2010). Indeed under multiple disease resistance in sorghum, one locus or several loci may confer resistance to the different diseases has been reported (Ali *et al*., 2013). Using F_2 progeny derived from diallel crosses, resistance to dual infection of TLB and maize

streak virus resistance in maize have been investigated (Okori *et al*., 2001; Opio, 2012). Resistance under concomitant infection was found to be largely due to general combining ability for TLB and specific combining ability for maize streak virus disease (Okori *et al*., 2001; Opio, 2012). Multiple resistance could in fact be supported by the coevolution of genomic segments that condition the same or similar metabolites associated with resistance cascades (Balint-Kurti and Johal, 2009). Occurrence of multiple resistance is thus conceivably more common than but requires elucidation especially for crop- pathogen systems that have long history of co-evolution such as those of sorghum and endemic fungal pathogens as was investigated in this thesis.

2.3 Molecular marker technology for anthracnose and TLB breeding and genetics

2.3.1 Disease resistance loci

Sorghum geneticists have long known that variation exists within sorghum genome for single resistance but breeding for multiple disease resistance has had limited success (Esele *et al*., 1993). In sorghum, resistance is to most foliar diseases are conditioned by both qualitative and quantitative loci (Mohan *et al*., 2010). For example several qualitatively inherited pericarp traits such as color and pigmented testa that influence the level of grain mold resistance have been reported (Esele *et al*., 1993). These resistance genes have been tagged for numerous diseases (Klein *et al*., 2005), insect pests (Nagaraj *et al*., 2001), and striga (Tao *et al*., 2003). Genes and or quantitative trait loci that condition tolerance and or resistance to abiotic stresses have been identified including drought tolerance (stay-green) (Haussmann *et al*., 2004), pre-harvest sprouting (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). Mohan *et al*. (2010) identified quantitative trait loci associated with multiple disease resistance for target leaf spot, zonate leaf spot and drechstera leaf blight resistances using sorghum recombinant inbred lines. 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).

2.3.2 Breeding for complex traits and use of markers

The completion of reference genome sequences for many important crops and the ability to perform high-throughput resequencing unlock opportunity for elucidating both crop evolution and well as the development of appropriate genetic tools for breeding (Morrell *et al*., 2012). For the most part, agronomically important traits in many crops are complex and breeding for such traits requires introgression of few to several QTL. Depending on the genome size and complexity this process may be slow or could be improved using both genetic (molecular) and physical phenotypes. The relatively small size of the sorghum genome 750 - 818 Mbp (Price *et al*., 2005) suggests that it is highly amenable to structural genomic transformation (Paterson, 2008). In deed, there is an increasing use of molecular markers for both genetic and breeding activities in sorghum since the 1990s (Ejeta *et al*., 2000; Babu *et al*., 2004; Mohan *et al*., 2010). The use of molecular markers has their greatest potential in accelerating the rate of genetic gain from selection for desirable traits and in the manipulation of quantitative trait loci. Diverse types of meolecular markers have been used through some are now rather old and rarely used. The past and recent molecular markers used have included random amplified polymorphism DNA, (RAPD), restricted fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), diversity array technology (DArT) and single nucleotide polymorphgism (SNPs) (Klein *et al*., 2000, 2003; Mullet *et al*., 2001; Trudy *et al*., 2009; Mace *et al*., 2009). Recent developments in high-throughput genotyping that allow for inexpensive genome-wide marker data to be rapidly collected in large numbers are unlocking opportunities for deployment of other approaches in breeding of complex traits. Genomic selection, a form of indexed, marker-assisted selection in which a marker data set is an example used to make phenotypic predictions (Meuwissen *et al*., 2001; Heffner *et al*., 2009).

The presence of high-density genetic markers such as SNPs are being used in genome-wide association studies (GWASs). GWAS refers to studies that search for a statistical association between a phenotype and a particular allele by screening loci (most commonly by genotyping SNPs) across the entire genome (Morrell *et al*., 2012). Overall these advances will accelerate the introgression of multiple favourable alleles into breeding populations. Other emerging approaches such as the use of targeted genome-editing technologies, such as zinc finger nucleases142 and transcription activator-like effector (TALE) nucleases offers exciting potential to resolve these issues (Bogdanove and Voytas, 2011; Morrell *et al*., 2012). Deployment of a combination of GWASs and next-generation- mapping populations especially in orphan crops such as sorghum have the potential to improve ability to connect phenotypes and genotypes, and underpin genomic selection to leverage data being generated by such systems or rapid selection and breeding (Morell *et al*., 2012).

2.3.3 Tools for studying genetic artitecture and deployment in breeding

Effective use of genetic variation for plant breeding requires an understanding of the genetic architecture of traits that have immediate applications to plant breeding. Current under-standing of genetic architecture is largely derived from quantitative trait locus (QTL) mapping (Mauricio, 2001; Bernardo, 2008). Methods for quantitative trait loci mapping have traditionally depended on the use of biparental mating involving parents with diverging traits that are crossed for a number of generations to form a population of recombinant homozygous lines. The F_1 generation is self-fertilized, but backcrossing and other strategies are also used (Bernardo, 2008; Morrell *et al*., 2012). Older methods used for mapping based on biparental derived populations include simple techniques such as single-marker analysis and more sophisticated methods such as interval mapping, joint mapping, multiple regression and composite interval mapping (Bernardo, 2008). The primary disadvantages of QTL mapping however is the time involved in creating these populations, the limited inference that can be made from alleles in populations that are generally treated as a fixed effects rather than random

effects. As such the development of high- throughput, dense genotyping has led to a shift from traditional QTL mapping to association or linkage disequilibia-LD mapping (Morrell *et al*., 2012).

Linkage disequilibria-mapping approaches assess the correlation between phenotype and genotype in populations of unrelated individuals. The mapping panels sample more genetic diversity and can take advantage of many more generations of recombination, avoiding the generations of time-consuming crosses that are necessary for QTL mapping (Mohan *et al*., 2010). These new approaches are also supported by so called next generation populations for genetic mapping. These populations are designed with the goal of overcoming many of the limitations of biparental QTL mapping and association mapping. They combine the controlled crosses of QTL mapping with multiple parents and multiple generations of inter-mating and are often larger than traditional QTL populations, and many lines are crossed in parallel (Morrell *et al*., 2012). These populations are also more effective for sampling of rare alleles than typical biparental populations and therefore provide enormous opportunity for both genetic and crop breeding.

2.4 Sectional conclusion

The foliar diseases anthracnose and turcium leaf blight are considered important constraints to sorghum production, particularly in the wetter humid tropics areas. Individually, each disease causes extensive defoliation especially during the grain filling period (from half bloom to physiological maturity), resulting in grain yield losses of up to 70%. Deployment of resistant varieties is the most cost effective way to manage both diseases which when integrated with appropriate agronomy, provide suitable protection levels. Unfortunately, most commercial varieties are mostly bred for anthracnose. Breeding for dual diseases is a challenging process that needs constant review of approaches and strategies which include lessons learnt from

other breeding programs of sorghum, cereals and plant species. Disease resistance in cereals to anthracnose and TLB have been reported to be explained by both additive and non additive genetic variances. The QTL have, in some cases, been mapped but rarely have genetic studies been conducted to elucidate resistance under dual infection, a phenomenon that is common in the tropics amnd most agroecosystems world over. This thesis explores various pathology and genetic studies to elucidate disease resistance to anthracnose and turcicum leaf blight in sorghum. Where as biparental derived populations are less informative compared to next generation mapping populations, the precision expecially for the target populations (fixed effects) makes them still informative. This thesis has thus used QTL mapping strategies that are based on biparental derived populations to eludicate resistance to anthracnose and turcium leaf blight. This way, the thesis research will contribute to the development of tools for sorghum breeding.

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Chapter Three

Severity and incidence of sorghum leaf blight in the sorghum growing areas of central Sudan

3.1 Introduction

Occurrence of *E. turcicum* foliar pathogen on sorghum has been very common in Kenya (Ngugi *et al*., 2001) and Uganda (Ramathani *et al*., 2011) for a long time however, in Sudan it was first reported in 1970s (ARC, 2012). Alternative hosts and volunteer crops also provide sanctuary for the pathogens. In case of maize, quick inoculum build up is not uncommon as sorghum and maize grow in the same ecologies at the same time and same season (Ngugi *et al*., 2000).

Under concomitant infection, TLB causes blighting of especially leaf tissues which may lead to coalescence of large patches of the leaf blade reducing the functional leaf area for photosynthesis (Rajeshwar *et al*., 2014) and ultimately resulting in 70% grain yield losses (Mittal and Boora, 2005). Hitherto, no report was available on presence of *E. turcicum* in Sudan and its extent damage to sorghum production. Ngugi *et al*. (2000) and Ramathani *et al*. (2011) found highly resistant genotypes for foliar diseases among the five sorghum races (*kafir*, *guinea*, *caudatum*, *bicolor* and *durra*) in East Africa. Therefore, high resistant genotypes are expected to exist in Sudan because it is believed to be one of the centers of diversity for sorghum race *bicolor* (Kimber, 2000). In East Africa, there are two mating type genes of *E. turcicum* namely MAT1-1 and MAT1-2. The pathogen has been characterized further into races; as race 0, 1, 2 and 3 assessed on fungal isolates deriving from both sorghum and maize (Ramathani *et al*., 2011).

Under severe epidemics, TLB causes significant grain yield losses as high as 70%, through reduced kernel weight (Rajeshwar *et al*., 2014). TLB is controlled by use of disease free seeds

or seeds treated with chemicals and hot water, following a two to three year crop rotation. Possible, application of fungicides and growing resistant varieties also getting rid of this pathogen (Ramathani *et al*., 2011). The distribution of TLB and pathotypes of *E. turcicum* have been identified in Uganda (Sserumaga *et al*., 2013) and Kenya (Ngugi *et al*., 2000) but work on this aspect was limited in Sudan. Therefore, a survey was undertaken in the major sorghum growing areas in central Sudan to examine the *E. turcicum* pathosystem in terms of disease incidence and severity on sorghum.

3.2 Materials and methods

3.2.1 Study area

The study was conducted in four districts namely Khartoum, Sennar, Gedarif and Gezira where sorghum is produced under both irrigation and rainfed systems. These four districts are the main areas for sorghum production in central Sudan (Ahmed, 2011). Disease incidence and severity were evaluated in 45 fields distributed in the nine sorghum-growing areas within the four districts. These areas included Elrahad, Doka and Gedarif in Gedarif district, Abu Naama in Sennar district, Wad Elhadad, Wad Elturabi and Wad Medani in Gezira district and Elfaki Hashim and Shambat in Khartoum district (Figure 3.1).

3.2.2 Field observations and material collection

A hierarchical surveillance structure was used to determine turcicum leaf blight intensity (incidence and severity) in 45 fields, about 10 - 20 days after flowering (Ramathani *et al*., 2011) during the rainy season of 2014. The surveillance structure consisted of two hierarchical levels; districts and location within district zones. From each location, at least 5 field each averaging one hectare in size was assessed at every 20 km along the main road.

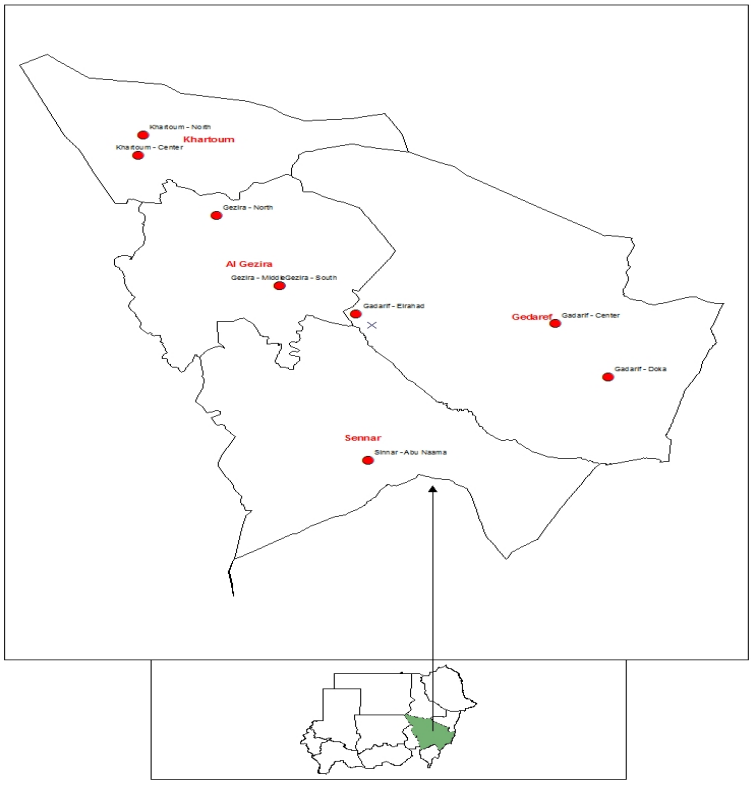

Figure 3. 1: The four main districts showing major sorghum growing agro-ecologies in central Sudan and the nine sampled locations.

The cultivated varieties assessed included improved varieties released for their high yields (Hageen Dura 1, Arfa Gadamak, Tabat and Wad Ahmed) drought tolerant varieties (Bashair, Butane and Yarwasha), and Kurulolu and Gadam Elhamam which are local varieties preferred by farmers. Leaf samples were collected to confirm pathogen identity in the laboratory.

3.2.3 Fungal isolation, culture and DNA isolation

Diseased leaves were collected and used for single spore isolation following sporulation of *E. turcicum* from leaf lesions under aseptic conditions as described by Carson (1995). The pure cultures were subsequently grown on potato dextrose agar (Difco), and the mycelia harvested by scrapping off the plate and directly used in DNA extraction (Ramathani *et al*., 2011).

3.2.4 *Exserohilum turcicum* species-specificity

The *E. turcicum* isolates were screened by PCR using the sequence information from the

internal transcribed spacer ribosomal DNA (ITS rDNA) of the 5.8S ribosomal RNA gene (GenBank accession number AF163067). The following primers were designed, forward: 5′ - GCAACAGTGCTCTGCTGAAA-3′, reverse: 5′-ATAAGACGGCCAACACCAAG-3′, following Ramathani *et al*. (2011) method generating a 344 bp fragment. PCR was carried out using 10 ng of template DNA, which was added to a 24 µl mix consisting of sterile H_2O, 2.5 mM $MgCl_2$, 2.5 µl Taq buffer, 0.2 mM of each dNTP, 0.25 µM of forward and reverse primers and 1 U of Taq polymerase. The PCR conditions used were 95°C for 4 min, 35 cycles of 30 s at 94°C, 30 s at 58°C, 1 min at 72°C and a final extension was set at 72°C for 10 min (Ramathani *et al*., 2011). The PCR products were separated on 1% agarose gels to confirm fragment size and consequently the identity of the isolate.

3.2.5 Data collection and analysis

In each field, disease incidence was assessed for cultivar as the proportion of plants showing symptoms in the field (Ramathani *et al*., 2011) at 10 - 20 days after flowering as TLB symptoms appear before flowering stage (Ngugi *et al*., 2000). Twenty plants in the middle of each field were randomly selected and the number of plants having *E. turcicum* symptoms were counted on whole plant basis and expressed as a percentage of the plant population (Ramathani *et al*., 2011). Disease severity was rated using a scale of 0, 3, 5, 10, 25, 50 and >75% leaf area affected (Adipala *et al*., 1993). Data were recorded on several components, including lesion colour. Means for the different parameters were determined from data collected from the experimental plots. All data were subjected to correlation and analysis of variance (Steel and Torrie, 1997). Data analyses were performed using GenStat 12^{th} Edition (VSN International Ltd., UK).

3.3 Results

3.3.1 Confirmation of occurrence of *Exserohilum turcuicum*

All diseased leaf samples showed positive reaction for species-specific using the internal transcribed spacer ribosomal DNA (ITS rDNA) PCR scoring indicating the presence of *E. turcuicum* across all locations studied (Figure 3. 2). Positive isolates gave a bright band at 344 base pairs. The results of analysis of variances for incidence and severity of TLB in the four districts in central Sudan are presented in Table 3. 1. The analysis of variance revealed non-significant influence of districts on disease incidence and severity. However non-significance was detected, nested ANOVA from districts and locations within districts confirmed the equal occurrence of TLB in central Sudan where sorghum is mainly grown.

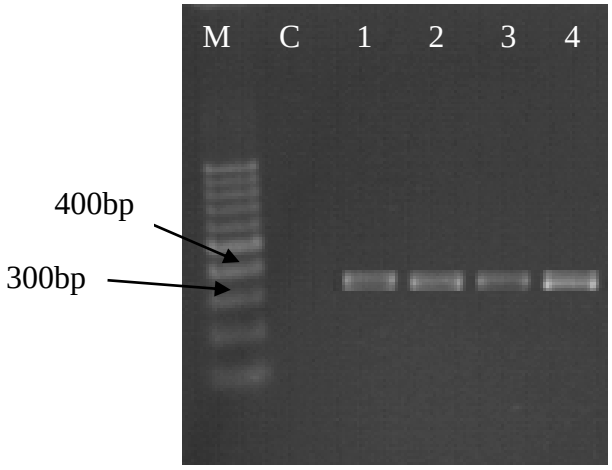

Figure 3. 2: Example of the DNA and PCR product. PCR amplicons of *Exserohilum turcicum* derived by amplification using rDNA ITS species specific primers: Lane descriptions 1 – Wad Medani, 2 – Shambat, 3 – Gadarif, 4 – Abu Naama, C – Control and M – 100bp DNA ladder.

Table 3. 1: Nested analysis of variance for incidence and severity of turcicum leaf blight on sorghum across districts.

Source	df	Severity			Incidence		
		SS	MS	F [a]	SS	MS	F [a]
District	3	3214.0	1071.3	2.13 [ns]	2187.5	729.2	1.59 [ns]
Location	9	6603.2	733.7	1.46 [ns]	3950.3	438.9	0.96 [ns]
Error	24	12073.5	503.1		11007.4	458.6	
Total	34	18676.7	549.3		15188.6	446.7	

[a] Statistical significant differences = P≤0.05; df degrees of freedom; SS Sum of squares; MS Mean square.

Mean of disease incidence and severity of TLB in major sorghum growing areas in central Sudan are presented in Table 3. 2. The result of the survey revealed that TLB was prevalent in all districts but with varying intensity on the different varieties. At the first level of hierarchy, the districts, disease incidence varied from 65% to 100% with the highest mean incidence in Gezira, Gedarif and Sennar (100%). At the second level of hierarchy, the locations, mean disease incidence was highest in Wad Medani, Wad Elhadad, Abu Naama, Doka, Gedarif and Elrahad (100%) and lower in Shambat and Elfaki Hashim (65%) but the lowest (45%) disease severity was recorded in Shambat (Khartoum district). In Gezira, a very high TLB disease incidence and severity were found in the sorghum variety Tabat (Table 3.2).

Table 3. 2: Mean of disease incidence and severity of turcicum leaf blight in major sorghum growing districts in central Sudan.

Locations	District	Letter	X	Y	Incidence [a]	Severity [a]
Abu Naama	Sennar	P	620185	1404190	100.0	53.8
Doka	Gedarif	P	798645	1494856	100.0	61.3
Elrahad	Gedarif	P	610363	1560701	100.0	100.0
Gedarif	Gedarif	P	758439	1551675	100.0	65.0
Wad Medani	Gezira	P	553265	1590542	100.0	85.0
Wad Elhadad	Gezira	P	553017	1590178	100.0	65.0
Wad Elturabi	Gezira	P	506540	1665986	75.4	51.8
Elfaki Hashim	Khartoum	P	452481	1751375	65.0	65.0
Shambat	Khartoum	P	448233	1729931	65.0	45.0
LSD (P≤0.05)					21.0	22.0
CV%					24.1	41.9

[a] Disease incidence and severity were computed as proportion of plants showing symptoms and percentage leaf area damaged, respectively.

3.3.2 Reaction of sorghum varieties to *Exserohilum turcicum*

Analysis of variances for incidence and severity of TLB among farmer's preferred sorghum varieties is presented in Table 3. 3. There was significant variation (P=0.00003) in disease severity while there was no significance in incidence indicating that reaction to TLB varied among what farmers prefer to plant every season.

Table 3. 3: Analysis of variance for incidence and severity of turcicum leaf blight on sorghum across districts.

Source	df	Incidence			Severity		
		SS	MS	F	SS	MS	F
Variety	14	11075.2	791.1	3.9^{ns}	11420.0	815.7	2.4***
Residual	22	4449.2	202.2		7418.8	337.2	
Total	36	18838.8			15524.3		

[a] Statistical significant differences = P≤0.05 DF degrees of freedom; SS sum of squares; MS Mean square.

The reaction of farmer's preferred varieties to TLB are presented in Table 3. 4. Hageen Durra 1 (HD1) showed the lowest severity (20%) and incidence (40%) while Yarwasha showed the highest incidence (100%) and severity (85%). Tabat, Wad Ahmed and Abu 70 were cultivated on a relatively large scale in Gezira and Sennar districts while Gadam Elhamam and Wad Ahmed were cultivated mostly in Gedarif district, and the fodder sorghum Abu 70 in Khartoum district. Overall, location effect was non- significant for disease incidence and severity.

Table 3. 4: Reaction farmer's preferred varieties of sorghum to *Exserohilum turcicum* across districts.

Variety	Incidence [a]	Severity [a]
Hageen Durra 1 (HD1)	40.0	20.0
Abu 70	76.2	61.0
Wad Ahmed	90.0	35.8
Arfa Gadamak	100.0	60.8
Bashair	100.0	45.0
Butana	100.0	35.0
Gadam Elhamam	100.0	45.0
Korakolu	100.0	45.0
Tabat	100.0	62.5
Wafir	100.0	55.0
Yarwasha	100.0	85.0
LSD (P≤0.05)	22.0	17.0
CV	20.1	25.9

[a] Disease incidence and severity were computed as proportion of plants showing symptoms and percentage leaf area damaged, respectively.

3.4 Discussion

The results of the laboratory analysis indicated that *E. turcuicum* was the causative agent of leaf blight observed in the study area of central Sudan. Furthermore, the TLB occurred in all the study locations with incidence and severity ranging from 45 to 100, and 65 to 100, respectively. Although location differences were non- significant, varietals differences were highly significant (P=0.00003). In spite of the fact that TLB was widely distributed in the studied area, farmer's knowledge about the disease was limited. In Khartoum district where sorghum is produced mainly as fodder using irrigation, TLB incidence and severity were lowest among the districts. The low incidence and severity levels of the disease in Khartoum district were attributed to high temperature and low humidity levels (Mahgoub, 2014) which are characteristic of Khartoum, unlike other districts.

In Gezira, Gedarif and Sennar, sorghum is normally largely produced for food grain under irrigation and rainfed conditions. These areas are characterized by lower temperature and higher humidity levels during the growing season of sorghum (Mahgoub, 2014), both conditions are conducive to the disease. This in turn explains the high observed levels in incidence and severity of the disease in these districts. Similar results were reported by Ngugi *et al*. (2000), Mohan *et al*. (2010) and Ramathani *et al*. (2011).

Currently in Sudan, about 90% of the total sorghum area is located in the rainfed belt which is characterized with low temperature and high humidity, both favourable to spread of the disease. This perhaps is a factor contributing to low rainfed belt yield levels to only 66% of the total sorghum production since about 90% of total sorghum area in Sudan is located in rainfed belt. Additionally, this study showed that the TLB disease, a yield depressant, was found to be more severe in Gezira district, which is the main irrigated sorghum producing area.

Additionally, the farmer's preferred sorghum varieties exhibited high TLB incidences and severities which rather explain why productivity is low in the country. Although the farmer's preferred varieties showed significantly high TLB incidences and severities, farmers continued to grow the varieties regardless of risks of high incidence and severity, which lead to reduced yield and income fluctuations from one year to another. Further work is still needed to gather important information on the detection of changes in the *E. turcicum*population and mating type distribution in order to eliminate this serious threat to sorghum production in Sudan as leading producer in the world.

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Chapter Four

Reaction of sorghum genotypes to dual infection by *Colletotrichum sublineolum* and *Exserohilum turcicum* in Uganda and Sudan agro-ecologies

4.1 Introduction

Turcicum leaf blight causes large, elongated, spindle-shaped spots, grey to tan lesions (Ramathani *et al*., 2011), whilst anthracnose symptoms appear on all above ground parts of the sorghum plant essentially as leaf spots (Dube *et al*., 2010). Under concomitant infection, the blighting of especially leaf tissues may lead to coalescence of large patches of the leaf blade reducing photosynthetic tissues and ultimately yield (Reddy and Prasad, 2013). Individually, each disease causes extensive defoliation especially during the grain filling period (from half bloom to physiological maturity), resulting in grain yield losses of up to 70% (Mittal and Boora, 2005). The pathogens that cause both diseases complete several life cycles during the cropping season and can survive from one season to another in various resting stages such as mycelia/ sclerotia or chlamydospores on infected crop debris (Frederiksen and Odvody, 2000). Alternative hosts and volunteer crops also provide sanctuary for the pathogens and in the case of leaf blight which also attacks maize, quick inoculum build up is not uncommon as both crops grow in the same ecologies at the same time (Ngugi *et al*., 2000).

Deployment of resistant varieties is the most cost effective way to manage both diseases which when integrated with appropriate agronomy, provide suitable protection levels. Unfortunately, most commercial varieties are mostly bred for either anthracnose (Tesso *et al*., 2012) or turcicum leaf blight (Reddy and Prasad, 2013) resistance. Resistance in sorghum to anthracnose is qualitatively inherited (Thakur and Mathur, 2000) and recent studies have mapped resistance to chromosomes 5, 8 and 9 (Biruma *et al*., 2012). Sorghum resistance to turcicum leaf blight is quantitatively and qualitatively inherited (Beshir, 2011) with resistance

loci mapped such as *St* mapped to chromosome 5 (Martin *et al*., 2011). Thus possibility for integral interactions that exploit inter loci effects cannot be precluded because both diseases are caused by necrotrophic fungi with similar pathogenicity properties. Indeed in multiple disease resistance, a form of host resistance in which one locus or several loci may confer resistance to the different diseases has been reported (Ali *et al*., 2013) but there are very limited studies of such phenomena. Sorghum varieties with multiple resistance to anthracnose and turcicum leaf blight would contribute to productivity increase as well as insights to the evolution of resistance to multiple infections, which is a fairly common phenomenon in nature. Previous studies have characterized incidence of *C. sublineolum* (Sserumaga *et al*., 2013) and *E. turcicum* (Ramathani *et al*., 2011) on sorghum and resistance to either disease in Uganda. The objective of this study was to determine the reaction of sorghum lines to dual infection by *C. sublineolum* and *E. turcicum* in Sudan and Uganda as well as document the occurrence of the phenomenon in the sorghum growing agro-ecologies in both countries.

4.2 Material and methods

4.2.1 Intensity of anthracnose and turcicum leaf blight in Sudan agro-ecologies

The studies were conducted in the districts of Khartoum, Gezira, Sennar and Gedarif, the main areas for sorghum production in Sudan (http://www.sudagric.gov.sd/ accessed on 1st March, 2015). The description for geographic information for sampling sites is illustrated in Table 4. 1. Small scale farmers in Khartoum and Gezira produce the crop under irrigation, while farmers in Gedarif and Sennar rely on rain-fed production (http://www.sudagric.gov.sd/ accessed on 1st March, 2015).

Table 4. 1: Geographic information for sampling sites in four main sorghum production districts in Sudan.

District	Location	Geographic information systems (GIS)		
		Letter	X	Y
Gedarif	Elrahad	P	610363	1560701
Gedarif	Doka	P	798645	1494856
Gedarif	Center	P	758439	1551675
Sennar	Abu Naama	P	620185	1404190
Gezira	Wad Elhadad	P	553017	1590178
Gezira	Wad Elturabi	P	506540	1665986
Gezira	Wad Medani	P	553265	1590542
Khartoum	Elfaki Hashim	P	452481	1751375
Khartoum	Shambat	P	448233	1729931

4.2.2 Field observations and material collection

A hierarchical surveillance structure was used to determine anthracnose and turcicum leaf blight incidence and severity from fields in nine sites within the four Sudan districts. The surveillance structure consisted of two hierarchical levels in agro-ecological zones and districts within agro-ecological zones. From each site, at least five fields each averaging one hectare in size were visited. Most fields were cultivated with Abu70, Arfa Gadamak, Tabat and Wad Ahmed as farmer's preferred sorghum varieties. In each field, disease incidence was assessed on 20 plants in the middle of each field which were randomly selected and the number of plants having *C. sublineolum* and *E. turcicum* disease symptoms were recorded from 100 m^2 based on whole plant basis and expressed as a percentage of the plant population (Ramathani *et al*., 2011). Disease severity on whole plant basis was rated using a scale of 0, 3, 5, 10, 25, 50 and >75% leaf area affected per plant (Ramathani *et al*., 2011). During assessment of disease incidence and severity**,** leaf samples were collected to confirm pathogen identity in the laboratory.

4.2.3 Fungal isolates, culture and DNA isolation

Sorghum turcicum leaf blight infected leaves were collected and used for single spore isolation following sporulation of *E. turcicum* from leaf lesions under aseptic conditions as described by

Ramathani *et al*. (2011). The pure isolates were subsequently grown on potato dextrose agar (Difco), and the mycelia harvested by scrapping off the plate was directly used in DNA extraction (Ramathani *et al*., 2011). Species-specific ITS based primers for *E. turcium* were used to confirm identity of the isolates. The primers are based on internal transcribed spacer 1 and 2 (ITS) of the 5.8S ribosomal RNA gene (GenBank accession number AF163067) (Ramathani 2009).

4.2.4 Experimental sites

In Sudan, field experiments were conducted at Gezira Research Station in Wad Medani and Wad Elturabi while in Uganda, at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) and National Semi-Arid Resources Research Institute (NaSARRI). Description of experimental sites is presented in Table 4. 2. Greenhouse experiments were conducted at Wad Medani and MUARIK during the first rains of 2012 at MUARIK and during the rainy season of 2014 at Wad Medani and Wad Elturabi.

Table 4. 2: Description and climate data during rainy seasons at experimental sites in Uganda and Sudan.

Location	Country	Longitude (E)	Latitude (N)	Altitude (m)	Temperature (C°)		Humidity		Reference
					2012	2014	2012	2014	
MUARIK	Uganda	32°37"	00°28"	1200	22°c	22°c	64%	64%	Beshir *et al*., 2012
NaSARRI	Uganda	33°33"	01°30"	1085	28°C	28°C	53%	53%	Wambi *et al*., 2014
Wad Elturabi	Sudan	32°31"	15°33"	382	34°C	35°C	12%	24%	ARC, 2014
Wad Medani	Sudan	33°05"	14°41"	414	30°C	30°C	54%	55%	ARC, 2014

4.2.5 Inoculation

Inoculum was prepared using single spore isolation following sporulation of *C. sublineolum* and *E. turcicum* from sorghum infected leaf lesions under aseptic conditions (Ramathani *et al.*, 2011). Twenty infested air – dried sorghum kernels of both *C. sublineolum* and E. turcicum were placed into the leaf whorls at vegetative growth stage two (five leaf stage) (Vanderlip, 1993) as described by Ramathani (2009). Twenty five seedlings of each genotype were

inoculated this way in the evening when dew and ambient temperature were optimal to successful infection (Carson, 1995) and inoculation was repeated three times at six day intervals to ensure successful infection. Seedlings were incubated in a humidity chamber at 22^{o}C for 48 hours before observations were made.

4.2.6 Genetic materials and experimental design

Fourteen cultivars were planted in the greenhouses and fields of MUARIK and Wad Medani during the first rains of 2012 and the rainy season of 2014, respectively. Cultivars were planted following a randomized complete block design (RCBD) (Apendix 1). Fourty four $F_{8:9}$ lines developed from a cross of MUC007/009 (resistant to turcicum leaf blight) (Ramathani, 2009) and Epuripuri (farmer's prefered variety) were planted in fields of MUARIK and NaSARRI in Uganda and in Wad Medani and Wad Elturabi in Sudan; $F_{8:9}$ were planted following an alpha lattice design (Apendix 2). Due to the variation in diseases pressure all experiments in Uganda and greenhouse experiment at Wad Medani in Sudan were inoculated artificially while field experiments at Wad Medani and Wad Elturabi in Sudan were left for natural infestation. During the surveillance studies, it was decided that where very high intensity of the disease was noted no further increase in disease pressure was added. All cultural practices recommended for the crop were followed.

4.2.7 Data collection and analysis

In both cultivars and $F_{8:9}$ lines experiments; disease assesments commenced 40 days after planting based on the proportion of infected green area per leaf. Turcicum leaf blight and anthracnose severities were recorded at weekly intervals from growth stage for four scores till scenesence using a quantitative scale of 0 to 75% (Ramathani *et al.*, 2011). Data on lesion number, days to 50% flowering and 1000 seed weight (g) were collected across locations in Uganda and Sudan. However, 1000 seed weight (g) data from Uganda was not collected due to

attack of birds during grain fillings while it was successfully done in Sudan. Area under disease progress curves (AUDPC) were computed using the weekly severity ratings (Madden *et al*., 2007). All data were subjected to analysis of variance with mean comparison performed using Fisher's protected least significant difference test (LSD) at $P \leq 0.05$ (Steel and Torrie, 1997). Least square means for all $F_{8:9}$ lines were generated using the linear mixed model (REML) option of GenStat 12th Edition (VSN International Ltd., UK) with genotypes being considered as fixed effects and replications and blocks within replications as random effects. Genotype main effect (G) plus genotype-by-environment (GE) interaction (GGE) were analysed and principal components (PC) 1 and 2 computed using Breeding Management Systems (BMS). Correlation analysis was performed using combined means across locations for anthracnose and turcicum leaf blight severities at 40 days after inoculation, AUDPC, lesion number, days to 50% flowering and 1000 seed weight (g).

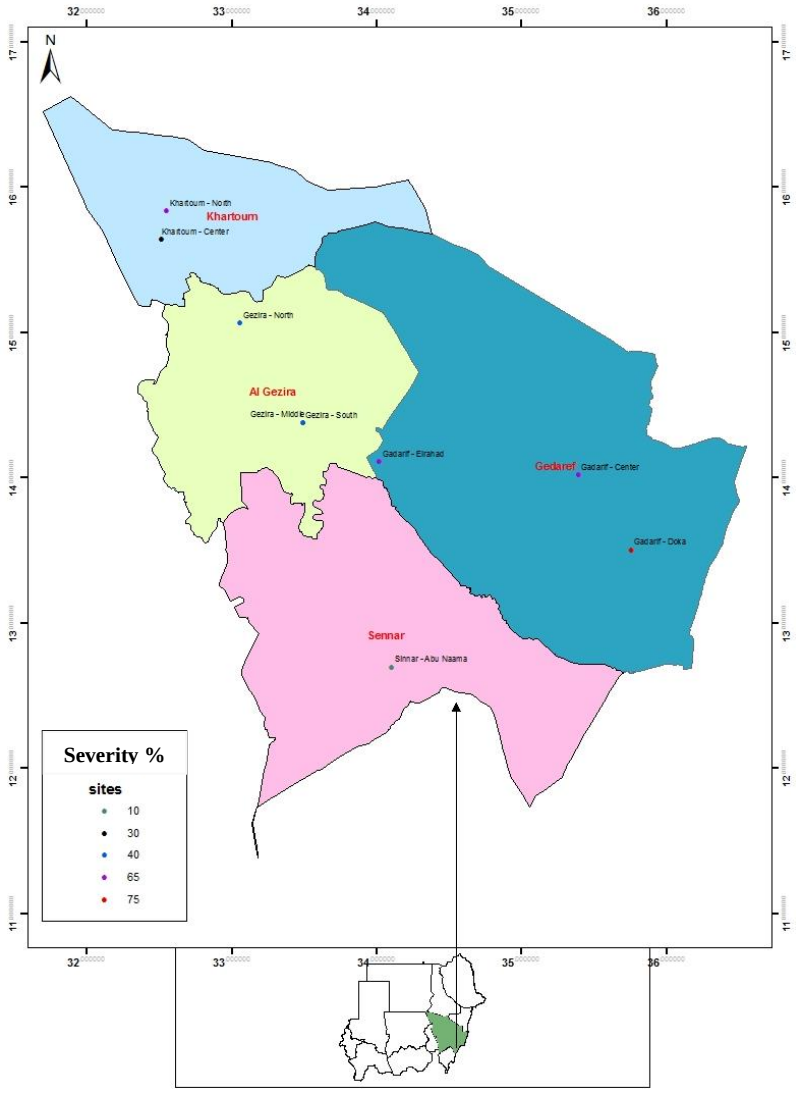

Figure 4. 1: Presence of *Exserohilum turcicum* in four main districts in major sorghum growing agro-ecologies in Sudan (rains of 2014).

4.3 Results

4.3.1 Intensity of turcicum leaf blight in sorghum growing regions of Sudan

Severity and AUDPC of anthracnose and turcicum leaf blight in sorghum growing regions of Sudan is presented in Figure 4. 1. Besides, the age of sorghum plants across districts and sites varied during sampling and turcicum leaf blight symptoms appeared before flowering stage, when field activities were carried out, while anthracnose symptoms did not develop at sampling time. The DNA of all isolates was positive to the species-specific ITS 1 and 2 derived primers assayed using PCR. These results indicated that *E. turcicum* was distributed in all study sites. Wad Medani, Wad Elhadad and Wad Elturabi in Gezira district preceded by

Sennar district had the highest disease severity, while Gedarif district had the lowest disease severity.

4.3.2 Reaction of sorghum cultivars and lines to *C. sublineolum* and *E. turcicum* infection in Uganda and Sudan

Reactions of sorghum cultivars to dual infection by *C. sublineolum* and *E. turcicum* are presented in Table 4. 3. Sorghum cultivars exhibited significant ($P \leq 0.05$) different reactions to both pathogens across locations. Both diseases had high severity environments. Sekedo had the lowest anthracnose severity and therefore was considered resistant to anthracnose, whilst Arfa Gadamak had the highest severity and therefore was considered susceptible. Jesu 91-104DL had the lowest turcicum leaf blight severity with few lesions and therefore was considered resistant to turcicum leaf blight whilst Tabat had the highest severity with many lesions and therefore was considered susceptible. However, Arfa Gadamak had significantly ($P \leq 0.05$) higher anthracnose and turcicum leaf blight severities indicating that this cultivar was susceptible to both diseases. Jesu 91-104DL and KARI mtama cultivars had significantly ($P \leq 0.05$) lower anthracnose and turcicum leaf blight severities, indicating that both cultivars were resistant to both diseases. Cultivars Wad Ahmed, Butana, HD1, Tabat, Yarwasha, GA06/18 and Sekedo had low severities for turcicum leaf blight but had high severities for anthracnose indicating that these cultivars are resistant to turcicum leaf blight but susceptible to anthracnose.

Table 4. 3: Reactions of sorghum cultivars means for *C. sublineolum* and *E. turcicum* at experimental sites in Uganda and Sudan.

Cultivar	Anthracnose									Turcicum leaf blight									Lesion nunber			Yield related traits	
	Severity at 40 days after inoculation					AUDPC[a]				Severity at 40 days after inoculation					AUDPC[a]				Initial	Final	AUDPC[a]	100 seed weight (g)	Days to 50% flowering
	MUARIK		Wad Medani			MUARIK		Wad Medani		MUARIK		Wad Medani			MUARIK		Wad Medani						
	Green-house	Field	Green-house	Field		Green-house	Field	Green-house	Field	Green-house	Field	Green-house	Field		Green house	Field	Green-house	Field					
Arfa Gdamak	8.0	25.0	15.0	20.0	MR	280.0	450.0	350.0	375.0	5.0	10.0	20.0	5.0	MR	154.9	150.0	273.3	150.0	15.5	139.5	$1.5x10^4$	3.7	54.4
Butana	5.4	12.8	6.8	0.0	MR	98.7	395.5	101.2	0.0	13.8	5.8	32.9	11.5	S	186.4	174.9	691.0	137.5	14.4	139.5	$1.7x10^4$	3.3	66.6
Epuripuri	7.0	10.2	1.7	10.0	R	77.1	322.3	40.8	241.7	5.0	5.5	22.5	4.3	MR	154.9	139.4	496.4	143.3	17.3	142.2	$2.0x10^4$	2.3	74.5
GA06/106	4.1	16.1	7.0	3.2	MR	42.4	386.4	124.0	87.5	5.6	6.2	32.4	23.3	MR	155.9	169.2	572.5	273.3	12.6	138.7	$1.5x10^4$	3.3	65.4
GA06/18	5.8	8.7	7.0	0.4	R	76.8	238.1	124.0	55.1	7.0	10.0	32.4	6.0	S	153.4	284.6	572.5	169.2	12.5	157.8	$1.5x10^4$	3.0	74.8
Gadam elhaman	4.9	12.8	15.1	8.3	MR	74.6	400.9	224.9	143.3	4.2	6.0	26.7	18.5	MR	146.0	185.5	511.0	227.5	12.6	128.1	$1.2x10^4$	3.6	68.6
HD1	3.6	10.9	5.3	12.0	R	89.7	327.9	131.3	165.0	7.5	6.2	37.2	5.0	S	161.6	154.7	672.8	150.0	11.8	134.6	$1.4x10^4$	3.4	66.4
Jesu 91-104DL	2.7	11.5	5.0	2.7	R	64.1	329.3	64.2	143.3	6.8	6.1	21.7	10.2	MR	178.1	206.4	539.6	215.8	14.5	136.1	$1.7x10^4$	4.3	72.6
KARI Mtama	10.2	13.0	13.3	5.0	MR	109.3	335.6	239.2	120.0	6.6	8.2	19.2	24.0	MR	170.0	243.7	347.1	425.0	16.6	155.2	$1.7x10^4$	3.2	71.0
MUC007/009	5.4	8.7	20.7	4.7	MR	82.3	263.6	337.8	100.5	5.3	10.2	26.7	20.0	MR	163.1	302.1	376.3	302.3	13.1	166.6	$1.6x10^4$	3.9	65.9
Sekedo	4.6	10.5	3.3	0.0	R	73.3	275.3	64.2	0.0	3.5	8.6	38.3	3.0	S	156.7	236.6	615.4	140.0	9.3	133.8	$1.2x10^4$	3.7	73.9
Tabat	6.6	13.3	7.3	4.3	MR	101.5	410.0	118.8	88.8	5.5	7.7	32.9	30.3	S	141.5	199.0	452.9	431.4	11.0	130.7	$1.3x10^4$	3.8	67.0
Wad Ahmad	8.6	11.6	1.7	18.5	MR	116.9	336.7	30.6	277.5	9.3	9.3	40.8	9.5	S	170.9	263.0	707.3	223.3	12.7	138.1	$1.5x10^4$	3.7	69.5
Yarwasha	2.0	10.0	5.0	7.5	R	58.1	279.0	122.5	112.5	4.9	9.2	38.3	20.0	S	125.9	309.0	672.6	425.0	9.3	139.0	$1.2x10^4$	4.8	63.7
SED(P≤0.05)	2.5	2.8	16.6	21.7		23.6	1.0	212.4	156.2	2.9	2.7	30.5	85.1		18.2	79.7	430.6	68.8	7.8	13.9	$0.1x10^4$	1.1	7.9
LSD(P≤0.05)	5.0	2.7	8.3	21.2		46.9	1.9	106.9	324.3	5.7	5.4	15.4	35.1		36.1	157.8	216.8	429.9	2.8	27.5	$0.08x10^4$	2.2	12.0

a= Area under disease progress curve. R= resistant (0-10%), MR= moderately resistant (10-25%) and S= susceptible (>25%).
Disease severity scales were reported by Ramathani 2009.

The 44 lines clustered into three groups based on their reaction to *C. sublineolum* and *E. turcicum* presented in Table 4.4. However, only twelve lines were selected to represent the three groups presented in Table 4.4. All lines exhibited significantly ($P<0.05$) different reactions to both pathogens. The check MUC007/009 had higher anthracnose severity than the check Epuripuri. There was high anthracnose severity in MUARIK and NaSARRI in Uganda than in Wad Medani and Wad Elturabi in Sudan. The leaf blight susuecptible check Epuripuri had higher turcicum leaf blight severity than the resistanct check MUC007/009 under field conditions. There was high turcicum leaf blight severity in MUARIK and NaSARRI compared to Wad Medani and Wad Elturabi. Lines MUTLB01003, MUTLB01020, MUTLB01092 and MUTLB01006 showed resistant response to both diseases across environments. MUTLB01016 and MUTLB01102 showed resistance to turcicum leaf blight and susceptibility to anthracnose. MUTLB01120, MUTLB01018, MUTLB01068, MUTLB01066, MUTLB01010 and MUTLB01069 showed susceptible response to turcicum leaf blight but resistance to anthracnose. MUTLB01003 showed low severity to both diseases with few lesions across environments. Across locations, genotypes planted in Uganda showed early flowering and maturing while genotypes evaluated in the fields were late flowering and maturing compared to greenhouse. However, the lines flowered late, on average 72.4 days across environments in comparison to Epuripuri (62 days) and MUC007/009 (63 days). Across locations, $F_{8:9}$ genotype means for lesion numbers showed non-significant differences among all locations. Lines which showed low turcicum leaf blight severities had few lesions of turcicum leaf blight while lines which showed high severities had many lesions.

Table 4. 4: Means of reaction of selected 12 sorghum $F_{8:9}$ lines (Out of 44) to *Colletotrichum sublineolum* and *Exserohilum turcicum* evaluated at four locations (first rains of 2012 in Uganda and rainy season of 2014 in Sudan).

Lines	Anthracnose									Turcicum leaf blight									Lesion number	Yield related traits	
	Severity at 40 days after inoculation					AUDPC[a]				Severity at 40 days after inoculation					AUDPC[a]				AUDPC[a]	100 seed weight (g)	Days to 50% flowering
	MUARIK	NaSARRI	Wad Elturabi	Wad Medani		MUARIK	NaSARRI	Wad Elturabi	Wad Medani	MUARIK	NaSARRI	Wad Elturabi	Wad Medani		MUARIK	NaSARRI	Wad Elturabi	Wad Medani			
Group one:																					
MUTLB01016	18.5	35.8	25.6	9.1	S	336.0	583.5	11.1	482.6	18.5	9.4	4.3	4.0	R	178.0	222.3	2.1	149.3	$9x10^5$		73.9
MUTLB01003	12.7	11.4	0.6	0.6	R	245.2	319.5	0.8	0.8	12.7	14.6	4.3	4.3	R	165.7	275.1	8.3	8.3	$8 x10^5$	3.7	63.1
MUTLB01006	15.3	19.6	1.8	3.7	MR	200.7	401.0	2.1	99.6	15.3	19.6	8.1	3.9	MR	159.6	353.1	7.8	91.3	$3 x10^5$	1.9	75.0
MUTLB01020	26.4	17.5	5.0	0.0	MR	279.7	357.0	5.1	0.0	26.4	19.5	20.0	1.4	MR	282.6	323.9	10.4	53.8	$1 x10^6$	2.3	77.9
Group two:																					
MUTLB01102	21.6	23.7	25.6	9.0	S	285.8	511.0	17.0	90.8	21.6	16.2	4.3	25.6	MR	230.6	347.3	3.3	314.3	$6 x10^5$	2.5	68.7
MUTLB01092	24.8	25.0	0.3	5.1	MR	240.9	510.7	0.8	292.6	24.8	27.2	27.7	4.0	MR	190.9	355.0	20.3	329.3	$7 x10^5$	2.5	70.9
MUTLB01120	21.7	24.5	19.5	1.5	MR	416.9	359.5	10.0	78.3	21.7	17.2	20.6	25.9	S	215.5	198.8	8.8	438.1	$6 x10^5$	2.7	65.6
Group three:																					
MUTLB01018	37.5	5.4	4.5	2.6	S	174.2	182.6	0.7	67.3	37.5	16.7	75.6	2.9	S	230.4	284.5	27.8	110.3	$4 x10^5$	1.3	76.7
MUTLB01068	22.3	11.4	12.5	1.5	MR	274.6	245.4	5.0	88.3	22.3	10.2	62.5	40.6	S	259.7	210.8	17.7	489.3	$7 x10^5$	1.8	78.7
MUTLB01066	24.0	21.4	0.0	4.0	MR	252.6	436.8	0.0	100.8	24.0	19.3	50.0	45.6	S	265.9	392.5	14.5	476.8	$8 x10^5$	1.3	71.5
MUTLB01010	38.1	18.0	2.5	3.2	S	224.9	342.7	3.6	137.1	38.1	19.0	40.2	43.4	S	243.1	314.8	8.5	583.8	$7 x10^5$	1.8	73.2
MUTLB01069	24.6	13.6	27.5	2.1	MR	248.0	326.4	10.8	22.6	24.6	17.0	25.0	74.5	S	221.5	242.2	5.3	921.8	$9 x10^5$	2.5	72.5
Epuripuri	1.2	12.5	0.0	1.7		12.5	145.8	0.0	40.8	2.6	39.4	7.5	32.9		1.0	616.9	265.0	496.4	$5 x10^5$	3.9	62.6
MUC007/009	4.8	44.7	75.0	20.7		44.7	734.4	760.2	337.8	3.3	5.3	2.5	26.7		1.0	120.9	262.8	376.3	$9x10^4$	2.3	63.3
SED (P≤0.05)	23.9	18.8	37.1	14.2		158.7	313.1	11.4	208.1	23.9	16.7	50.6	19.3		59.3	362.0	8.3	253.7	$5 x10^5$	1.1	17.7
LSD (P≤0.05)	12.2	9.6	18.6	28.2		80.7	158.7	5.7	414.9	12.2	8.4	25.4	38.5		80.8	183.5	16.5	505.9	$1 x10^5$	2.2	4.9

a= Area under disease progress curve. R= resistant (0-10%), MR= moderately resistant (10-20%) and S= susceptible (>25%) (Ramathani *et al.*, 2011).

Correlation of anthracnose and turcicum leaf blight severities, AUDPC and incidence with yield related traits is presented in Table 4. 5. Negative correlation was observed between anthracnose and turcicum leaf blight severities combined means across locations, AUDPC and between 1000 seed weight (g). This indicates that genotypes with less anthracnose and turcicum leaf blight severities had higher 1000 seed weight and vice versa.

Table 4. 5: Correlations of *Colletotrichum sublineolum* and *Exserohilum turcicum* severities and AUDPC evaluated under four locations under field conditions (first rains of 2012 in Uganda and rains of 2014 in Sudan).

Trait		100 seed weight (g)	Days to 50% flowering
Anthracnose			
	Initial severity	0.03	-0.11
	Final severity	0.14	-0.30^{+}
	AUDPC	0.21	-0.14
Turcicum leaf blight			
	Initial severity	0.12	0.01
	Final severity	-0.16	0.06
	AUDPC	-0.09	-0.01
Lesion number			
	Initial No	0.06	-0.32^{+}
	Final No	-0.12	-0.02
	AUDPC	0.04	-0.27^{+}

$^{+}$= Significantly different at P≤0.1.

Polygons of cultivars and $F_{8:9}$ lines for anthracnose and turcicum leaf blight severities based on symmetrical scaling are presented in Figure 4. 2. Polygons for anthracnose for cultivars and lines are presented in Figure 4. 2 (A) and Figure 4. 2 (B), respectively. The polygons showed that test lines at MUARIK performed similarly to Wad Medani, while testing at Wad Elturabi gave similar results to NaSARRI. Polygons for turcicum leaf blight for cultivars and lines are presented in Figure 4. 2 (C) and Figure 4. 2 (D). The polygons showed that MUARIK, Wad Elturabi and NaSARRI performed differently from Wad Medani. These results indicated that the locations in Uganda performed similarly while locations in Sudan performed differently. The results of genotypes and genotype x environment interaction (GGE) analysis of cultivars and $F_{8:9}$ lines reactions are presented in Figure 4. 2. Results showed that environments had high

significant (P<0.001) influence on PC1 and PC2 for both diseases severities and across all locations. This indicates that the performance of the genotypes varied across environments.

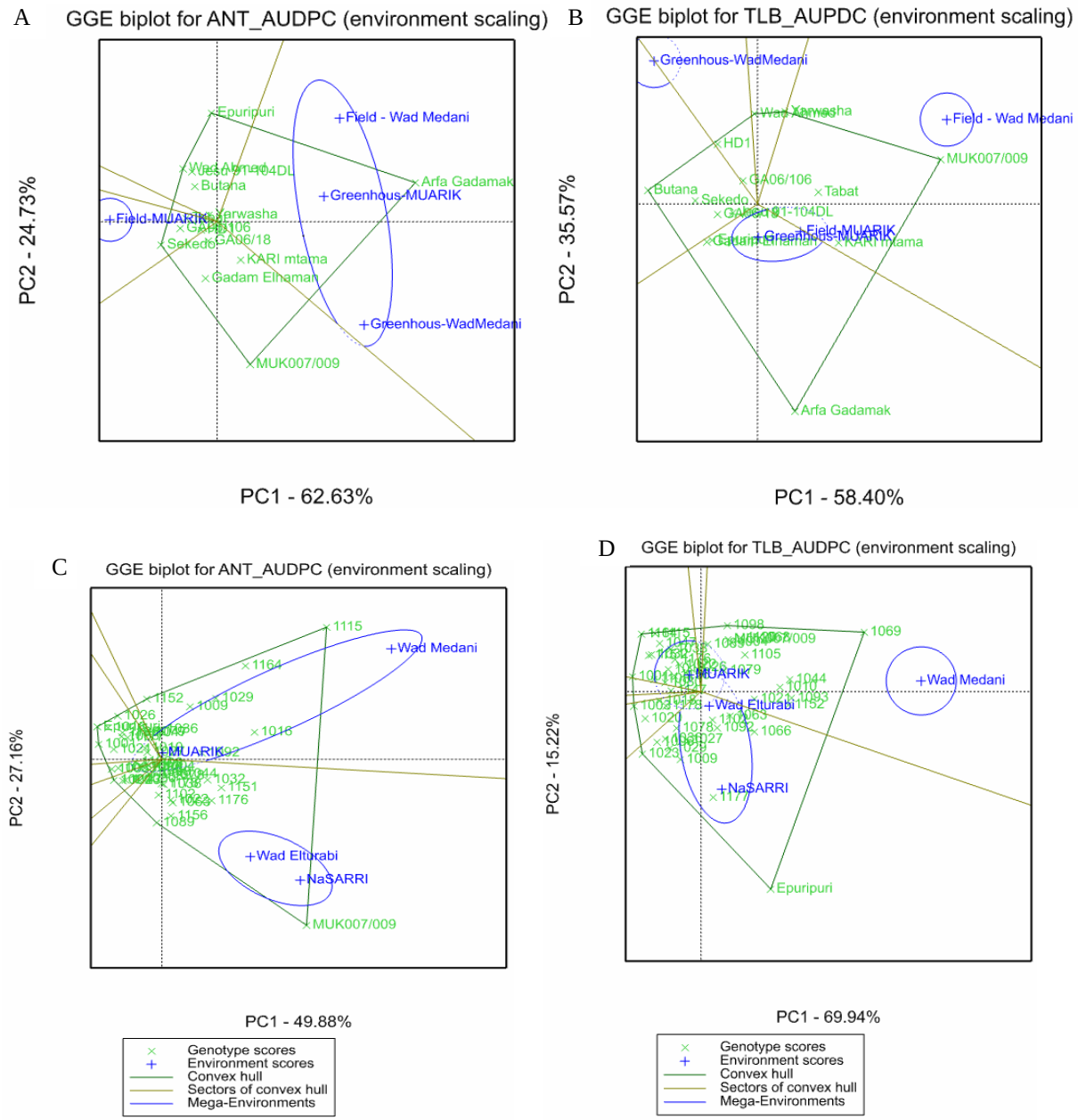

Figure 4. 2: Polygon views of the GGE biplot analysis based on symmetrical scaling for genotypes and environments using area under disease progress curve (AUDPC) (first rains of 2012 in Uganda and rains of 2014).

A = Polygon for cultivar responses to anthracnose AUDPC and B= Polygon for cultivar responses for turcicum leaf blight AUDPC. C= Polygon for $F_{8:9}$ line responses for anthracnose AUDPC and D= Polygon for $F_{8:9}$ line responses for turcicum leaf blight AUDPC. PC= principal component.

4.4 Discussion

Results from this study clearly demonstrated the presence of *E. turcuicum* in the four districts where sorghum is grown in Sudan. Among Sudanese farmer's preferred varieties, Wad Ahmed showed significantly ($P<0.001$) low turcicum leaf blight severity and incidence. Among Sudanese locations, Wad Medani showed the highest turcicum leaf blight severity and incidence among the locations. This could be due to the intensive plantation of sorghum every season, which could have increased the presence of *E. turcicum*. This high inoculum presence in Wad Medani specifically and Gezira generally makes the areas good sites for screening for turcicum leaf blight resistance. This research focused on *E. turcium* since epidemics of *E. turcium* always start earlier than those of *C. sublineolum* (Ngugi *et al*., 2000). Reddy and Prasad (2013) reported that development of fungal disease symptoms is increased during the sorghum plant lifecycle. Anthracnose symptoms appeared at late stages on sorghum (Ngugi *et al*., 2000, Dube *et al*., 2010) while turcicum leaf blight symptoms appeared before flowering especially in susceptible cultivars (Reddy and Prasad, 2013).

Both *C. sublineolum* and *E. turcicum* were sensitive to environmental conditions including (or specially) high humidity weather and the sorghum genotypes exhibited high severity reactions under field than under greenhouse in both Uganda and Sudan. Results from this research agreed with other results reported earlier that environment has significant effect on anthracnose (Ngugi *et al*., 2000) and turcicum leaf blight (Ramathani *et al*., 2011) severities. Under greenhouse conditions, young plants were inoculated and exhibited high level of resistance unlike those under field conditions which were inoculated at older stages and exhibited low level of resistance. Similar results were reported by Julian *et al*. (1994) and Ngugi *et al*. (2000) however, there is little definitive information on the mechanisms underlying these observations.

Ngugi *et al*. (2000) characterised sorghum genotype performance for either turcicum leaf blight or anthracnose infections, but not for dual infections under natural and uncontrolled conditions. This study characterised some sorghum cultivars based on dual infections by *C. sublineolum* and *E. turcicum* as resistant to anthracnose and turcicum leaf blight across environments. Similarly Druga (1999) evaluated 30 Indian genotypes for leaf blight resistance and classified them as resistant, moderately resistant and susceptible. Across locations in Uganda and Sudan, Jesu 91-104DL, KARI mtama and Epuripuri were resistant to dual infection of anthracnose and turcicum leaf blight. These three genotypes could be considered as possible sources of resistance to both diseases for sorghum breeding. Ramathani (2009) characterised Epuripuri as susceptible to turcicum leaf blight however, results from this study characterised it as moderately resistant based on pooled mean across locations in Uganda and Sudan. This was attributed to the presence of both *C. sublineolum* and *E. turcicum* and to environmental effects on plants performance. Negative significant correlation was observed between anthracnose and turcicum leaf blight severities across sites in Sudan and Uganda. This could be attributed to pleiotropy. The results further showed that the genotypes that flowered and matured early showed less anthracnose and turcicum leaf blight severities than those, which flowered and matured late. Therefore this research highlighted the importance of selecting for early flowering and maturing sorghum genotypes which are expected to have low anthracnose and turcicum leaf blight severities.

Genotype and Genotype x Environment analysis was used to elucidate performance across environments among groups. Ahmadi *et al*. (2012) defined ideal resistant genotypes as those showing the lowest disease severity and absolutely stable across test environments. Cultivar Jesu 91104DL and inbred line $F_{8:9}$ MUTLB1003 had the lowest anthracnose and turcicum leaf blight severities and were the most stable and therefore characterised as ideal genotypes for resistance to the two diseases. Jesu 91104DL and MUTLB01003 could be utilised for dual diseases resistance in sorghum especially in East and Central Africa. Concentric circles were drawn to

help visualize the distance between each genotype and the ideal genotype. Large proportion of the total variation was explained by the first two principal components in all environments and also in the combined analysis. The reaction of sorghum genotypes to anthracnose and turcicum leaf blight varied across environments in Uganda and Sudan, while selecting for dual diseases resistance was equally effective across locations in Uganda but not in Sudan. This could be attributed to dominance of mating types of *E. turcicum* in Uganda (Ramathani *et al*., 2011) unlike Sudan where the dominant mating types are unknown.

Sorghum genotypes Jesu 91104DL and MUTLB01003 carried dual resistance genes to anthracnose and turcicum leaf blight but with low yield compared to Epuripuri. Disease resistance is often assumed to be costly and traits associated with resistance to pathogens may reduce plant fitness (Ayala *et al*., 2001). Brown (2002) has suggested that the lack of understanding of the cost of disease resistant genes on yield performance may hamper the selection of commercially successful resistant cultivars. Efforts are needed to understand and determine the true cost of anthracnose and turcicum leaf blight resistance in sorghum.

In this study, fungal isolates from Sudan were used in Sudan and isolates from Uganda were used in Uganda and therefore, variability and virulence of the different isolates may also have been partly responsible for different genetic reaction of sorghum genotypes evaluated in this study. Further research in the area of race determination in *C. sublineolum* and *E. turcicum* perhaps should take advantages of the low cost DNA sequencing. The use of molecular markers could be used to identify and combine different sources of anthracnose and turcicum leaf blight resistance which may promulgate the useful genotypes and increase resistance to both diseases. The complete understanding of the basis of *C. sublineolum* and *E. turcicum* resistance is still limited and needs to be fully elucidated.

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Chapter Five

Gene action conditioning resistance to anthracnose and turcicum leaf blight in dual infection of sorghum

5.1 Introduction

Identification of the mode of inheritance to the two pathogens is highly required. Mode of inheritance and several sources of resistance to TLB (Reddy and Prasad, 2013) and anthracnose (Ngugi *et al*., 2000) have been identified separately. Genetic resistance to TLB has been reported to be controlled by partial dominance effects (Welz and Geiger, 2000) and qualitative and quantitative effects (Beshir *et al*., 2012), impling that additive effects with genotypes x environment interaction are contributing factor towards the variation in genotype reaction to the pathogen (Ngugi *et al*., 2000). Anthracnose resistance is controlled by dominant genes and the proportions of resistant and susceptible plants in the segregating populations conform to the frequencies expected under the hypothesis of gene-for-gene resistance and dominant gene action (Singh *et al*., 2006). Matiello *et al*. (2012) reported that both anthracnose and TLB resistances were controlled by dominance and additive gene effects.

Breeders often use combining ability to obtain genetic information about a trait of interest from a fixed or randomly chosen set of inbred lines mated in diallel designs (Sleper and Poehlman, 2006). In this study combining ability was used to detect gene actions and to identify parents with high general combining ability (GCA) and hybrids with high specific combining ability (SCA) effects. To develop efficient resistant cultivars with dual resistance, the gene action involved in dual resistance for anthracnose and TLB must be understood. The purpose of this study was, therefore, to understand the dual effects of simultaneous *C. sublineolum* and *E. turcicum* infection on sorghum genotypes and the gene action conditioning resistance to both pathogens.

5.2 Material and methods

Experiments were conducted in the greenhouse and field at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) in central of Uganda during the first rains (April – August) of 2012 and in the greenhouse at Gezira Research Station, Wad Medani, Sudan during the rainy season (August – November) of 2014. MUARIK, a disease pressure site for both diseases (Beshir *et al*., 2012) is at an elevation of 1200 m above sea level (O°28'N and 32°37'E) and Wad Medani is at an elevation of 414 m above sea level (14º41'N and 33º05'E).

Six sorghum cultivars namely HD1, Epuripuri, Sekedo, GA06/106, GA06/18 and MUC007/009 from East Africa were subjected to anthracnose and TLB fungi in order to study their reaction to dual pathogen infection. The cultivars were planted in a split plot design with three replications and at three weeks after planting (Biruma *et al*., 2012) each cultivar was inoculated with four treatments (main plots); *C. sublineolum* only, *E. turcicum* only, both *C. sublineolum* and *E. turcicum*; and the un-inoculated control. The diseases incidence and severity were assessed from five leaf stage (stage 2) (Vanderlip, 1993) till physiological maturity (Dube *et al*., 2010).

Colletotricum sublineolum and *E. turcicum* inoculum was prepared as described by Ramathani *et al*. (2011). Isolates for both pathogens were obtained from Sudan for use in Sudan and from Uganda for use in Uganda. In the case of simultaneous infection, the plants were first inoculated with *E. turcicum* and immediately inoculated with *C. sublineolum*. For the greenhouse experiment, twenty five seedlings of each genotype were incubated in a humid chamber at 22ºC for 48 hours after which the observations were taken (Mittal and Boora, 2005).

Twelve segregating F_2 populations developed using Griffing's method 4 (Griffing, 1956) were used for studying gene action conditioning resistance to both pathogens using randomised

complete block design (RCBD) in a greenhouse at Gezira Research Station, Wad Medani, Sudan. Each genotype was replicated 15 times. Humidity conditions were maintained in the greenhouse using overhead sprinklers. Innoculation was done using infested sorghum grains colonised by the pathogenes, as described by Ramathani (2009). In the case of dual infection, the plants were inoculated by placing a mixture of 20 – 25 air-dried sorghum grains colonised by *C. sublineolum* and *E. turcicum* into the leaf whorls of each plant at vegetative growth stage two (five leaf stage) under greenhouse conditions and at stage three in the field (Vanderlip, 1993). Inoculation was done in the evening hours when dew and ambient temperature were optimal for successful infection (Beshir *et al*., 2012) and was repeated three times at six day intervals to ensure successful infection (Carson, 1995). All required agronomic practices for the crop were followed.

Cultivars and segregating populations were assessed for disease severity two weeks after innoculation and continued until physiological maturity at a weekly interval. Disease severity was computed based on the scale suggested by Ramathani *et al*. (2011). Severity data were subjected to analysis of variance using GenStat 12th Edition (VSN International Ltd., UK). Means were compared using the Fisher's Protected least significant difference test (LSD) at P$\leq$0.05) (Steel and Torrie, 1997). Area under disease progress curves (AUDPC) were computed using the weekly severting ratings (Madden *et al*., 2007). Correlation analysis was performed using combined means of anthracnose and TLB final disease severities, AUDPC and lesion colour number and size. Lesion type was rated as for: (a) chlorotic or tan lesion type; (b) for heterozygote and (c) for pigmented or red lesion type (Beshir, 2011).

F-values for combining ability analysis were computed according to Owolade *et al*. (2006). GCA mean square was tested against SCA mean square and SCA mean square was tested against error mean squares and crosses mean square were tested against error mean square (Vivek *et al*., 2009). These components were used to decide whether GCA or SCA would account for

anthracnose and TLB resistance. Variance components were estimated to determine genetic and environmental effects. Additive ($\sigma^2 A$), dominance ($\sigma^2 D$) and phenotypic ($\sigma^2 P$) variances were calculated from expected mean squares of analysis of variance according to Singh and Chaudhary (2004) as follows:

$\sigma^2 A = 4\ \sigma^2 GCA$
$\sigma^2 D = 4\ \sigma^2 SCA$
$\sigma^2 P = 2\ \sigma^2 GCA + \sigma^2 SCA + \sigma^2 E$

Heritability estimates on plot and entry mean bases were determined based on fixed effects model (Baker, 1978). Broad sense heritability on entry mean basis was referred to as broad sense coefficient of genetic determination, and narrow sense heritability on plot basis was referred to as narrow sense coefficient of genetic determination as follows:

BS-CGD (H^2)= Broad sense coefficient of genetic determination=

$2\sigma^2 GCA + \sigma^2 SCA / 2\sigma^2 GCA + \sigma^2 SCA + \sigma^2 E$

NS-CGD (h^2)= Narrow sense coefficient of genetic determination=

$2\ \sigma^2 GCA / 2\ \sigma^2 SCA + \sigma^2 E$

Where:

$\sigma^2 GCA$ = General combining ability variance.

$\sigma^2 SCA$ = Specific combining ability variance.

$\sigma^2 E$= Environmental error variance component.

GCA effects were calculated and tested for significance from zero using a t-test at 90 degrees of freedom for the error mean square according to Singh and Chaudhary (2004) as follows:

$t - test_{GCA\ effects} = (GCA - 0) / SEM$

Where:

GCA = General combining ability value.

SEM = Standard error of means.

Baker's ratio was used to determine the progeny performance based on the relative importance of GCA and SCA mean squares according to fixed effects model 1 (Baker, 1978). Significance of variance components was determined using t-tests using the standard error of means and standard error of differences according to Dabholkar (1992) as follows:

Baker's Ratio= $2gi^2/ (2gi^2 + sij^2)$

Where:

gi and sij= GCA and SCA mean squares.

5.3 Results

5.3.1 Reaction to dual infection

The ANOVA for reaction to both diseases based on initial and final severities of both diseases were highly significant ($P \leq 0.001$) under greenhouse, but not significant under field conditions (Table 5.1).

Table 5.1: Mean square of combined means for severity of leaf anthracnose and turcicum leaf blight under greenhouse and field conditions at MUARIK (first and second rains of 2012).

Sources of variation	Greenhouse condition					Field condition				
	df	Anthracnose		Turcicum leaf blight		df	Anthracnose		Turcicum leaf blight	
		[a]Severity	[b]AUDPC	[a]Severity	[b]AUDPC		[a]Severity	[b]AUDPC	[a]Severity	[b]AUDPC
Rep	2	139*	8591^{+}	887***	20079***	2	314**	489556***	247**	184388**
Inoculation	3	194^{+}	3564	72	4215	3	23.8	61313^{+}	39	91663
Genotype	16	91	7875^{+}	111	3192	16	53.3	46798^{+}	34	34846
Interaction	48	71	4594^{+}	79***	3306**	47	37.9	29990	29	25491
Residual	126	38	3335	49	1977	87	46.3	35268	44	38143

$^{+}$, *, **, ***= significantly different at $P \leq 0.1$, $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.
a= Final severity was taken 40 days after inoculation; b= Area under disease progress curve.

ANOVA of traits related to anthracnose and TLB is presented in Table 5. 2. Significant differences ($P<0.1$) were observed among genotypes for small lesion area under greenhouse but not under field conditions. Number of large lesions differed significantly ($P<0.01$) among genotypes under field conditions but not under greenhouse conditions. Lesion colour did not vary

significantly among genotypes under greenhouse and field conditions. Under field conditions, simultaneous application with both pathogens' inoculum caused significant ($P<0.1$) differences in lesion colour and large lesion area.

Under greenhouse conditions applying both pathogens increased anthracnose and TLB severities by increasing the size of large lesion indicating that genotypes showed more diseases symptoms because of increased pathogen pressure (Table 5. 3). Anthracnose severity and AUDPC varied significantly ($P<0.05$) when only *C. sublineolum* was applied under greenhouse, while severity and AUDPC were not significantly different under field condition. Inoculation with both pathogens caused significant ($P<0.05$) variation in large lesion area under greenhouse conditions and field conditions and also small lesion area under field conditions.

Table 5. 2: Mean square of combined means for lesion colour and size of turcicum leaf blight and leaf number and area for genotypes under greenhouse and field conditions at MUARIK (first and second rains of 2012).

Sources of variation	Greenhouse conditions								Field conditions							
	df	[a]Lesion colour	Lesion area (cm^2)		Number			Leaf area (cm^2)	df	[a]Lesion colour	lesion area (cm^2)		Number			Leaf area (cm^2)
			Small	Large	Small lesion	Large lesion	Leaf				Small	Large	Small lesion	Large lesion	Leaf	
Rep	2	2.8	$1.3x10^{-5}$	140***	5174	887	12	2750	2	3.0**	$6.8x10^{-5}$	599**	97384***	22146***	13***	31327***
Inoculation	3	0.7^{+}	$1.3x10^{-4+}$	38*	777	118	6^{+}	2903	3	0.3	$3.0x10^{-4}$**	213	8380^{+}	18370***	1	1475
Genotype	16	1.4	$8.2x10^{-5}$	10	445	74	4	2338	16	0.5	$8.9x10^{-5}$	148	4838	6173**	1	6014
Interaction	48	0.9	$5.7x10^{-5}$	13*	455	134	3	2127	47	0.5	$5.7x10^{-5}$	171	4455	2408	2*	4414
Residual	126	0.5	$5.5x10^{-5}$	8	441	93	5	1714	87	0.6	$7.5 x10^{-5}$	127	4036	2255	1	4379

*, **, ***= significantly different at P≤0.05, P≤0.01 and P≤0.001.

a= Rating of chlorotic or tan lesion type.

Table 5. 3: Reaction of sorghum genotypes to *C. sublineolum* and *E. turcicum* dual infection under greenhouse and field conditions at MUARIK (first and second rains of 2012).

Trait	Greenhouse conditions						Field conditions					
	ANT	TLB	ANT and TLB	Control	SED (P≤0.05)	LSD (P≤0.05)	ANT	TLB	ANT and TLB	Control	SED (P≤0.05)	LSD (P≤0.05)
ANT [a] final severity (%)	4.16	1.38	1.74	0.74	1.54	3.06	3.66	3.73	3.43	3.75	0.42	0.82
ANT [b] AUDPC	0.66	0.47	0.53	0.38	0.13	0.27	0.82	0.84	0.91	0.99	0.09	0.18
TLB [a] final severity (%)	2.11	2.17	2.94	2.03	0.61	1.20	1.94	1.89	2.08	1.75	0.45	0.89
TLB [b] AUDPC	0.82	0.84	1.03	0.89	0.08	0.16	0.41	0.50	0.51	0.56	0.09	0.18
Small TLB lesion area (cm^2)	0.35	0.44	0.34	0.36	0.07	0.13	0.01	0.01	0.00	0.00	0.00	0.00
Large TLB lesion area (cm^2)	1.12	2.30	4.22	3.47	1.05	2.08	3.11	4.68	3.20	4.28	0.90	1.78
Large lesion number	26.2	18.4	25.1	19.4	4.50	8.94	2.95	3.04	3.23	3.28	9.40	18.6

ANT= Anthracnose inoculum; TLB= Turcicum leaf blight inoculum.

a= Final severity was taken 40 days after inoculation; b= Area under disease progress curve.

5.3.2 Correlation analysis

Correlation of anthracnose and TLB severities, AUDPC and their related disease components are presented in Table 5. 4. Anthracnose severity and AUDPC was negatively correlated though not significant, with small lesion number while it positively significantly ($P \leq 0.01$) correlated with large lesion number and leaf area indicating that genotypes which showed anthracnose symptoms had few small lesions. Negative but non-significant correlation was found between anthracnose and TLB severities and AUDPC. There was significant ($P<0.05$) correlation between TLB severity and AUDPC and between small lesions and large lesions area. Small lesion number correlated significantly ($P<0.05$) negatively with large lesion number indicating that genotypes which showed small lesions had few large lesions. There was no significant correlation between lesion colour and anthracnose and TLB severity and AUDPC indicating that high anthracnose severity and AUDPC had no significant relationship with tan or red lesion colour. The lesion colour was significantly ($P<0.001$) positively correlated with TLB disease severity and AUDPC indicating that genotypes showing high severity and high AUDPC were tan lesion coloured.

Table 5. 4: Correlation of anthracnose and turcicum leaf blight disease severity, AUDPC, leaf area, lesion colour, number and size evaluated under greenhouse and field conditions at MUARIK (first and second rains of 2012).

		Anthracnose			Turcicum leaf blight			Related traits				
		[a]Initial severity	[b]Final severity	[c]AUDPC	[a]Initial severity	[b]Final severity	[c]AUDPC	[d]Lesion colour	Small lesion area (cm^2)	Large lesion area (cm^2)	Small lesion No	Large lesion No
Leaf anthracnose	[a] Initial severity	1.00										
	[b] Final severity	0.85***	1.00									
	[c] AUDPC	0.96***	0.91***	1.00								
Turcicum leaf blight	[a] Initial severity	0.52**	0.33	0.45**	1.00							
	[b] Final severity	0.01	-0.15	-0.03	0.26^{+}	1.00						
	[c] AUDPC	0.45**	0.33	0.41*	0.62***	0.51**	1.00					
Related traits	[d] Lesion colour	0.25	0.11	0.20	0.32^{+}	0.51**	0.43**	1.00				
	Small lesion area	0.54***	0.48**	0.56***	0.03	0.03	0.19	-0.02	1.00			
	Large lesion area	0.79***	0.70***	0.84***	0.56***	0.15	0.62***	0.30	0.43**	1.00		
	Small lesion No	-0.17	-0.01	-0.11	-0.20	-0.03	-0.41*	-0.13	-0.04	-0.25^{+}	1.00	
	Large lesion No	0.91***	0.75***	0.87***	0.40*	0.08	0.59***	0.29^{+}	0.63***	0.82***	-0.27^{+}	1.00
	Leaves No	-0.25	-0.23	-0.20	-0.13	-0.18	-0.07	-0.43**	-0.26^{+}	-0.10	0.02	-0.19
	Leaf area (cm^2)	0.92***	0.81***	0.92***	0.45**	0.00	0.52**	0.28	0.58***	0.84***	-0.31^{+}	0.93***

+, *, **, ***= significantly different at P≤0.1, P≤0.05, P≤0.01 and P≤0.001.

a, b= Initial and final severity was taken 14 and 40 days after inoculation respectively. c= Area under disease progress curve; d= Rating of chlorotic or tan lesion type.

5.3.3 Estimates of combining ability and heritability

The estimate of broad sense heritability for anthracnose (0.73) and TLB (0.88) were high indicating that greater proportion of the total phenotypic variance observed among the genotypes for resistance was due to genetic action and low environmental influences (Table 5. 5). However, the narrow sense heritability for anthracnose was low (0.42) and for TLB was moderate (0.65), indicating that dominance or epitasis played a major role in the inheritance of the resistance. The results also showed that the mean square due to GCA and SCA were positively significant ($P<0.05$) for anthracnose suggesting that the parents and their populations performed differently for resistance. These results indicated that under high pathogen pressure, there was variable performance for populations and their parents. High non - significant GCA and SCA mean squares were observed among crosses for TLB severity thus indicating that the parents and their populations performed similarly for resistance. Higher SCA variance component (σ^2SCA) among populations was observed than σ^2GCA for anthracnose severity while the opposite was observed for TLB severity. Additive variances were significant ($P<0.001$) for anthracnose severity, and not significant for TLB. Dominance variances for anthracnose were higher than for TLB. Baker's ratio for turcicum leaf blight (0.59) was higher than the one of anthracnose (0.40). Baker's ratio, broad and narrow sense heritability for anthracnose were less than those for TLB.

Estimation of GCA effects indicated that cultivars HD1 and Epuripuri had the lowest significant ($P<0.01$) but negative GCA effects for anthracnose, indicating resistance to anthracnose, while the cultivars GA06/106 and MUC007/009 had the highest significant ($P<0.05$) but positive GCA (Table 5. 6.).

Table 5. 5: Mean squares, variance components, Baker's ratio and heritability of F_2 populations for resistance to anthracnose and turcicum leaf blight evaluated at Wad Medani under greenhouse condition (rains of 2014).

Source of variation	df	Anthracnose severity[a]		Turcicum leaf blight severity[a]	
		Mean squares	Variance components	Mean squares	Variance components
Population	11	134.5***		266.7	
GCA	5	20.6***	3.288	72.0	13.5
SCA	6	8.9*	4.800	14.3	9.5
Residual	90	4.1	4.100	4.8	4.8
[b] σ^2 A		13.15		53.79	
[c] σ^2 D		19.17		38.16	
[d] σ^2 P		15.51		41.20	
[e] BS-CGD		0.733		0.884	
[f] NS-CGD		0.424		0.652	
Baker's Ratio		0.407		0.585	

* and ***= significantly different at P≤0.05 and P≤0.001.

a= Final severity was taken 40 days after inoculation. b=Variance due to additive effects. c= Variance due to non-additive (Dominance) effects. d= Phenotypic variance. e= Broad sense coefficient of genetic determination. f= Narrow sense coefficient of genetic determination.

b, c, d, e and f were computed according to fixed effect model.

The cultivars GA06/106 and MUC007/009 had the lowest GCA for TLB severity indicating resistance to *E. turcicum*. Contrastingly, the cultivars HD1 and Sekedo had positive and significant (P<0.05) GCA effects for indicating susceptibility to *E. turcicum*.

Table 5. 6: Estimates of general combining ability (GCA) effects for reactions to anthracnose and turcicum leaf blight of F_2 populations evaluated in Wad Medani under greenhouse condition (rains of 2014).

Parent	Anthracnose		Turcicum leaf blight	
	Final severity[a]	GCA	Final severity[a]	GCA
Epuripuri	5.8	-2.3*	14.5	1.7
GA06/106	14.7	3.1***	17.9	-5.3
GA06/18	5.4	-0.8	9.0	-2.1
HD1	5.1	-2.7**	12.4	6.8
MUC007/009	22.2	2.4*	13.5	-3.4
Sekedo	7.5	0.3	14.4	6.7
SEij	0.6	1.1	0.7	57.1
SED (P≤0.05)	0.9		0.9	
LSD (P≤0.05)	1.7		1.8	

*, **, *** Significantly different at P≤0.05, P≤0.01 and P≤0.001.

a= Final severity was taken 40 days after inoculation.

Cultivars GA06/18 had non – significant and negative GCA effects for anthracnose and TLB suggesting resistance to both diseases. Non – significant but negative SCA estimates among F_2 segregating populations were observed on seven populations for anthracnose and five populations for TLB out of 12 populations studied (Table 5. 7). The Populations deriving from the crosses GA06/106 x MUC007/009 and GA06/18 x HD1 showed significant ($P<0.05$) positive SCA estimates for anthracnose severity indicating susceptibility to *C. sublineolum*. Based on the lowest anthracnose severity and non-significant negative SCA estimates, two superior populations GA06/106 x HD1, and MUC007/009 x HD1 were found. Relatedly, for TLB, non-significant negative SCA estimates were obtained in two populations GA06/18 x HD1, and GA06/106 x Epuripuri. Overall, the F_2 populations MUC007/009 x Epuripuri and MUC007/009 x HD1 showed non – significant negative SCA estimates for both diseases severities.

Table 5. 7: Estimates of specific combining ability for resistance to dual infection of anthracnose and turcicum leaf blight in F_2 populations evaluated at Wad Medani (rains of 2014).

Segregating population (F_2)	Anthracnose severity[a]	Turcicum leaf blight severity[a]
Epuripuri x GA06/18	0.0	2.4
Epuripuri x HD1	2.1	2.8
Epuripuri x Sekedo	-1.0	0.6
GA06/106 x Epuripuri	-0.4	-4.9
GA06/106 x GA06/18	-1.9	1.2
GA06/106 x HD1	-2.9	3.7
GA06/106 x MUC007/009	4.2**	0.6
GA06/106 x Sekedo	1.0	-0.6
GA06/18 x HD1	3.2*	-5.2
MUC007/009 x Epuripuri	-0.6	-0.9
MUC007/009 x GA06/18	-1.2	1.6
MUC007/009 x HD1	-2.4	-1.3

*, **= Significantly different at $P\leq0.05$ and $P\leq0.01$.
a= Final severity was taken 40 days after inoculation.

5.4 Discussions

5.4.1 Reaction to dual infection

The results indicated that resistant genotypes for anthracnose showed resistant symptoms for TLB indicating that loci conditioning resistance to both diseases could be collocated together. Genotypes with small leaves, a few and small lesion showed less anthracnose and TLB severities compared to those with large leaves, many and large lesions. This indicated that lesion size was correlated with diseases severities and that few and small lesions were linked with diseases resistance. Thus, small lesion trait could be used to characterise and select for resistance to anthracnose and TLB in sorghum. Lesion size was used in previous studies to characterise resistance to TLB in maize (Welz and Geiger, 2000) and sorghum (Reddy and Prasad, 2013). Genotypes did not show significant variations for lesion colour under both conditions. However, sorghum leaves and stalks of some genotypes for example MUC007/009, GA06/106 and GA06/18 accumulated red pigments upon wounding while others did not. Correlation of lesion colour and diseases resistance showed no evidence that the red - pigmented plants were better protected against pathogen attack or were more resistant than tan plants as was previously suggested by Dykes *et al*. (2005) and Funnell-Harris *et al*. (2013).

5.4.2 Estimates of combining ability and heritability

The study also showed that additive and non – additive nature (dominance and epitasis) gene actions conditioned resistance to both diseases. The role of non – additive gene action for anthracnose resistance was further confirmed by low Baker's ratio (0.4) (Falconer and Mackay, 1996). Additive gene action also played a significant ($P<0.05$) role in the inheritance of resistance to anthracnose in this study and was more important than non – additive gene action in conditoining resistance to TLB. It was observed that there is relationship between physiological race type of and the components of resistance as incubation period, lesion

expansion rate, lesion number, lesion size (Ramathani, 2009). In Uganda and Sudan, no studies on *C. sublineolum* race type were reported hence there is limited information of the pathogen in sorghum (Sserumaga *et al*., 2013). While studies on *E. turcicum* reported that race 0 (Adipala *et al*., 1993) existed in Uganda however, Ramathani *et al*. (2011) reported that new races might have evolved from race 0. Predominance of race 0 in East and Central Africa for which additive gene action is critical. The role of additive gene action was confirmed by the moderately high Baker's ratio (Falconer and Mackay, 1996). Similar results were previously reported on sorghum (Reddy and Prasad, 2013) and maize (Sigulas *et al*., 1988).

Parent GA06/18 had negative GCA for anthracnose and TLB though non – significant suggesting that this genotype could be used in sorghum breeding. Some F_2 populations exhibited resistance to anthracnose and others to TLB. However, GA06/106 x Epuripuri and MUC007/009 x Epuripuri showed negative SCA effects for both diseases indicating that these two crosses would yield populations for selection for resistance to anthracnose and TLB and could be utilized for selecting dual resistant cultivars.

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Chapter Six

Simple sequence repeat markers associated with anthracnose and turcicum leaf blight resistance in sorghum

6.1 Inroduction

There is paucity of information on dual resistance in sorghum to both diseases (Ngugu *et al*., 2000). However studies conducted separately for each disease show that both qualitative and quantitative resistance mechanisms condition resistance (Paterson, 2008; Tesso *et al*., 2012; Rajeshwar *et al*., 2014). Deployment of quantitative resistance for management of both diseases will invariably reduce pressure for evolution of new pathogen races (McDonald and Linde, 2002; Okori *et al*., 2015). However breeding for quantitative resistance is fraught with challenges of uneven disease pressure and or erratic epidemics especially under natural field conditions. Thus the use of molecular markers has been suggested as a better approach to breed for such complex traits. Mohan *et al*. (2010) show that markers linked to resistance loci can improve precision in selection for resistance loci when resistance loci have been mapped and associated molecular markers. By mapping quantitative trait loci (QTL), the roles of specific resistance loci can be accessed, race-specificity of partial resistance loci assessed (Mittal and Boora, 2005) and interactions between resistance loci and plant development (Paterson, 2008) and the role of environment elucidated (Mohan *et al*., 2010).

In different crop species, QTL associated with disease resistance have been mapped and are being introgressed using line conversion breeding strategies such as backcross breeding (Varshney *et al*., 2014). In cereals molecular markers have been used

successfully for the isolation of a number of important plant loci, including loci for resistance to target leaf spot, zonate leaf spot and drechslera leaf blight in sorghum (Mohan *et al*., 2009); turcium leaf blight, grey leaf spot and southern leaf blight in maize (Ali *et al*., 2013). Given that both diseases are endemic in many tropical countries and indeed germplasm either or both diseases observed, the presence of dual resistance to both diseases cannot be precluded. Presence of and the co-localization of QTL that confer resistance to anthracnose and TLB is desirable and the identification of molecular markers linked to them could improve breeding for resistance. The aim of the study reported in this paper was to identify SSR markers associated with anthracnose and TLB resistance in sorghum for future use in markers assisted introgression.

6.2 Material and methods

6.2.1 Experimental sites

Molecular characterisation of $F_{8:9}$ sorghum population was carried out at the Biotechnology and Biosafety Research Centre, Agricultural Research Corporation (ARC), Sudan. Whilst, phenotypic characterisation was conducted under field conditions in Uganda at Makerere University Agricultural Research Institute Kabanyolo (MUARIK), the National Semi-Arid Resources Research Institute (NaSARRI) and at Gezira Research Station of ARC at Wad Medani and Wad Elturabi in Sudan. All these locations are hotspots for both anthranose and TLB (Ramathani *et al*., 2011; Beshir *et al*., 2015).

6.2.2 Phenotypic characterisation

6.2.2.1 Development of the recombinant inbred lines

One hundred and twenty six $F_{8:9}$ recombinant inbred lines were developed from a cross of MUC007/009 (a Ugandan accession and source of resistance to TLB but susceptible to anthracnose) and Epuripuri (a commercial variety and source of resistance to anthracnose but susceptible to TLB). The $F_{8:9}$ lines were planted using an alpha lattice design with three replications in all locations.

6.2.2.2 Field techniques for inoculation and disease evaluation

On average 20 to 25 infested air-dried sorghum kernels containing *C. sublineolum* and *E. turcicum* inoculum were placed into the leaf whorls (Ramathani, 2009) at vegetative growth stage two (five leaf stage) (Vanderlip, 1993). Artificial inoculation was used in MUARIK and NaSARRI, while materials were subjected to natural infestation in Wad Medani and Wad Elturabi using the infector rows of Epuripuri and MUC007/009 suseptible varieties planted two weeks befor planting the experiments.

6.2.2.3 Phenotypic data characterisation

Disease severities were assessed at weekly interval starting two weeks after innoculation until physiological maturity based on the scale described by Ramathani (2009). Area under disease progress curves (AUDPC) were computed using the weekly ratings (Madden *et al.*, 2007). Data were also taken on lesion type (Beshir, 2011). MUC007/009 and Epuripuri had distinctly different lesion types from each other (Beshir, 2011); MUC007/009 had narrow lesions with a red border and Epuripuri had wider lesions without a red border. The frequency of the resistant lesion type and the susceptible lesion type was assessed among the 126 RILs. Plot means were

calculated and used for the statistical analysis of the field data. Means were computed and subjected to analysis of variance using GenStat 12th Edition (VSN International Ltd., UK) (Steel and Torrie, 1997).

6.2.3 Genotypic data characterisation and analysis

6.2.3.1 SSR selection

DNA was isolated from two week old leaf tissues of the plants (Edwards *et al*., 1991). Fifty eight SSRs, obtained from the consensus genetic map of sorghum obtained through Diversity Array Technology (DArT) was used (Mace *et al*. (2009). These SSRs were synthesized at the Department of Molecular and Cellular Biology, University of Cape Town, South Africa. All SSRs were screened for polymorphism between the parental lines. Twelve of the 58 SSRs were polymorphic between the two parents, representing 21% of the tested SSRs. Only 7 SSRs i.e. Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95 had clearly distinguishable bands on agarose gels the media available and were therefore used for further genetic analysis (Apendix 3). These seven SSRs were polymorphic between Epuripuri and MUC007/009. PCR amplification was performed as described by Beshir (2011). For each SSR, the polymorphic information content (PIC) values was used to calculate the genetic diversity according to the formula:

$$PIC = 1 - \sum_{i=1}^{k} xi^2$$

Where k is the total number of alleles detected for an SSR and *xi* is the frequency of the i^{th} allele of the SSR loci (Abu Assar *et al*., 2005).

6.2.3.2 Genotypic scoring

A qualitative score scheme was used to score for presence or absence of resistance

alleles in homozygous or heterozygous state in the progeny. Recombination inbred lines carrying the susceptibility alleles at the polymorphic SSR loci were given a zero scored, while those carrying the resistant allele were given score of 1. Segregating progeny carrying alleles from both parents (heterozygote) were given a score of 2.

6.2.4 Data analysis

The relationship between molecular markers and phenotypic scores was assayed using single marker analysis to identify SSR that were significantly associated with anthracnose and TLB symptoms like lesion type and severity scores. Chi square ($\varkappa^2$) was used to test the goodness of fit of observed resistance segregation patterns for anthracnose and TLB to expected segregation ratios among RILs genotypic data at $p \leq 0.05$. ANOVA and regression analysis were used to detect the significance of the three allelic groups of each SSR (Steel and Torrie, 1997). The allelic groups of RILs which carried the allele from Epuripuri (the source of resistance to anthracnose) were scored as 0, those carrying the allele from the MUC007/009 (the source of resistance to TLB) were given a genotypic score as 1 and those carrying alleles from both parents (heterozygote) were given a genotypic score as 2. All data were analysed using GenStat Discovery Edition 12.

6.3 Results

6.3.1 Phenotypic data: Reaction to anthracnose and TLB

Frequency distributions of the final severity of anthracnose and TLB (taken at 80 days after planting), AUDPC and lesion type of 126 RILs are presented in Figure 6. 1. Segregation patterns for anthracnose were discontinuous with evidence of

transgressive segregation for resistance to anthracnose (Figure 6. 1A and B). AUDPC for anthracnose a product of integration of the epidemics, was similar to final lesion severity ratings except that mots progeny were moderately to highly resistant (Figure 6. 1B). In the case of TLB, was also discontinuous with but less skewered than was the case for anthracnose (Figure 6. 1C). AUDPC for TLB interestingly, exhibited a continuous variation, although slightly skewed for resistance (Figure 6. 1C and D). There was no strong evidence of transgressive segregation against the resistant parent MUC007/009 background. The lesion type i.e. wild type- tan and non-wild type- have reddish halo around each lesion had on main peak for the number of individual RILs the vast majority of individual belonging to the class having a score of 1.6 to 2.0. This class had a mixture of tan to reddish lesions (Figure 6. 1E). Analysis of variances of AUDPC, final severity ratings for anthracnose and TLB and AUDPC, as well as lesion type are presented in Table 6. 1. No significant differences ($P>0.05$) were observed among RILs for final severity ratings and AUDPC of both diseases. However, highly significant differences among RILs ($P\leq0.01$), were observed for lesion colour. ANOVA revealed significant ($P\leq0.001$) effect of environments on the development of both TLB and anthracnose but not for lesion colour.

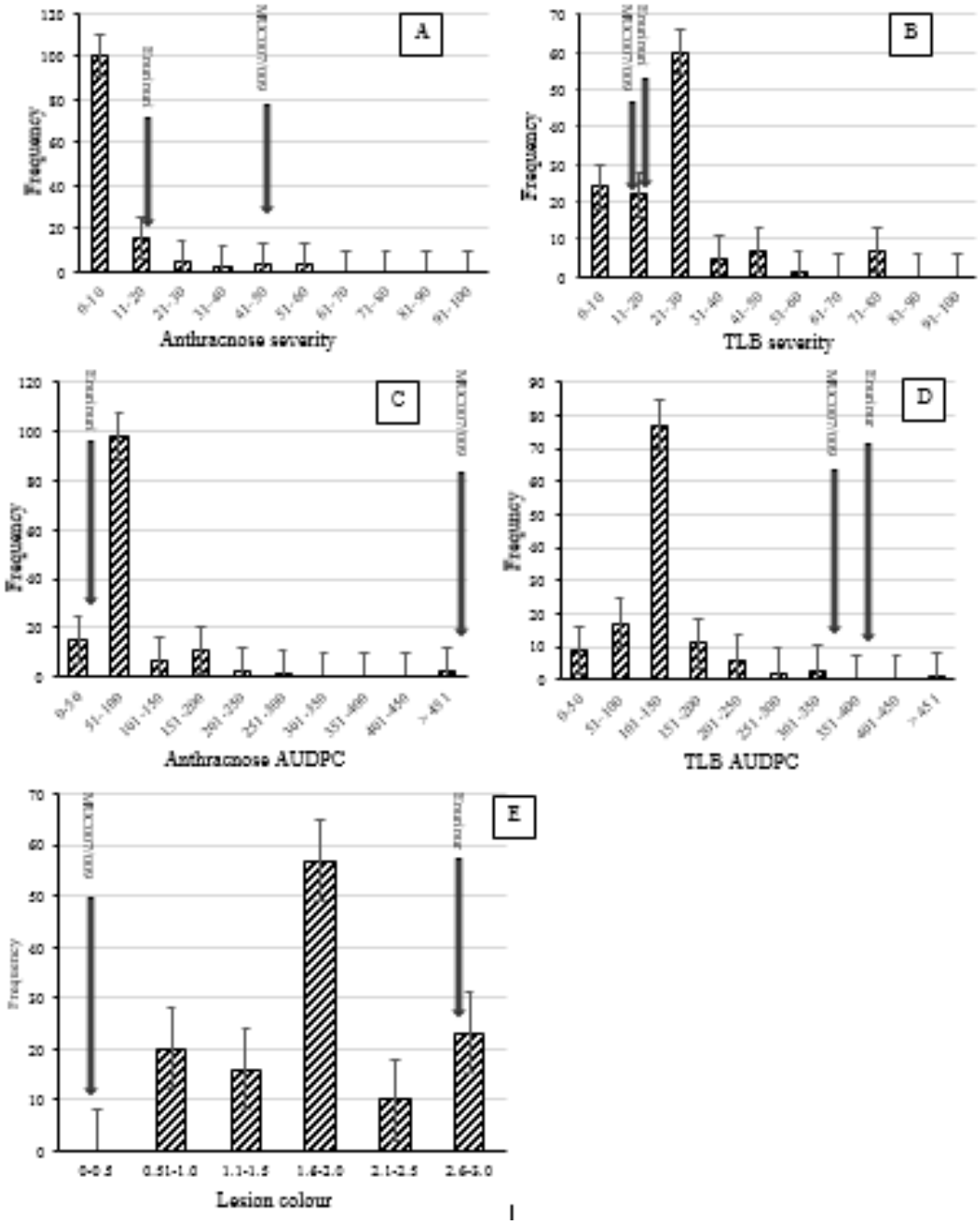

Figure 6. 1: Frequency distribution of mean disease scores in recombinant inbred lines derived from parental lines MUC007/009 and Epuripuri across Uganda and Sudan (rains of 2012 and2014).

Bars show the standard errors (±SE). A= Anthracnose severity, B= TLB severity, C= Anthracnose AUDPC, TLB AUDPC and E= Lesion colour.

Table 6. 1: Descriptive statistics anthracnose and TLB in 126 RIL mapping population under Uganda and Sudan field conditions (rains of 2012 and 2014).

Trait	Parents		Check	RILs (n= 126)			Mean squares			
	Epuripuri	MUK0 07/ 009	Butana	Min.	Max	SED (P<0.05)	Genotype	Environment	GxE	Residual
Anthracnose										
Severity[a]	0.83	47.8	5.0	0.0	52.9	17.2	176.6	920.8***	105.8	13.0
AUDPC[b]	20.4	549.0	247.0	0.0	549.0	178.3	28338	178666***	22527	3362
Turcicum leaf blight										
Severity[a]	20.2	14.6	15.9	0.0	72.2	19.6	88.0	1168.6***	79.8	53.3
AUDPC[b]	380.7	319.5	181.0	0.0	498.3	168.1	11946.0	290572.0***	15210.0	6091
Lesion colour[c]	3.0	1.0	2.0	0.7	3.0	0.9	0.8**	0.4	0.4	0.3

RILs= Recombinant inbred lines. a= Final severity was taken 40 days after inoculation; b= Area under disease progress curve; c= Rating of chlorotic or tan lesion type.
** and ***= significantly different at P≤0.01 and P≤0.001.

6.3.2 Genotypic data: Segregation of polymorphic simple sequence repeat markers

Polymorphic bands, total bands, major allele frequency and PIC frequencies per primer pairs are presented in Table 6. 2. Polymorphic SSRs were located in linkage groups 1, 2, 4, 5, 6 and 7 based on sorghum map reported by Mace *et al*. (2009). Seven SSRs pairs yielded 1046 alleles, and total number of alleles per marker varied between 159 and 132. Primer Xtxp201 had the highest PIC value (0.59) while Xtxp95 had the lowest (0.44). The major allele frequency varied between 0.39 and 0.61 and gene diversity ranged between 0.54 and 0.66.

Distribution of RILs carrying MUC007/009, heterozygote and Epuripuri alleles, respectively, among the polymorphic SSRs (Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95) are presented in Figure 6. 2. The SSRs Xtxp25 and Xtxp95 had the least frequency for heterozygote alleles among the RILs while Xtxp201 had the highest frequency. The SSR Xtxp303 had the highest homozygote allele frequency for Epuripuri (source of resistance to anthracnose but susceptible to TLB), while Xtxp177 had the highest homozygote allele frequency for MUC007/009 (source of resistance to TLB). The genotypic segregation patterns of MUC007/009, Epuripuri and heterozygote alleles showed that resistance to TLB was quantitative (continuous), whilst resistance to anthracnose was mainly qualitative (discontinuous) similar to what was observed for the phenotypic data.

Table 6. 2: Summary of 7 polymorphic SSR markers used to screen 126 RIL mapping population (MUC007/009 x Epuripuri).

RILs (n= 126)	Xtxp302	Xtxp25	Xtxp201	Xtxp177	Xtxp303	Xtxp295	Xtxp95
SB Linkage Group [a]	1	2	2	4	5	7	6
No of RILs with MUK007/009 allele	35	62	49	77	31	43	65
No of RILs with Epuripuri allele	62	56	34	31	68	50	55
No of RILs with Heterozygote alleles	29	8	43	18	27	33	6
No of total alleles	155	134	169	144	153	159	132
Polymorphic information contents	0.56	0.46	0.59	0.48	0.54	0.58	0.44
Major allele frequency	0.49	0.49	0.39	0.61	0.53	0.40	0.52
Gene diversity	0.63	0.56	0.66	0.55	0.61	0.66	0.54

a= Linkage groups were based on the sorghum genome map published by Mace *et al*., 2009.

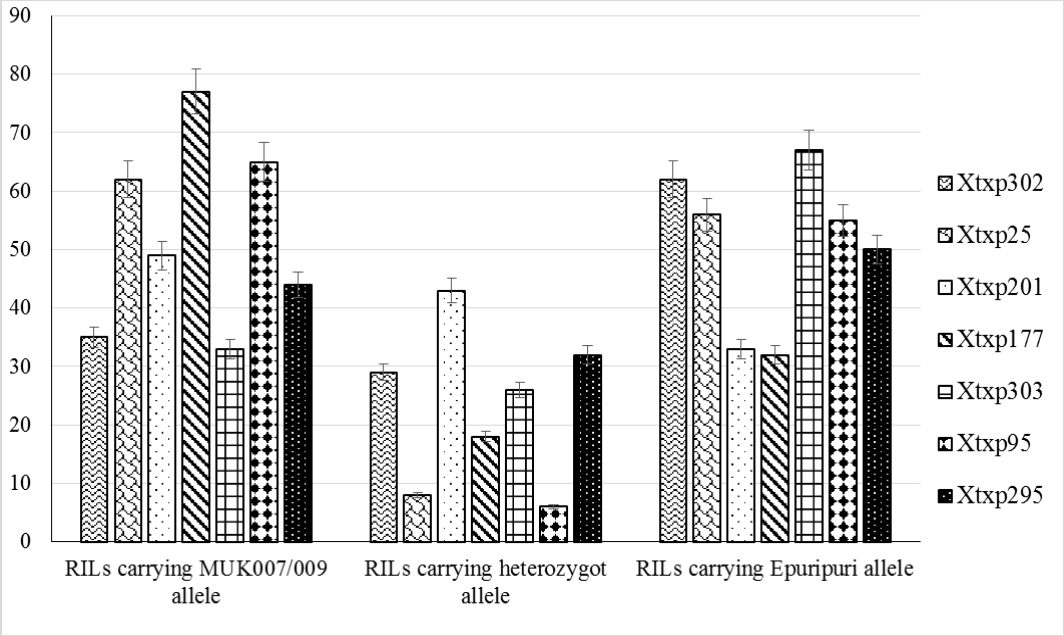

Figure 6. 2: Distribution of frequencies of recombinant inbred lines carrying MUC007/009, heterozygote and Epuripuri alleles, respectively, of the polymorphic SSRs Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95.
Bars show the standard errors (±SE) for SSRs markers at p<0.05.

6.3.3 Single marker analysis for polymorphic SSR markers

Single marker analysis and segregation pattern for SSR Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95 are presented in Table 6. 3. There was a highly significant association (P<0.01) among the SSRs Xtxp201, Xtxp177 and Xtxp303 and anthracnose severity. The SSRs Xtxp25, Xtxp201, Xtxp303, Xtxp295 and Xtxp95 were significantly associated with TLB severity at (P<0.1). The SSRs Xtxp201 and Xtxp303 were significantly ($P \leq 0.05$) associated with both anthracnose and TLB resistance. Single marker analysis for SSRs Xtxp201 and Xtxp303 showed significant (P<0.05) association among the allelic groups for anthracnose and TLB severities in the RILs. SSR Xtxp302 did not show association either with anthracnose or TLB severity.

Table 6. 3: Single marker analysis and segregation pattern of the seven polymorphic SSR markers among 126 RIL mapping population (MUC007/009 x Epuripuri) under Uganda and Sudan field conditions (rains of 2012 and 2014).

Source of variation	Anthracnose				Turcicum leaf blight			
	AUDPC [a]		Severity [b]		AUDPC [a]		Severity [b]	
	MS	F test	MS	F test	MS	F test	MS	F test
Xtxp302	103.0	0.1	52.4	0.6	5009.0	1.3	166.2	0.6
Xtxp25	215.0	0.1	71.5	0.9	19657.0**	5.3**	456.1	1.8
Xtxp201	3967.0	1.1	222.5*	2.9*	7303.0+	1.9+	256.0	1.0
Xtxp177	16480.0**	4.9**	407.3**	5.3**	4659.0	1.2	293.4	1.1
Xtxp303	3714.0	1.1	284.5*	3.6*	10539.0+	2.7+	754.5*	3.0*
Xtxp295	1740.0	0.5	69.0	0.8	2023.0	0.5	1383.4**	5.7**
Xtxp95	35.0	0.1	4.6	0.1	6223.0	1.6	697.1+	2.8+

[+], *, **, ***= significantly different at P≤0.1, P≤0.05, P≤0.01 and P≤0.001.
a=Area under disease progress curve; b= Final severity was taken 40 days after inoculation.

Goodness of fit results of the polymorphic SSRs to the expected segregation ratios among RILs genotypic data at $p<0.05$ using chi square test are presented in Table 6. 4. Only SSRs Xtxp25 and Xtxp201 had non-significant chi square results with two loci having a segregation ratio of 9:7, indicative of two loci in epistasis dominant gene action for anthracnose. While SSRs Xtxp302, Xtxp25, Xtxp295 and Xtxp95 were non-significant with for the two loci segregation ratio of 9:7, similarly indicative of two loci epistasis dominant gene action for TLB. Other, SSRs such as Xtxp177 and Xtxp303 had high significant ($p<0.001$) chi square results implicating more than two loci in dual resistance to TLB and anthracnose. In general, all polymorphic SSRs had no goodness of fit with the genotypic segregation ratios for both diseases.

Table 6. 4: Chi squares and segregation patterns of the seven polymorphic SSR loci among 126 RILs under Uganda and Sudan field conditions (rains of 2012 and 2014).

No of loci	Segregation pattern	Segregation ratio	Xtxp302	Xtxp25	Xtxp201	Xtxp177	Xtxp303	Xtxp295	Xtxp95
Anthracnose (source of resistance was Epuripuri):									
One locus	Dominant	1R:2H:1S	48. 27***	96.60***	16.14***	97.87***	61.81***	31.08***	104.73***
	Recessive	1S:2H:1R	48.27***	96.60***	16.14***	97.87***	61.81***	31.08***	104.73***
Two locus	Dominant epistasis	9R:7S	13.06***	1.52	1.05	14.83***	15.79***	3.99*	3.14*
	Dominant epistasis	15R:1S	99.66***	396.80***	229.79***	646.05***	85.50***	176.76***	442.01***
	Recessive epistasis	9R:4H:3R	6.79*	82.99***	51.69***	147.70***	4.89*	23.73***	96.66***
	Recessive epistasis	9R:3H:4S	74.97***	212.16***	96.35***	714.59***	77.77***	63.00***	288.78***
Turcicum leaf blight (source of resistance was MUC007/009):									
One locus	Dominant	1R:2H:1S	48.27***	96.60***	16.14***	97.87***	61.81***	31.08***	104.73***
	Recessive	1S:2H:1R	48.27***	96.60***	16.14***	97.87***	61.81***	31.08***	104.73***
Two locus	Dominant epistasis	9R:7S	1.52	0.02	15.18***	17.91***	4.55*	0.85	0.01
	Dominant epistasis	15R:1S	396.80***	313.70***	85.94***	78.43***	473.50***	240.36***	300.80***
	Recessive epistasis	9R:4H:3R	80.69***	63.01***	14.67***	9.28**	100.84***	39.64***	62.80***
	Recessive epistasis	9R:3H:4S	200.84***	128.65***	66.39***	71.70***	315.34***	73.86***	127.95***

R is resistant; H is heterozygote and S is susceptible.

6.4 Discussion

6.4.1 Reactions to anthracnose and TLB

Inheritance of resistance to dual infection by *C. sublineolum* and *E. turcicum* in sorghum is less understood. Resistance to either disease have however been described as either qualitative or quantitative (Tesso *et al*., 2012; Reddy and Prasad, 2013). In this study, we investigated the reaction of sorghum to dual infection by *C. sublineolum* and *E. turcicum* in recombinant inbred lines segregating for resistance and susceptibility to both diseases. Analysis of variance revealed that highly significant differences ($P \leq 0.001$) among environments i.e. MUARIK and NaSARRI in Uganda and Wad Medani and Wad Elturabi in Sudan suggesting that the RILs performed differently and that the environments were effective in reducing experimental errors. However, sensitivity of genotype by environmental interactions are common especially when quantitative inheritance is present (Geiger and Heun, 1989). Analysis of the segregation patterns of RILs provide strong evidence for additive gene action for both diseases with transgressive segregation for resistance to both diseases. Segregation patterns as shown in the histograms show that both parents MUC007/009 and Epuripuri carry minor loci or alleles for resistance, different from each other but that in an additive manner contribute to resistance. This is positive given that selection pressure could be easily deployed to identify novel material with dual resistance to both foliar diseases. The general shape of the histograms confirm resistance to anthracnose as qualitative as previously reported (Costa *et al.*, 2011) and quantitative for TLB (Reddy and Prasad, 2013).

6.4.2 Association of SSRs to anthracnose and TLB

In this study, four SSRs had PIC higher than 0.5 and the rest of SSRs had a PIC between 0.44 and 0.48. Polymorphism of loci are considered high if values are greater than 0.5 or

between 0.5 - 0.25 (Abu Assar *et al.*, 2005). Thus nearly all of the SSRs used in this study i.e. Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95 can provide contribute substantial information for the genetics and breeding of sorghum. These SSRs multiple loci to diseases have genetic mapped (Kong *et al*., 2000; Mohan *et al*., 2010). In this study we find that the SSRs Xtxp25 and Xtxp201 segregated in a manner consistent with two loci with (epistasis and dominant) for anthracnose and the SSRs Xtxp302, Xtxp25, Xtxp295 and Xtxp95 were associated with segregation of two loci (epistasis and dominant) for TLB. Interestingly, the marker Xtxp95 had highly significant association between the allelic groups for anthracnose and TLB. It has been suggested that SSR Xtxp95 among others in the sixth linkage group could harbour a cluster of disease resistance to diverse fungal pathogens (Mohan *et al*., 2009). The same region is in synteny with linkage group four of rice and group two of maize linkage (Young, 1996). Thus, the information gained from this study and others, suggests that this is a highly conserved locus among gramineae essential for protection against especially fungal pathogen. These SSRs markers could this provide additional tools for marker assisted breeding for anthracnose and TLB and map-based isolation of multiple disease resistant loci in sorghum.

6.4.3 Co-segregation of anthracnose and TLB resistant loci

The SSR Xtxp25 exhibited dominant epistasis for resistance to anthracnose and TLB. This particular was selected from linkage group two of sorghum that has been reported to have the resistant loci for TLB in sorghum (Martin *et al*., 2011). In general, disease resistance loci cluster on different chromosomes as observed in other crops such as maize (Ali *et al.*, 2013). In deed the SSRs Xtxp25 and Xtxp201 for anthracnose and Xtxp302, Xtxp25, Xtxp295 and Xtxp95 for TLB were associated

with dominant epistasis and were distributed across sorghum genome with high PIC and gene diversity frequencies. Other studies show a high association for the same SSRs to resistance in maize to the foliar diseases TLB and grey leaf spot on maize (Paterson, 2008; Mace *et al*., 2009; Mohan *et al*., 2010; Ali and Yan, 2012). In maize resistance QTL associated with TLB, grey leaf spot and southern leaf blight are randomly distributed in maize genome, but clustered at different regions of the chromosomes (Ali *et al.*, 2013). Clustering of resistance loci, invariably can permit inheritance of such loci, and with molecular markers that co-segregate for these loci, their deployment in resistance breeding will pave way for more effective breeding schemes for both diseases in sorghum (Mohan *et al*., 2010).

Taken together, this study has confirms the prevalence of anthracnose and TLB of sorghum in Uganda and Sudan with plant reaction being invariably affected by environment. Resistance was confirmed as qualitative for anthracnose and quantitative for TLB with resistance exhibiting dominance epistasis associated with SSR markers that co-segregate for resistance to both diseases. These SSRs when coupled with phenotypic data will improve screening for dual resistance and the development of novel sorghum. Identification of additional SSR markers may improve

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Chapter Seven

General discussions, conclusions and recommendations

7.1 Discussions

7.1.1 Prevalence of turcicum leaf blight in Sudan

While Sudan is a major producer of sorghum and indeed a center of diversity for the crop, there is limited evidence of studies on leaf blight on sorghum. Yet symptoms akin to leaf blight have been observed on the crop. Thus the objectives of this study were to investigate the occurrence and intensity of TLB in central Sudan, a major sorghum growing area in the country. The results showed presence of leaf blight in all locations with incidence of 65-100% and severity ratings of between 45-85% in the 45 fields inspected. The highest disease incidence (100%) was observed in Sennar, Gedarif and Central and South Gezira districts, while the lowest was observed in Khartoum district. Disease severity did not follow the same trend and the lowest (45%) was recorded in Shambat (Khartoum district), North Gezira and Sennar. The highest severity was observed in Elrahad (Gedarif district) (100%). All 11 sorghum varieties preferred and grown by farmers in central Sudan are susceptible to by *E. turcicum*. These results showed that turcicum leaf blight is indeed an important disease in Sudan and deployment of resistance to manage it are critical. Host plant resistance is the most economical and ecologically acceptable way to address this disease. But given the limited studies it is possible that very limited effort has been done to deploy resistant lines already. In the other chapters of this thesis I also examined the potential for dual infection with the endemic anthracnose a deadly fungal disease and the potential for breeding for dual resistance.

7.1.2 Sorghum reaction to dual infection by *C. sublineolum* and *E. turcicum*

In this study, four field and two greenhouse experiments were carried out in sorghum growing regions of Sudan and Uganda to evaluate reaction to *C. sublineolum* and *E. turcicum* dual infection. Breeding lines for TLB resistant had varied reaction with some susceptibility found in some environments and not others sugessting potential role of either pathotype variation due to gene for gene reactions or the role of environments in attenuating epidemics. In the case of anthracnose, progeny expected to be resistant were generally stable for the trait. Sorghum genotypes exhibited considerable variations to dual infection by *C. sublineolum* and *E. turcicum.* Cultivar Jesu 91-104DL and $F_{8:9}$ line MUTLB1003 was resistant to both pathogens across environments and were identified as new sources for dual resistance to both diseases. The results of GGE interaction revealed that breeding for anthracnose resistance was equally effective across environments but was not effective against TLB. In Uganda and Sudan, no studies on *C. sublineolum* race type have been reported hence there is limited information of the pathogen in sorghum (Sserumaga *et al*., 2013). While studies on *E. turcicum* reported that race 0 (Adipala *et al*., 1993; Weltz, 1998) were found to exist in Uganda however, Ramathani *et al*. (2011) reported that new races might have evolved from race 0. Therefor, the relationship between physiological race types of both pathogenes and the components of resistance could be used to develop sorghum lines with dual resistance.

Diverse sources of qualitative and quantitative resistance to either anthracnose or TLB in sorghum have been reported (Singh *et al*., 2006; Reddy and Prasad, 2013), however, resistrance to dual infection is poorly understood. In this study the effects of dula infection were investigated under controlled (greenhouse) and field conditions.

Highre severities of both diseases were observed under field than greenhouse conditions. No evidence of correlation between of lesion type and pigmentation due to (anthocyanin) production, a trait associated with phenolics that is implicated in host resistance was found. Small lesion sizes were however correlated with low severities and, therefore, the trait could be used in identifying resistant genotypes. Genotype GA06/18 had resistant genes for dual pathogen infection. Two crosses; GA06/106 x Epuripuri and MUC007/009 x Epuripuri showed high heterosis and resistance to both pathogens indicating that they were good materials for sorghum breeding programmes. The results demonstrated that the sorghum genotypes used contained resistant alleles for dual pathogen infection with *C. sublineolum* and *E. turcicum*. Additive and non – additive (dominance) variance components were almost equally reflected by equal contribution of both variances towards the anthracnose resistance suggesting that both additive and dominance gene effects control anthracnose resistance. Contribution of additive gene effects towards TLB resistance was greater than non – additive gene effects revealing that additive gene effects were more important in controlling TLB resistance.

The high genetic variability observed in sorghum is expected to have a strong impact on sorghum breeding in Sudan (Abu Assar *et al*., 2005). Ramathani (2009) indicated that sorghum collections in Uganda could be source of TLB resistance. Prom *et al*. (2012) indicated that Sudan, and Uganda sorghum collections are important sources of anthracnose resistance. Similarly, evidence of northern leaf blight, sourthen leaf blight and grey leaf blight (multiple foliar disease) resistance and its implication was reported on maize. This indicates that similar disease resistance approaches are also expected to exist in sorghum (Ali *et al*., 2013). While varied disease severities could

be used to identify segregating progeny, both epidemics of anthracnose and TLB are influenced weather (Ngugi *et al*., 2000). Therefore it is critical to use platforms like greenhouses and chambers to phenotype sorghum plants rather than field-based. Thus breeding for anthracnose and TLB resistance requires quantifying these traits in heterogeneous plant populations and genotyping the plants to identify the traits' genetic bases using molecular markers. The use of next generation populations for mapping and consequently breeding will harness the rich allele diversity of the crop and its relatively small genome renders it amenable to genetic manipulation. In the next section discusions for breeding for complex traits such as resistance that in sorghum is generally conditioned by addiutive gene action as evidenced in this study is made.

7.1.3 Simple sequence repeat associated with anthracnose and TLB resistance

Mapping of resistance to anthracnose and TLB was undertaken in 126 $F_{8:9}$ sorghum recombinant inbred lines derived from a cross between MUC007/009 and Epuripuri. The $F_{8:9}$ lines were evaluated for field resistance to anthracnose and TLB in Uganda and Sudan. ANOVA showed significant differences among locations ($P<0.001$) suggesting a strong influence of environments on the expressivity of both diseases. Transgressive segregation was observed indicating that both parents carried minor genes or alleles for resistance that differed from each other. SSRs Xtxp25 and Xtxp201 and Xtxp302, Xtxp25, Xtxp295 and Xtxp95 were associated respectively with anthracnose and TLB genotypic segregation ratio of two loci in epistasis dominant. However, SSRs Xtxp201 and Xtxp303 were associated significantly ($P<0.05$) with both anthracnose and TLB phenotypic characterisation. High polymorphic information content (0.44 to 0.59) and gene diversity (0.54 to 0.66) were observed. This suggested that these

SSRs could be used to detect the dual resistant genotypes and therefore contributed substantive information to multiple disease resistance research of sorghum.

Evolution of genotyping technologies has resulted in unique possibilities for evaluating collections of germplasm, characterizing of segregating populations, and finding markers cosegregate with specific alleles of disease resistance (Kassa *et al*., 2014). Information gained from this study could be used in deploying marker assisted breeding for dual diseases infection of anthracnose and TLB. In this study, only seven SSR markers were used to locate the resistant loci associated with multifactorial inherited diseases anthracnose and TLB. Recently, simple sequence polymorphism (SNP) markers are being used to detect QTL associated with fungal diseases in sorghum (Upadhyaya *et al*., 2013), maize (Kassa *et al*., 2014), tomato (Víquez-Zamora *et al*., 2013) and cabage (Lee *et al*., 2015). Furthermore, findings and methods from this study may be used in this study applicable to other complex traits in sorghum but also in other cereals.

7.2 General conclusions and recommendations

Taking together this study has confirmed the presence of turcicum leaf blight in sorghum producing regions in Sudan resulting in great loss for small and large scale farmers and the farmer's preferred varieties. Therefore, there is urgent need to develop risk management model to manage the severe TLB outbreaks. Also there is the need to identify and combine different sources of anthracnose and TLB resistance in order to transfer useful genes and increase resistance to both diseases. In this thesis study, sorghum cultivars and lines with resistance to anthracnose and TLB were found. For example cultivar Jesu 91-104DL and RILs MUTLB1003 offered resistance

to dual infection and are therefore were recommended for sorghum breeding programmes.

Resistance was confirmed as qualitative for anthracnose and quantitative for TLB with resistance exhibiting dominance epistasis associated with SSR markers that co-segregate for resistance to both diseases. However, there is need to investigate more on multiple foliar diseases resistance in Sudan and Uganda. The biparental progeny usedfor QTL mapping have limitation. And as such there is need to use next generation mapping approaches such as nested association mapping derivatives (QWAS and nested association mapping). THis would explore the rich genetic diversity in the region as well as the rapid advanced in genomics now becoming available.

In this study, $F_{8:9}$ sorghum RILs derived from a cross between MUC007/009 (resistant to TLB and susceptible to anthracnose) and Epuripuri (resistant to anthracnose and susceptible to TLB) to map both anthracnose and TLB resistance. Two loci found to be co-segregating with anthracnose and TLB resistance while generally resistant loci for both diseases segregated together. Further, this study yielded SSR markers i.e. Xtxp302, Xtxp25, Xtxp201, Xtxp177, Xtxp303, Xtxp295 and Xtxp95 that are related to both diseases resistance and could be used in marker assisted breeding. However, studies are needed to identify QTLs related to dual resistance by saturating the genetic map using more polymorphic markers. More advanced mothodes suggested above could be usfel.

7.3 Research gaps

It is crucial to initiate screening and characterizing of the sensitivity of the widely

grown sorghum varieties to establish national breeding programme for anthracnose and TLB resistance. Therefore, high-throughput phenotyping system is required to quantify complex traits associated with disease resistance in large plant populations. Indeed, some platforms collect data in greenhouses or growth chambers while others are field-based especially when phenotyping anthracnose and TLB. However, studies on the detection of changes in the *C. sublineolum* and *E. turcicum* population and mating type distribution are still lacking and should be undertaken. This needs to be investigated further by studying the expression of these genes for multiple foliar diseases across different agrological zones.

The complete understanding of the basis of resistance of sorghum - *C. sublineolum* and *E. turcicum* is still lacking and needs to be fully elucidated. The variability of *C. sublineolum* and *E. turcicum* isolates need to be tested for the possibility of cross infection between isolates from sorghum, maize and other Gramineae species. This study indicated that there was co-segregation between genes coding for resistant to anthracnose and TLB in sorghum. In cereals, majority of resistant genes encode nucleotide binding site and a leucine-rich-repeat region. The role of such resistance in the case of dual infection and specifically its deployment in crop improvement is still vague especiallt for tropical cereals such as sorghum. THis is a study area for the future that would require linkage disequilibria based methods and larger popultations.

Successful utilization of whole-genome sequencing for large-scale SNP identification and development of molecular markers for identifying novel QTLs that cosegregate with both anthracnose and TLB resistant phenotypic traits. The high-density genetic

map will promote QTL analysis for other important agricultural traits and marker-assisted breeding of sorghum. To detect QTL for dual disease resistance, there is need for performing whole-genome sequencing of the parent MUC007/009 and Epuripuri and genome-wide SNP identification using the recently published sorghum genome sequences as reference.

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List of Apendices

Apendix 1: Means of farmer's preferred varieities evaluated in Uganda and Sudan (year 2012 and 2014)

Country	Condition	Locaion	Genotype	ANT initial severity	ANT final severity	ANT AUDPC	TLB initial severity	TLB final severity	TLB AUPDC	Days to 50% flowering	Lesion colour
Sudan	Greenhouse	WadMedani	Arfa Gadamak	5.00	20.00	375.00	5.00	5.00	150.00	68.00	3.00
Sudan	Greenhouse	WadMedani	Butana	0.00	6.75	101.21	0.00	32.92	690.96	82.50	1.00
Sudan	Greenhouse	WadMedani	Epuripuri	0.00	1.67	40.83	3.33	22.50	496.42	78.67	3.00
Sudan	Greenhouse	WadMedani	GA06/106	0.74	6.98	123.96	2.11	32.43	572.46	80.71	1.51
Sudan	Greenhouse	WadMedani	GA06/18	0.74	6.98	123.96	2.11	32.43	572.46	80.71	1.51
Sudan	Greenhouse	WadMedani	Gadam Elhaman	2.50	15.08	224.88	4.17	26.67	511.00	84.33	1.00
Sudan	Greenhouse	WadMedani	HD1	0.00	5.28	131.25	3.33	37.22	672.78	87.33	1.00
Sudan	Greenhouse	WadMedani	Jesu 91-104DL	0.00	5.00	64.17	0.00	21.67	539.58	84.00	1.67
Sudan	Greenhouse	WadMedani	KARI Mtama	0.00	13.33	239.17	5.00	19.17	347.08	82.67	1.00
Sudan	Greenhouse	WadMedani	MUK007/009	4.17	20.67	337.75	0.00	26.67	376.25	70.50	1.00
Sudan	Greenhouse	WadMedani	Sekedo	1.67	3.33	64.17	0.00	38.33	615.42	71.83	1.00
Sudan	Greenhouse	WadMedani	Tabat	0.00	7.28	118.81	1.67	32.94	452.86	75.56	3.00
Sudan	Greenhouse	WadMedani	Wad Ahmed	0.42	1.67	30.63	2.08	40.83	707.29	85.50	1.00
Sudan	Greenhouse	WadMedani	Yarwasha	3.33	5.00	122.50	4.17	38.33	672.58	75.33	1.00
Sudan	Field	Wad Elturabi	Arfa Gadamak	12.75	38.25	467.50	2.50	5.00	112.50	47.50	1.00
Sudan	Field	Wad Elturabi	Butana	2.50	0.00	12.50	10.25	15.25	302.50	55.50	3.00
Sudan	Field	Wad Elturabi	Epuripuri	0.00	0.00	0.00	5.50	7.50	265.00	72.00	3.00
Sudan	Field	Wad Elturabi	GA06/106	2.67	43.33	431.52	3.33	5.00	142.08	58.33	1.00
Sudan	Field	Wad Elturabi	GA06/18	2.50	5.25	44.49	2.88	6.54	86.96	59.50	1.00

Sudan	Field	Wad Elturabi	Gadam Elhaman	3.25	37.50	483.75	2.50	2.50	57.50	49.50	1.00
Sudan	Field	Wad Elturabi	HD1	0.25	2.75	40.00	0.00	2.50	125.00	44.50	1.00
Sudan	Field	Wad Elturabi	Jesu 91-104DL	0.00	0.00	0.00	5.25	5.00	153.75	58.50	3.00
Sudan	Field	Wad Elturabi	KARI Mtama	8.00	42.50	777.50	8.00	10.00	275.00	61.50	1.00
Sudan	Field	Wad Elturabi	MUK007/009	5.25	75.00	760.17	2.50	2.50	262.79	56.00	1.00
Sudan	Field	Wad Elturabi	Sekedo	3.25	25.25	555.00	2.50	5.00	137.50	60.50	2.00
Sudan	Field	Wad Elturabi	Wad Ahmed	3.00	41.25	296.25	5.00	5.00	235.00	61.50	1.00
Sudan	Field	Wad Elturabi	Yarwasha	2.75	25.00	598.75	0.00	2.50	115.00	64.50	1.00
Sudan	Field	Wad Medani	Arfa Gadamak	5.00	20.00	375.00	5.00	5.00	150.00	68.00	3.00
Sudan	Field	Wad Medani	Epuripuri	1.67	10.00	241.67	5.00	4.33	143.33	101.00	1.67
Sudan	Field	Wad Medani	GA06/106	2.00	3.17	87.50	5.00	23.33	273.33	72.20	1.01
Sudan	Field	Wad Medani	GA06/18	0.00	0.36	55.09	5.00	6.04	169.22	99.60	3.98
Sudan	Field	Wad Medani	Gadam Elhaman	0.25	3.25	77.50	3.50	12.50	125.00	72.00	1.67
Sudan	Field	Wad Medani	HD1	1.00	3.17	80.83	5.00	18.67	253.33	85.00	1.00
Sudan	Field	Wad Medani	Jesu 91-104DL	2.67	2.67	143.33	3.33	10.17	215.83	89.00	1.67
Sudan	Field	Wad Medani	KARI Mtama	3.00	5.00	120.00	5.00	24.00	425.00	89.00	1.00
Sudan	Field	Wad Medani	MUK007/009	1.00	1.00	5.00	5.00	45.00	740.00	74.30	2.01
Sudan	Field	Wad Medani	Sekedo	0.00	0.00	0.00	5.00	3.00	140.00	108.00	1.00
Sudan	Field	Wad Medani	Tabat	2.21	4.25	88.75	4.18	30.25	431.39	86.48	2.27
Sudan	Field	Wad Medani	Wad Ahmed	1.67	8.33	103.33	5.00	28.33	360.00	81.50	1.67
Sudan	Field	Wad Medani	Yarwasha	0.00	7.50	112.50	5.00	20.00	425.00	75.00	1.33
Uganda	Greenhouse	MUARIK	Arfa Gadamak	4.62	10.79	307.84	4.58	9.29	285.98	41.44	1.71
Uganda	Greenhouse	MUARIK	Butana	1.49	5.40	98.73	2.57	13.83	186.35	40.77	1.56
Uganda	Greenhouse	MUARIK	Epuripuri	1.08	6.98	77.05	2.72	5.00	154.91	41.84	1.22
Uganda	Greenhouse	MUARIK	GA06/106	0.42	4.08	42.43	2.58	5.65	155.93	43.75	1.39
Uganda	Greenhouse	MUARIK	GA06/18	1.30	5.84	76.83	2.94	7.02	153.38	43.29	1.50
Uganda	Greenhouse	MUARIK	Gadam Elhaman	0.83	3.73	60.50	2.67	7.18	151.12	44.31	1.50

Uganda	Greenhouse	MUARIK	HD1	1.99	3.63	89.72	3.51	7.46	161.64	43.25	1.39
Uganda	Greenhouse	MUARIK	Jesu 91-104DL	0.66	2.69	64.11	2.82	6.77	178.07	42.00	1.26
Uganda	Greenhouse	MUARIK	KARI Mtama	1.65	10.19	109.27	2.88	6.57	169.97	44.86	1.27
Uganda	Greenhouse	MUARIK	MUK007/009	1.52	5.41	82.25	2.92	5.30	163.08	41.42	1.05
Uganda	Greenhouse	MUARIK	Sekedo	1.55	0.40	19.73	4.01	13.11	176.09	40.48	2.34
Uganda	Greenhouse	MUARIK	Tabat	2.41	6.59	101.49	2.39	5.46	141.47	41.92	2.05
Uganda	Greenhouse	MUARIK	Wad Ahmed	2.49	8.61	116.92	3.23	9.26	170.91	38.94	1.23
Uganda	Greenhouse	MUARIK	Yarwasha	1.49	1.96	58.15	2.14	4.89	125.87	43.95	0.95
Uganda	Field	MUARIK	Arfa Gadamak	0.83	7.92	107.92	5.00	24.17	392.50	78.25	1.50
Uganda	Field	MUARIK	Butana	5.52	12.84	395.47	3.64	5.78	174.86	40.77	1.33
Uganda	Field	MUARIK	Epuripuri	4.28	10.24	322.28	3.51	5.47	139.36	41.84	1.50
Uganda	Field	MUARIK	GA06/106	5.61	16.09	386.44	2.88	6.18	169.24	43.75	1.69
Uganda	Field	MUARIK	GA06/18	4.09	8.67	238.10	2.54	9.97	284.58	43.29	1.70
Uganda	Field	MUARIK	Gadam Elhaman	4.97	12.82	400.86	3.88	5.96	185.52	44.31	1.40
Uganda	Field	MUARIK	HD1	4.78	10.86	327.88	2.81	6.21	154.70	43.25	1.58
Uganda	Field	MUARIK	Jesu 91-104DL	5.92	11.47	329.32	6.49	6.08	206.42	42.00	1.32
Uganda	Field	MUARIK	KARI Mtama	4.15	13.01	335.59	4.36	8.23	243.68	44.86	1.79
Uganda	Field	MUARIK	MUK007/009	4.47	8.72	263.65	5.65	10.24	302.11	41.42	1.92
Uganda	Field	MUARIK	Sekedo	4.39	9.32	281.26	4.38	8.13	215.15	40.48	1.51
Uganda	Field	MUARIK	Tabat	5.42	13.35	410.02	3.78	7.75	199.04	41.92	1.26
Uganda	Field	MUARIK	Wad Ahmed	4.75	11.63	336.67	4.90	9.33	263.02	38.94	1.90
Uganda	Field	MUARIK	Yarwasha	4.49	9.96	279.00	4.25	9.24	308.95	43.95	1.52

Apendix 2: Means of recombinant inbred lines evaluated in Uganda and Sudan (year 2012 and 2014)

Country	Location	Code	ANT initial severity	ANT final severity	ANT AUDPC	TLB initial severity	TLB final severity	TLB AUDPC	ANT incidence	TLB incidence	Lesion colour	Lesion number	Days to 50% flowering
Sudan	Wad Elturabi	MUTLB01001	5.83	5.56	7.32	5.62	24.27	12.12	56.51	43.49	1.10	50.00	71.16
Sudan	Wad Elturabi	MUTLB01003	0.83	0.56	0.82	6.12	4.27	8.34	62.47	37.53	1.00	104.00	58.16
Sudan	Wad Elturabi	MUTLB01004	3.05	37.54	17.47	5.05	13.45	8.40	58.17	41.83	1.00	34.50	58.71
Sudan	Wad Elturabi	MUTLB01006	1.77	1.77	2.12	4.47	8.13	7.78	42.43	57.57	2.91	27.50	68.21
Sudan	Wad Elturabi	MUTLB01009	0.00	0.00	0.00	4.97	50.63	12.96	50.10	49.90	0.91	16.00	60.71
Sudan	Wad Elturabi	MUTLB01010	2.55	2.54	3.61	2.80	40.20	8.50	67.69	32.31	1.00	70.00	64.93
Sudan	Wad Elturabi	MUTLB01016	6.33	25.56	11.10	0.62	4.27	2.12	48.49	51.51	1.10	12.50	65.16
Sudan	Wad Elturabi	MUTLB01018	0.00	4.51	0.70	4.97	75.63	27.81	59.67	40.33	0.90	76.00	80.00
Sudan	Wad Elturabi	MUTLB01020	5.05	5.05	5.08	2.55	19.95	10.40	50.15	49.85	3.00	22.50	67.93
Sudan	Wad Elturabi	MUTLB01021	2.80	5.04	4.94	12.55	27.45	18.97	59.31	40.69	2.00	27.50	69.71
Sudan	Wad Elturabi	MUTLB01022	12.80	62.54	26.90	10.05	2.70	11.18	53.85	46.15	1.00	102.00	52.43
Sudan	Wad Elturabi	MUTLB01023	0.12	1.53	0.76	5.02	10.18	7.87	58.68	41.32	2.00	54.50	76.71
Sudan	Wad Elturabi	MUTLB01026	0.05	7.54	4.33	6.55	14.95	10.97	47.60	52.40	2.00	139.00	67.43
Sudan	Wad Elturabi	MUTLB01027	4.27	19.51	7.84	4.47	25.63	9.39	79.19	20.81	2.91	52.00	69.71
Sudan	Wad Elturabi	MUTLB01029	0.83	0.56	4.39	1.12	28.02	6.80	64.90	35.10	3.10	75.00	66.16
Sudan	Wad Elturabi	MUTLB01032	5.48	24.38	14.42	20.57	1.72	21.71	63.22	36.78	1.04	121.50	60.68
Sudan	Wad Elturabi	MUTLB01036	4.42	10.04	7.04	6.42	10.45	9.67	50.95	49.05	1.50	123.50	66.93

Sudan	Wad Elturabi	MUTLB01038	8.12	1.53	8.83	1.69	22.84	7.61	44.99	55.01	2.97	195.00	63.93
Sudan	Wad Elturabi	MUTLB01044	0.30	5.29	1.04	5.05	24.95	10.22	67.86	32.14	3.00	10.50	74.93
Sudan	Wad Elturabi	MUTLB01047	0.83	0.56	0.96	5.62	6.27	8.77	74.58	25.42	3.10	35.50	68.16
Sudan	Wad Elturabi	MUTLB01063	2.55	5.04	3.97	6.30	25.20	15.29	40.39	59.61	1.00	257.00	66.43
Sudan	Wad Elturabi	MUTLB01066	0.05	0.04	0.04	5.24	49.95	14.47	50.56	49.44	3.10	383.00	69.71
Sudan	Wad Elturabi	MUTLB01068	2.55	12.54	5.04	5.05	62.45	17.72	54.39	45.61	2.00	164.00	68.43
Sudan	Wad Elturabi	MUTLB01069	2.55	27.54	10.76	0.30	24.95	5.29	43.35	56.65	2.00	101.00	69.43
Sudan	Wad Elturabi	MUTLB01078	3.55	2.79	4.36	1.55	15.95	10.68	49.77	50.23	1.00	210.00	66.43
Sudan	Wad Elturabi	MUTLB01079	2.55	2.55	3.33	2.55	49.95	12.18	61.98	38.02	3.00	157.00	66.93
Sudan	Wad Elturabi	MUTLB01080	2.55	2.79	3.76	5.30	12.45	8.50	51.92	48.08	2.00	248.50	71.93
Sudan	Wad Elturabi	MUTLB01089	0.05	2.79	0.79	1.55	14.95	5.79	64.61	35.39	1.00	58.50	65.93
Sudan	Wad Elturabi	MUTLB01092	0.30	0.29	0.76	10.05	27.70	20.29	57.21	42.79	1.00	114.00	65.93
Sudan	Wad Elturabi	MUTLB01093	0.00	19.51	2.12	-0.53	20.63	7.96	60.58	39.42	2.91	53.00	63.71
Sudan	Wad Elturabi	MUTLB01098	4.77	49.51	7.44	4.47	5.63	8.03	44.59	55.41	0.91	53.50	69.71
Sudan	Wad Elturabi	MUTLB01102	11.33	25.56	17.03	1.12	4.27	3.34	77.19	22.81	1.10	28.50	65.16
Sudan	Wad Elturabi	MUTLB01105	0.05	12.54	2.19	6.97	5.95	6.43	62.59	37.41	2.00	78.50	64.93
Sudan	Wad Elturabi	MUTLB01108	2.80	0.29	3.19	4.47	24.95	9.50	48.98	51.02	2.00	70.00	68.93
Sudan	Wad Elturabi	MUTLB01115	0.83	6.06	20.89	5.62	24.27	9.34	59.14	40.86	1.10	18.00	66.16
Sudan	Wad Elturabi	MUTLB01120	0.00	19.51	9.98	0.00	20.63	8.81	53.62	46.38	0.91	147.00	55.71
Sudan	Wad Elturabi	MUTLB01147	Missing	5.56	8.46	Missing	24.27	5.70	48.76	51.24	3.10	34.00	67.16

Sudan	Wad Elturabi	MUTLB01151	6.33	35.04	16.01	0.62	25.20	9.00	51.06	48.94	1.00	97.00	73.93
Sudan	Wad Elturabi	MUTLB01152	Missing	4.51	6.41	Missing	50.63	34.74	36.24	63.76	2.91	49.50	67.71
Sudan	Wad Elturabi	MUTLB01156	4.27	7.11	92.62	19.47	4.53	166.80	50.63	49.37	1.00	52.00	101.01
Sudan	Wad Elturabi	MUTLB01164	19.27	40.04	9.83	4.47	12.45	5.75	37.72	62.28	1.00	Missing	72.71
Sudan	Wad Elturabi	MUTLB01176	1.33	22.11	247.62	5.03	4.03	174.30	62.16	37.84	1.17	117.00	69.83
Sudan	Wad Elturabi	MUTLB01177	3.30	2.79	4.40	5.05	32.45	11.83	59.28	40.72	2.00	47.50	65.93
Sudan	Wad Elturabi	MUTLB01178	0.05	0.04	0.40	7.55	49.95	17.72	45.72	54.28	1.00	224.00	Missing
Sudan	Wad Elturabi	Epuripuri	0.00	0.00	0.00	5.50	7.50	265.00	145.84	5.05	3.00	Missing	72.00
Sudan	Wad Elturabi	MUK007/009	5.25	75.00	760.17	2.50	2.50	262.79	734.41	0.00	1.00	Missing	56.00
Sudan	Wad Medani	MUTLB01001	5.83	5.56	7.32	5.62	24.27	12.12	71.99	28.01	1.10	51.00	71.16
Sudan	Wad Medani	MUTLB01003	0.83	0.56	0.82	6.12	4.27	8.34	85.27	14.73	1.00	108.00	58.16
Sudan	Wad Medani	MUTLB01004	4.48	4.81	139.64	1.08	22.85	399.85	97.81	2.19	1.25	43.00	80.48
Sudan	Wad Medani	MUTLB01006	6.08	3.67	99.55	4.90	3.87	91.34	94.84	5.16	2.63	27.00	95.51
Sudan	Wad Medani	MUTLB01009	4.38	9.11	342.62	5.03	4.03	179.30	76.40	23.60	1.17	20.00	80.51
Sudan	Wad Medani	MUTLB01010	6.08	3.17	137.06	0.90	43.37	583.84	88.98	11.02	2.63	86.00	95.51
Sudan	Wad Medani	MUTLB01016	4.38	9.11	482.62	0.03	4.03	149.30	85.58	14.42	1.17	15.00	95.51
Sudan	Wad Medani	MUTLB01018	0.23	2.64	67.34	4.96	2.95	110.32	84.85	15.15	0.90	70.00	97.76
Sudan	Wad Medani	MUTLB01020	1.08	-1.83	-12.90	4.90	1.37	53.84	81.72	18.28	2.63	20.00	Missing
Sudan	Wad Medani	MUTLB01021	0.23	2.64	42.34	4.96	31.20	479.07	73.65	26.35	2.63	35.00	88.76
Sudan	Wad Medani	MUTLB01022	3.31	8.36	119.77	5.00	5.25	193.50	74.15	25.85	1.02	94.00	85.74

Sudan	Wad Medani	MUTLB01023	6.08	-1.83	12.05	0.90	3.37	33.84	73.52	26.48	2.63	50.00	103.51
Sudan	Wad Medani	MUTLB01026	2.73	5.14	117.34	3.46	11.20	234.07	73.29	26.71	1.90	158.00	102.76
Sudan	Wad Medani	MUTLB01027	6.08	3.17	137.06	4.90	10.37	208.84	81.62	18.38	2.63	46.00	75.51
Sudan	Wad Medani	MUTLB01029	1.78	21.49	433.35	4.99	5.61	139.35	84.32	15.68	1.94	80.00	86.48
Sudan	Wad Medani	MUTLB01032	4.23	12.64	259.84	1.46	3.95	87.82	95.67	4.33	1.90	130.00	79.76
Sudan	Wad Medani	MUTLB01036	3.50	13.32	216.59	3.48	4.28	126.08	84.87	15.13	1.67	120.00	86.67
Sudan	Wad Medani	MUTLB01038	3.31	5.03	124.77	5.00	10.08	166.00	87.45	12.55	1.68	198.00	97.33
Sudan	Wad Medani	MUTLB01044	4.43	13.46	191.13	1.56	23.94	622.07	76.67	23.33	1.21	10.00	96.54
Sudan	Wad Medani	MUTLB01047	2.38	7.11	172.62	2.03	4.53	121.80	99.90	0.10	1.17	46.00	Missing
Sudan	Wad Medani	MUTLB01063	1.65	3.36	118.11	5.00	28.41	384.33	77.50	22.50	1.68	250.00	97.33
Sudan	Wad Medani	MUTLB01066	2.78	3.99	100.85	4.49	45.61	476.85	84.91	15.09	1.94	378.00	90.50
Sudan	Wad Medani	MUTLB01068	2.78	1.49	88.35	1.99	40.61	489.35	92.57	7.43	0.94	208.00	99.00
Sudan	Wad Medani	MUTLB01069	0.00	2.11	22.60	3.03	74.53	921.80	83.45	16.55	Missing	114.00	94.01
Sudan	Wad Medani	MUTLB01078	2.38	7.11	147.62	5.03	4.03	174.30	79.51	20.49	1.17	180.00	96.01
Sudan	Wad Medani	MUTLB01079	4.38	7.11	172.62	5.03	19.03	369.30	65.47	34.53	1.17	170.00	79.01
Sudan	Wad Medani	MUTLB01080	1.65	3.36	61.44	4.00	5.41	134.33	69.95	30.05	1.68	258.00	95.67
Sudan	Wad Medani	MUTLB01089	0.28	1.49	35.85	4.99	25.61	314.35	84.86	15.14	1.94	67.00	110.00
Sudan	Wad Medani	MUTLB01092	4.38	5.11	292.62	5.03	4.03	329.30	83.02	16.98	1.17	114.00	95.01
Sudan	Wad Medani	MUTLB01093	2.93	3.46	96.13	3.06	45.94	624.57	77.15	22.85	2.21	50.00	89.39
Sudan	Wad Medani	MUTLB01098	-0.52	4.81	114.64	5.08	22.85	389.85	97.75	2.25	1.25	50.00	Missing

Sudan	Wad Medani	MUTLB01102	1.78	8.99	90.85	4.99	25.61	314.35	96.37	3.63	0.94	35.00	85.50
Sudan	Wad Medani	MUTLB01105	2.48	2.81	99.64	5.58	47.85	452.35	86.07	13.93	1.25	85.00	82.48
Sudan	Wad Medani	MUTLB01108	1.08	3.17	112.06	1.90	3.87	101.34	61.62	38.38	0.63	60.00	123.51
Sudan	Wad Medani	MUTLB01115	6.08	43.17	887.06	4.90	3.37	103.84	86.25	13.75	0.63	16.00	Missing
Sudan	Wad Medani	MUTLB01120	2.78	1.49	78.35	4.49	25.86	438.10	91.62	8.38	0.94	146.00	82.51
Sudan	Wad Medani	MUTLB01147	-0.62	2.11	22.60	3.03	4.53	156.80	77.17	22.83	1.00	38.00	99.31
Sudan	Wad Medani	MUTLB01151	2.48	14.81	284.64	1.08	7.85	74.85	94.94	5.06	1.25	86.00	96.48
Sudan	Wad Medani	MUTLB01152	4.28	23.49	230.85	3.99	45.61	611.85	79.50	20.50	0.94	53.00	92.00
Sudan	Wad Medani	MUTLB01156	4.38	7.11	92.62	5.03	4.53	166.80	101.13	-1.13	1.00	54.00	101.01
Sudan	Wad Medani	MUTLB01164	4.08	43.17	577.06	4.90	1.37	53.84	103.22	-3.22	0.63		114.51
Sudan	Wad Medani	MUTLB01176	4.38	22.11	247.62	5.03	4.03	174.30	71.09	28.91	1.17	116.00	69.83
Sudan	Wad Medani	MUTLB01177	1.93	5.96	103.63	4.06	11.19	305.82	66.94	33.06	1.21	50.00	90.74
Sudan	Wad Medani	MUTLB01178	4.38	6.61	120.12	1.03	4.03	134.30	82.66	17.34	3.17	216.00	102.01
Sudan	Wad Medani	Epuripuri	0.00	1.67	40.83	3.33	32.94	496.42	0.00	100.00	3.00	250.00	78.67
Sudan	Wad Medani	MUK007/009	4.17	20.67	337.75	0.00	26.67	376.25	96.95	3.05	1.00	3.05	70.50
Uganda	MUARIK	MUTLB01001	3.61	36.29	305.96	3.35	36.29	291.90	56.51	43.49	0.95	49.00	62.13
Uganda	MUARIK	MUTLB01003	0.12	12.69	245.16	2.68	12.69	165.66	62.47	37.53	2.95	100.00	67.85
Uganda	MUARIK	MUTLB01004	3.58	10.16	215.87	1.79	10.16	166.33	58.17	41.83	1.00	26.00	67.34
Uganda	MUARIK	MUTLB01006	3.85	15.27	200.68	2.91	15.27	159.58	42.43	57.57	0.97	28.00	67.86
Uganda	MUARIK	MUTLB01009	2.51	10.78	250.72	2.81	10.78	153.13	50.10	49.90	1.72	12.00	71.44

Uganda	MUARIK	MUTLB01010	3.49	38.07	224.92	4.40	38.07	243.10	67.69	32.31	1.34	54.00	66.01
Uganda	MUARIK	MUTLB01016	2.86	18.50	336.00	2.58	18.50	178.03	48.49	51.51	1.01	10.00	67.13
Uganda	MUARIK	MUTLB01018	1.03	37.51	174.20	3.90	37.51	230.41	59.67	40.33	1.03	82.00	64.21
Uganda	MUARIK	MUTLB01020	0.81	26.41	279.71	1.80	26.41	282.63	50.15	49.85	1.02	25.00	73.44
Uganda	MUARIK	MUTLB01021	2.69	15.27	266.62	3.15	15.27	168.19	59.31	40.69	1.68	20.00	69.67
Uganda	MUARIK	MUTLB01022	1.63	16.09	190.87	1.62	16.09	155.23	53.85	46.15	1.02	110.00	67.24
Uganda	MUARIK	MUTLB01023	3.23	16.86	252.08	1.44	16.86	202.82	58.68	41.32	1.54	59.00	73.02
Uganda	MUARIK	MUTLB01026	0.80	19.64	142.32	2.12	19.64	192.08	47.60	52.40	1.04	120.00	68.91
Uganda	MUARIK	MUTLB01027	2.52	21.76	153.09	3.55	21.76	262.49	79.19	20.81	1.02	58.00	66.38
Uganda	MUARIK	MUTLB01029	1.99	18.31	214.04	3.59	18.31	141.08	64.90	35.10	3.00	70.00	62.66
Uganda	MUARIK	MUTLB01032	3.52	23.11	315.20	1.44	23.11	230.03	63.22	36.78	2.35	113.00	65.43
Uganda	MUARIK	MUTLB01036	2.75	30.26	161.54	1.90	30.26	206.02	50.95	49.05	3.00	127.00	62.28
Uganda	MUARIK	MUTLB01038	4.34	26.08	261.21	5.23	26.08	299.91	44.99	55.01	1.18	192.00	70.17
Uganda	MUARIK	MUTLB01044	3.45	15.29	157.64	1.36	15.29	195.14	67.86	32.14	1.32	11.00	73.59
Uganda	MUARIK	MUTLB01047	2.23	24.27	410.59	3.08	24.27	217.38	74.58	25.42	1.46	25.00	62.09
Uganda	MUARIK	MUTLB01063	1.08	24.49	222.83	2.23	24.49	239.50	40.39	59.61	2.36	264.00	61.80
Uganda	MUARIK	MUTLB01066	2.74	23.96	252.63	3.41	23.96	265.91	50.56	49.44	3.01	388.00	62.65
Uganda	MUARIK	MUTLB01068	3.96	22.31	274.63	2.83	22.31	259.72	54.39	45.61	1.02	120.00	73.36
Uganda	MUARIK	MUTLB01069	2.01	24.58	248.04	3.01	24.58	221.48	43.35	56.65	1.02	88.00	62.96
Uganda	MUARIK	MUTLB01078	4.07	32.72	278.21	4.57	32.72	280.03	49.77	50.23	2.99	240.00	61.73

Uganda	MUARIK	MUTLB01079	2.93	34.78	202.21	3.93	34.78	253.98	61.98	38.02	1.00	144.00	60.48
Uganda	MUARIK	MUTLB01080	2.95	21.67	213.34	2.64	21.67	179.84	51.92	48.08	1.03	239.00	67.36
Uganda	MUARIK	MUTLB01089	3.47	38.44	178.36	3.18	38.44	357.23	64.61	35.39	1.02	50.00	69.04
Uganda	MUARIK	MUTLB01092	1.02	24.78	240.93	1.76	24.78	190.90	57.21	42.79	0.99	114.00	61.07
Uganda	MUARIK	MUTLB01093	3.80	25.19	324.07	2.90	25.19	167.07	60.58	39.42	1.69	56.00	60.07
Uganda	MUARIK	MUTLB01098	2.94	23.21	269.96	2.58	23.21	243.38	44.59	55.41	1.03	57.00	61.83
Uganda	MUARIK	MUTLB01102	4.09	21.65	285.76	2.43	21.65	230.63	77.19	22.81	1.03	22.00	61.85
Uganda	MUARIK	MUTLB01105	2.96	21.25	187.28	3.22	21.25	219.66	62.59	37.41	0.95	72.00	62.71
Uganda	MUARIK	MUTLB01108	1.14	38.28	243.10	3.06	38.28	434.07	48.98	51.02	0.99	80.00	67.71
Uganda	MUARIK	MUTLB01115	3.15	14.87	174.93	2.09	14.87	155.37	59.14	40.86	1.63	20.00	67.16
Uganda	MUARIK	MUTLB01120	3.87	21.67	416.91	2.87	21.67	215.47	53.62	46.38	1.70	148.00	61.86
Uganda	MUARIK	MUTLB01147	2.82	23.03	199.84	2.12	23.03	206.16	48.76	51.24	1.00	30.00	68.56
Uganda	MUARIK	MUTLB01151	4.86	13.19	367.24	0.04	13.19	155.73	51.06	48.94	1.05	108.00	62.92
Uganda	MUARIK	MUTLB01152	3.39	16.39	236.96	2.35	16.39	198.98	36.24	63.76	0.98	46.00	62.08
Uganda	MUARIK	MUTLB01156	4.17	16.62	314.89	1.42	16.62	176.46	50.63	49.37	1.01	50.00	63.54
Uganda	MUARIK	MUTLB01164	5.01	21.85	306.89	1.51	21.85	260.35	37.72	62.28	0.95	118.00	69.15
Uganda	MUARIK	MUTLB01176	1.95	24.12	374.19	1.42	24.12	194.93	62.16	37.84	1.01	118.00	69.83
Uganda	MUARIK	MUTLB01177	-0.18	59.27	290.64	2.59	59.27	317.48	59.28	40.72	3.02	45.00	62.24
Uganda	MUARIK	MUTLB01178	0.81	23.22	222.61	3.54	23.22	189.69	45.72	54.28	0.99	232.00	64.09
Uganda	MUARIK	Epuripuri	51.58	1.22	12.46	0.00	2.57	1.00	145.84	5.05	616.94	250.00	49.58

Uganda	MUARIK	MUK007/009	65.17	4.76	44.72	96.95	3.28	1.00	734.41	0.00	120.85	3.05	63.17
Uganda	NaSARRI	MUTLB01001	3.69	22.05	206.94	3.26	14.72	239.35	71.99	28.01	1.00	53.00	64.13
Uganda	NaSARRI	MUTLB01003	5.20	11.40	319.53	6.62	14.61	275.07	85.27	14.73	1.66	100.00	69.85
Uganda	NaSARRI	MUTLB01004	3.87	19.49	419.94	3.93	7.38	194.62	97.81	2.19	1.00	40.00	69.34
Uganda	NaSARRI	MUTLB01006	4.73	19.56	401.03	4.69	19.55	353.10	94.84	5.16	1.00	26.00	69.86
Uganda	NaSARRI	MUTLB01009	5.13	15.12	340.29	4.69	22.35	394.36	76.40	23.60	1.01	17.00	73.44
Uganda	NaSARRI	MUTLB01010	5.31	18.02	342.67	3.71	19.01	314.76	88.98	11.02	1.00	48.00	68.01
Uganda	NaSARRI	MUTLB01016	3.14	35.80	583.52	4.69	9.39	222.27	85.58	14.42	1.00	19.00	69.13
Uganda	NaSARRI	MUTLB01018	5.11	5.37	182.58	5.25	16.67	284.55	84.85	15.15	1.01	80.00	66.21
Uganda	NaSARRI	MUTLB01020	4.57	17.49	356.96	3.75	19.53	323.94	81.72	18.28	3.00	16.00	75.44
Uganda	NaSARRI	MUTLB01021	4.81	10.49	263.13	3.60	22.04	311.85	73.65	26.35	1.33	38.00	71.67
Uganda	NaSARRI	MUTLB01022	4.82	22.93	548.08	4.90	13.71	213.87	74.15	25.85	1.00	144.00	69.24
Uganda	NaSARRI	MUTLB01023	5.03	16.45	383.57	4.07	20.85	376.83	73.52	26.48	3.00	51.00	75.02
Uganda	NaSARRI	MUTLB01026	2.62	7.43	169.40	4.77	15.52	229.47	73.29	26.71	1.00	165.00	70.91
Uganda	NaSARRI	MUTLB01027	3.55	17.71	354.85	3.38	21.89	378.41	81.62	18.38	1.00	38.00	68.38
Uganda	NaSARRI	MUTLB01029	4.72	13.42	378.57	3.82	19.84	367.21	84.32	15.68	1.34	82.00	64.66
Uganda	NaSARRI	MUTLB01032	4.60	37.70	598.81	4.70	7.48	193.16	95.67	4.33	0.99	96.00	67.43
Uganda	NaSARRI	MUTLB01036	6.93	18.93	321.72	4.22	19.47	358.17	84.87	15.13	1.00	112.00	64.28
Uganda	NaSARRI	MUTLB01038	4.77	28.60	498.40	3.92	10.13	205.13	87.45	12.55	1.00	200.00	72.17
Uganda	NaSARRI	MUTLB01044	4.27	21.30	512.17	4.14	13.25	295.72	76.67	23.33	1.83	12.00	75.59

Uganda	NaSARRI	MUTLB01047	4.52	17.05	315.88	2.94	8.41	175.03	99.90	0.10	1.01	39.00	64.09
Uganda	NaSARRI	MUTLB01063	4.93	29.93	579.56	2.06	26.66	346.87	77.50	22.50	1.00	248.00	63.80
Uganda	NaSARRI	MUTLB01066	4.19	21.44	436.80	4.44	19.34	392.49	84.91	15.09	2.00	356.00	64.65
Uganda	NaSARRI	MUTLB01068	3.19	11.37	245.42	5.72	10.20	210.78	92.57	7.43	1.00	112.00	75.36
Uganda	NaSARRI	MUTLB01069	4.43	13.57	326.40	4.19	17.02	242.17	83.45	16.55	0.99	100.00	64.96
Uganda	NaSARRI	MUTLB01078	2.96	24.31	484.35	3.35	24.43	355.26	79.51	20.49	1.00	208.00	63.73
Uganda	NaSARRI	MUTLB01079	4.94	15.92	299.90	4.67	12.67	260.61	65.47	34.53	1.01	196.00	62.48
Uganda	NaSARRI	MUTLB01080	6.86	17.62	415.67	5.21	14.20	245.67	69.95	30.05	1.00	220.00	69.36
Uganda	NaSARRI	MUTLB01089	6.55	32.62	598.60	3.97	8.11	221.93	84.86	15.14	1.01	58.00	71.04
Uganda	NaSARRI	MUTLB01092	5.06	24.98	510.69	3.69	27.24	355.01	83.02	16.98	0.99	104.00	63.07
Uganda	NaSARRI	MUTLB01093	3.88	16.28	399.19	4.16	19.15	327.42	77.15	22.85	0.99	53.00	62.07
Uganda	NaSARRI	MUTLB01098	6.33	25.38	435.04	3.72	8.72	174.48	97.75	2.25	1.00	64.00	63.83
Uganda	NaSARRI	MUTLB01102	4.86	23.65	511.00	2.58	16.20	347.32	96.37	3.63	1.66	10.00	63.85
Uganda	NaSARRI	MUTLB01105	3.62	12.12	234.94	3.08	11.44	234.21	86.07	13.93	2.01	98.00	64.71
Uganda	NaSARRI	MUTLB01108	3.92	16.33	393.66	3.57	28.24	279.24	61.62	38.38	3.00	36.00	69.71
Uganda	NaSARRI	MUTLB01115	4.10	21.40	446.09	4.82	5.65	143.70	86.25	13.75	1.00	20.00	69.16
Uganda	NaSARRI	MUTLB01120	4.36	24.51	359.45	3.45	17.19	198.80	91.62	8.38	1.00	144.00	63.86
Uganda	NaSARRI	MUTLB01147	5.00	23.93	381.81	4.04	17.51	268.87	77.17	22.83	1.00	46.00	70.56
Uganda	NaSARRI	MUTLB01151	5.64	29.82	659.96	4.49	6.62	182.40	94.94	5.06	0.99	64.00	64.92
Uganda	NaSARRI	MUTLB01152	6.44	9.92	196.79	1.33	18.60	340.87	79.50	20.50	1.00	60.00	64.08

Uganda	NaSARRI	MUTLB01156	4.76	31.15	552.26	4.03	11.74	211.78	101.13	-1.13	1.00	50.00	65.54
Uganda	NaSARRI	MUTLB01164	4.82	13.23	359.53	5.31	7.91	156.94	103.22	-3.22	1.00	Missing	71.15
Uganda	NaSARRI	MUTLB01176	4.11	14.99	500.36	3.67	10.19	183.88	71.09	28.91	1.00	56.00	71.83
Uganda	NaSARRI	MUTLB01177	4.27	9.59	265.92	5.03	28.37	509.92	66.94	33.06	3.00	43.00	64.24
Uganda	NaSARRI	MUTLB01178	2.20	33.67	495.69	4.71	9.13	291.51	82.66	17.34	0.99	220.00	66.09
Uganda	NaSARRI	Epuripuri	1.22	12.46	145.84	5.05	39.42	616.94	0.00	100.00	1.00	250.00	51.58
Uganda	NaSARRI	MUK007/009	4.76	44.72	734.41	0.00	5.28	120.85	96.95	3.05	1.00	3.05	65.17

Apendix 3: Seven polymorphic simple sequence repeat (SSR) markers and their size and melting tempreture ºC (mT)

SSR locus	Linkage group	Forward Primer:	Reverse Primer:	mT °C	Size (bp)
Xtxp302	1	TAGGTTCTGGACCACTTTTCTTTTTGTGTT	GAATCAACTATGTGCTTGCATTGTGCT	55	180
Xtxp25	2	GCACATCCTCTAAAACTACTTAGT	GAACAGGACGATGTGATAGAT	50	283
Xtxp201	2	GCGTTTATGGAAGCAAAAT	CTCATAAGGCAGGACCAAC	55	222
Xtxp177	4	GCCGGTTGTGACTTG	TTAAAGCGATGGGTGTAG	55	169
Xtxp303	5	AATGAGGAAAATATGAAACAAGTACCAA	AATAACAAGCGCAACTATATGAACAATAAA	55	160
Xtxp95	6	TCTCCGTTTGCCCGCCAG	CACCGTACCGCCTCCCGAATC	65	100
Xtxp295	7	AAATCATGCATCCATGTTCGTCTTC	CTCCCGCTACAAGAGTACATTCATAGCTTA	55	165