SCREENING SELECTED SORGHUM \textit{[Sorghum bicolor (L.) Moench]} LINES FOR POST-ATTACHMENT RESISTANCE AGAINST WITCH WEED \textit{[Striga hermonthica (Del.) Benth]}

DIANA WANJA N. MANENE (B.Ed. Sc.)

I56/CE/14599/2009

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science (Genetics) in the School of Pure and Applied Sciences of Kenyatta University.

September, 2015
DECLARATION

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

Diana Wanja N. Manene
Department of Plant Sciences
Signature Date 1/9/2015

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Dr. Fredrick M. Njoka
Dean, School of Agriculture
Embu University College
Signature Date 2/9/15

Dr. Steven M. Runo
Department of Biotechnology and Biochemistry
Kenyatta University
Signature Date 2/9/15
DEDICATION
This thesis is dedicated to my family; my beloved husband Peter Wesonga and my children Yvette Nanzala, Leon Wesonga and Alexis Murimi who have been a constant joy in my life.
ACKNOWLEDGEMENT

I give glory to God for giving me the intellect to pursue education. Special thanks to
my supervisors Dr. Fredrick Njoka and Dr. Steven Runo for their guidance, encour-
agement and mentorship. My sincere gratitude go to Professor Julie Scholes of the
University of Sheffield for giving me an opportunity to carry out my research work in
her laboratory and for all the academic, moral and material support she gave me
throughout the period I was in the UK. I am immensely indebted to the Association
for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA)
and Biotechnology and Biological Sciences Research Council (BBSRC) and the De-
partment for International Development (DFID) UK, for the financial support without
which this work could not be possible. I appreciate the moral and technical support of
my colleagues at the Department of Plant Sciences of Kenyatta University and the
Department of Animal and Plant Sciences, University of Sheffield. To Morris Muthini
who selflessly assisted during compilation of this work, God bless you. Thank you
also to my friend Eric Kuria, who provided excellent proof-reading of this thesis at
short notice. Lastly I acknowledge the special role played by my family; my husband
for his boundless support and especially for caring for the children while I was away
in the UK; my children for the sacrifice of time I spent away from them; my sister
Sharon Nyawira for her encouragement and support given to my children while I was
away and my mum Rose Ukima for taking me to school.
# TABLE OF CONTENTS

| TITLE | ................................................................. | i |
| DECLARATION | ................................................................. | ii |
| DEDICATION | ................................................................. | iii |
| ACKNOWLEDGEMENT | ................................................................. | iv |
| TABLE OF CONTENTS | ................................................................. | v |
| LIST OF TABLES | ................................................................. | vii |
| LIST OF FIGURES | ................................................................. | viii |
| ACRONYMS AND ABBREVIATIONS | ................................................................. | ix |
| ABSTRACT | ................................................................. | x |
| CHAPTER ONE | ................................................................. | 1 |
| INTRODUCTION | ................................................................. | 1 |
| 1.1 Background of the study | ................................................................. | 1 |
| 1.2 Problem statement and justification | ................................................................. | 3 |
| 1.3 Research questions | ................................................................. | 4 |
| 1.4 Hypotheses | ................................................................. | 4 |
| 1.5 Objectives | ................................................................. | 5 |
| 1.5.1 General objective | ................................................................. | 5 |
| 1.5.2 Specific objectives | ................................................................. | 5 |
| 1.6 Significance of the study | ................................................................. | 5 |
| CHAPTER TWO | ................................................................. | 6 |
| LITERATURE REVIEW | ................................................................. | 6 |
| 2.1 Origin and distribution of sorghum | ................................................................. | 6 |
| 2.2 Sorghum biology | ................................................................. | 6 |
| 2.3 Sorghum agronomy | ................................................................. | 7 |
| 2.4 Sorghum production and economic importance | ................................................................. | 8 |
| 2.5 Constraints to sorghum production | ................................................................. | 9 |
| 2.5.1 Striga as a constraint to sorghum production | ................................................................. | 10 |
| 2.5.1.1 Striga origin and distribution in Kenya | ................................................................. | 10 |
| 2.5.1.2 Economic importance of Striga | ................................................................. | 12 |
| 2.5.1.3 Biology and infection mechanism of Striga | ................................................................. | 12 |
| 2.6 Striga control | ................................................................. | 15 |
| 2.7 Host resistance | ................................................................. | 17 |
| CHAPTER THREE | ................................................................. | 21 |
| MATERIALS AND METHODS | ................................................................. | 21 |
| 3.1 Plant material | ................................................................. | 21 |
| 3.2 Methods | ................................................................. | 22 |
| 3.2.1 Seed sterilization | ................................................................. | 22 |
LIST OF TABLES

Table 2.1: Striga species occurrence and distribution in Kenya.................................11

Table 3.2: Striga hermonthica ecotypes used to infect the SRS sorghum lines.................................................................22

Table 4.1: Biomass, number and mean lengths of S. hermonthica (Mbita) at 21 DAI........................................................................30

Table 4.2: Biomass, number and mean lengths of S. hermonthica (Kibos at 21 DAI)..................................................................31

Table 4.3: Biomass, number and mean lengths of S. hermonthica (Alupe) at 21 DAI.................................................................32

Table 4.4: Biomass, number and mean lengths of S. hermonthica (Tanga) at 21 DAI.................................................................33

Table 4.5: Biomass, number and mean lengths of S. hermonthica (Mbita, Kibos, Alupe and Tanga) at 21 DAI..........................36
LIST OF FIGURES

Figure 2.1: The *Striga* life-cycle .................................................................14

Figure 3.1: Rhizotron setup used in the post-attachment sorghum resistance assays ..............................................................25

Figure 3.2: A typical rhizotron set-up in a growth room .................................................................25

Figure 4.1: Percentage germination of *Striga hermonthica* seeds in the presence of the artificial stimulant GR 24 .................28

Figure 4.2: *Striga hermonthica* (Sh-Mbita) growing on the roots of sorghum cultivars (in rhizotrons) 21 DAL ............................................................37

Figure 4.3: Light micrographs of transverse sections of *Striga hermonthica* haustoria penetrating sorghum roots .................39

Figure 4.4: Sorghum roots with attached Striga seedlings showing the phenotype of the resistance interaction between SRS 3308/5 and ShTanga ..................................................................................40
# ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ASARECA</td>
<td>Association for Strengthening Agricultural Research in Eastern and Central Africa</td>
</tr>
<tr>
<td>BBSRC</td>
<td>Biotechnology and Biological Sciences Research Council</td>
</tr>
<tr>
<td>CER</td>
<td>Controlled environment room</td>
</tr>
<tr>
<td>CSH</td>
<td>Coordinated Sorghum Hybrid</td>
</tr>
<tr>
<td>DfID</td>
<td>Department for International Development</td>
</tr>
<tr>
<td>DAI</td>
<td>Days after infection</td>
</tr>
<tr>
<td>DAP</td>
<td>Days after planting</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>GCA</td>
<td>General combining ability</td>
</tr>
<tr>
<td>HR</td>
<td>Hypersensitive response</td>
</tr>
<tr>
<td>HSD</td>
<td>Honestly Significance Difference</td>
</tr>
<tr>
<td>ICIPE</td>
<td>International Centre for Insect and Pest Ecology</td>
</tr>
<tr>
<td>ICRISAT</td>
<td>International Crops Institute for the Semi-Arid Tropics</td>
</tr>
<tr>
<td>IR</td>
<td>Incompatible response</td>
</tr>
<tr>
<td>KARI</td>
<td>Kenya Agricultural Research Institute</td>
</tr>
<tr>
<td>LGS</td>
<td>Low germination stimulant</td>
</tr>
<tr>
<td>LHF</td>
<td>Low production of haustorial initiation factors</td>
</tr>
<tr>
<td>SRS</td>
<td>Striga resistant sorghum</td>
</tr>
<tr>
<td>SSA</td>
<td>Sub Saharan Africa</td>
</tr>
<tr>
<td>SSR</td>
<td>Simple sequence repeats</td>
</tr>
</tbody>
</table>
ABSTRACT

Sorghum (Sorghum bicolor L. Moench) is the fifth most important cereal crop worldwide. The crop provides food security in most rural African households and is becoming a suitable alternative in many places where maize crop fails. However, its production has been greatly reduced by the parasitic weed Striga hermonthica (Del.) Benth. The intricate biological association between this hemi parasite and its sorghum host makes it difficult to control. Most of the control methods currently in use are either too expensive or not consistent with the smallholder farming systems in East Africa. The use of Striga resistant varieties has been envisaged as the most feasible strategy to combat the Striga problem because it does not require additional inputs and intense labour that are expensive to the resource poor farmers. This study evaluated post attachment resistance levels of four SRS sorghum lines against four ecotypes of Striga from Kenya and Tanzania and also characterized the phenotype of resistance mechanisms. Sorghum seeds were grown in rhizotrons (root observation chambers) and the seedlings were inoculated with pre-germinated Striga seeds and on emergence the attached Striga plants were harvested from the roots of sorghum and scored for the number of attachments, length and dry biomass. To characterize the phenotype of resistance the anatomy of the host parasite interface was studied using histochemical techniques. All statistical data collected was analyzed using ANOVA at 95% confidence interval with SPSS statistical computer software (version 21). Mean separation was carried out using Tukey’s pairwise comparison test at 5% probability level. SRS 1208/2 had the lowest biomass of attached Striga seedlings of 3.86 mg, 11.04 mg, 2.14 mg and 0.975 mg for Mbita, Kibos, Alupe and Tanga Striga ecotypes respectively. There was a significant difference in the biomass and average length of attached Striga seedlings among the three Kenyan Striga ecotypes on all sorghum lines. The phenotype of a resistance mechanism was characterized by the inability of the parasite to penetrate host endodermis, necrosis and the browning and death of attached Striga seedlings. This study has therefore shown that SRS 1208/2 sorghum line exhibits very strong broad spectrum resistance whereas SRS 2408, SRS 2208 and SRS 3308/5 exhibit intermediate resistance to the Striga ecotypes used. The elucidation of the genetic basis of resistance in SRS 1208/2 is recommended for the development of sorghum cultivars with multiple and durable resistance for use in farmers’ fields in East Africa and this will have a significant impact on the livelihoods of some of the world’s poorest farmers helping them to alleviate poverty and improve food security.
CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Sorghum (Sorghum bicolor L. Moench) is the fifth most important cereal crop worldwide after wheat, rice, maize and barley with an annual production of 61 million tonnes over the past decade (FAO, 2004; Folkertsman et al., 2005). It is ranked second, after maize as the most important cereal crop in East Africa. The crop provides food security and is becoming a suitable alternative in many places where maize crop fails (FAO, 2004). Sorghum is unique due to its tolerance to drought, waterlogging, saline or infertile soils and high temperature (Ejeta et al., 2007). Sorghum is cultivated worldwide for fodder, grain and syrup production. Nutritionally sorghum contains higher protein levels than maize (Haussmann et al., 2000). Its flour is made into bread, porridge and brew for beer and other alcoholic beverages. The straw is often used for fencing and roofing material for huts. Sweet sorghum is used for production of ethanol which is used as a biofuel (Kristjanson and Zerbini, 1999). It is also grown as a cover crop or green manure. The starch from waxy sorghums is used in adhesives. According to Khan et al. (2007) sorghum has been identified as a cover crop in push pull technology against stem borers and as reservoirs for their natural enemies.

Sorghum is grown in at least 86 countries, on an area of 47 million hectares, with annual grain production of 69 million tonnes and average productivity of 1.45 tonnes per hectare (Hess et al., 1992). Sorghum yields in Africa however range between 500-800 kg per hectare compared with yield levels of up to 7,000 kg per hectare in the developed world. The bulk of African sorghum production is centered on the savannah areas of East, West and Central Africa, where it forms a major component of the daily
menu for millions of people, either as porridge or as traditional beer. Uganda is using sorghum for the beer industry. In addition, in many developing countries, sorghum stover is used to feed cattle (Olupot, 2011).

In Eastern Africa more than 70 % of sorghum is cultivated in the dry and lowlands with low production per unit area (Haussman et al., 2000). Notable factors limiting production of sorghum in tropical Africa include poor climatic conditions, low soil nutrients, insect pests and weeds (Voortman et al., 2003). Important among the weeds is the parasitic weed *Striga* (*Striga hermonthica* Del. Benth) whose estimated yield losses in infested sorghum range from 40-100 % (Haussman et al., 2000). Farmers in Africa lack the resources to purchase and apply inputs such as fertilizers, herbicides and mechanical tillage equipment that are common in the developed countries where *Striga* is under control (Sand et al., 1990). *Striga* infestation is extending in Africa because of the high pressure on land due to population pressure.

To achieve sustainable sorghum production there is need to solve the *Striga* problem. Controlling *Striga* is an enormous task considering the high seed production rate of 10000-100000 seeds per plant which can remain viable for 20 years (Voortman et al., 2003). Several methods have been recommended for the control of *Striga* including; cultural, biological, chemical and use of tolerant varieties. In East Africa as in many developing countries, most of the available methods to date have been costly and beyond the means of farmers (Ezeaku and Gupta, 2004). The diversity of farming systems in Africa and that of the parasite have rendered the use of a single control method ineffective. At present efforts are being made to alleviate the parasitic weed problem through plant host resistance and improvement of soil fertility (Esilaba, 2006).
1.2 Problem statement and justification

Striga is one of the major constraints to sorghum production in Africa. In terms of monetary value the annual cereal losses due to *Striga* are estimated at US$ 7 billion in SSA (Olupot, 2011). In East Africa as in many developing countries, most of the available *Striga* control methods to date have been costly and beyond the means of farmers, furthermore information on race specific association of sorghum cultivars and *Striga* ecotypes is lacking. In addition *Striga* resistant sorghum varieties have not been tested repeatedly to determine the durability of resistance. Selection for resistance to *Striga* is normally done under field or greenhouse conditions. Complex interactions between host, parasite and the environment influence germination, attachment and growth of the parasite on the host roots. It is also difficult to establish a uniform *Striga* infestation level at an appropriate intensity for reliable and reproducible results (Hess, 1992). Field screening for *Striga* is therefore difficult given the many confounding factors.

The use of resistant sorghum varieties has been identified as the most feasible and economical *Striga* control measure because it does not require any additional inputs (Haussman *et al.*, 2000; Atera *et al.*, 2012). Precise and reliable screening techniques are prerequisites for success when breeding for resistance to any biotic or abiotic stress factor (Taylor, 2001; Jamil *et al.*, 2011). Distinct defense mechanisms to *Striga* parasitism have been identified using agar gel and extended agar gel assays. One of the post-attachment mechanisms is the incompatible response where parasite development is arrested with no apparent necrosis to the host root. The actual host defense
mechanism underlying this response is not known. This study was carried out using rhizotrons (root observation chambers) in controlled environment growth rooms for efficient screening of post-attachment resistance levels and resistance mechanisms of the selected sorghum lines to *Striga*. Identification of *Striga* resistant sorghum cultivars in this study could significantly contribute in breeding for *Striga* resistant sorghum that will eventually be used in integrated *Striga* control strategies in East Africa.

1.3 Research questions

i. Do sorghum lines developed for field resistance show post attachment resistance to *Striga* under controlled conditions?

ii. Do sorghum lines show multiple resistance to different *Striga* ecotypes?

iii. Do sorghum lines exhibit similar phenotype of resistance mechanisms?

1.4 Hypotheses

i. Sorghum lines selected for field resistance do not show post attachment resistance to *Striga* under controlled conditions.

ii. Sorghum lines do not show multiple resistance to different *Striga* ecotypes.

iii. Sorghum lines do not exhibit similar phenotype of resistance mechanisms
1.5 Objectives

1.5.1 General objective

To screen post attachment resistance levels on selected field resistant sorghum lines to different ecotypes of *S. hermonthica* from different regions of Kenya and Tanzania.

1.5.2 Specific objectives

i. To determine post attachment *Striga* resistance in selected field resistant sorghum lines under laboratory conditions.

ii. To determine multiple resistance of selected field resistant sorghum lines to different *Striga* ecotypes.

iii. To determine the phenotype of resistance mechanisms of selected sorghum lines to different *Striga* ecotypes.

1.6 Significance of the study

The findings of this study will significantly contribute to the development of stable high yielding sorghum cultivars that have multiple and durable resistance to *Striga* and are acceptable to farmers. This will enable resource poor farmers to grow sorghum without additional inputs, helping them to improve food security and alleviate poverty.
CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of sorghum

Sorghum is believed to have its origin in the Ethiopian highlands and South Sudan (Dogget and Prasada, 1995). It was domesticated in Sudan about 1000 BC probably from Sorgum arudinaceum which was later called Sorghum Sudanese (ICRISAT, 2004). The crop is grown on 44 million hectares in 99 countries in Africa, Oceania, Asia and America, with annual grain production of 69 million tonnes and an average productivity of 1045 kg per hectare. The bulk of African sorghum production is centered on the savannah areas of East, West and Central Africa, with Nigeria being the leading producer of sorghum in Africa. It is the only cereal species indigenous to Kenya, therefore it is produced throughout much of the country even in areas with low agricultural potential. Most sorghum production is concentrated in Kenya’s Western and Nyanza regions.

2.2 Sorghum biology

Sorghum bicolor (L.) Moench is an annual C4 monocot with a genome size of 730 mega bases (Paterson et al., 2009). It is predominantly a self-pollinated species although outcrossing occurs. Genetically, S. bicolor is a functional diploid with \(2n = 20\) chromosomes although evidence of tetraploids has been reported (Ghaffari, 2009). Plant height vary from less than a meter to five meters while inflorescence types vary from open to compact with a wide range of dimensions. Basal plant color can be tan
colored, red or purple. The caryopsis is 4-8 mm in diameter rounded with a blunt tip. The grain shape and size depend on variety; colour too varies from white to yellow, brown, red and black depending on the specific genotype (Langer, 1991). Under subtropical conditions, even planting densities and optimum conditions sorghum hybrids take between 60-80 days to flower. Yanase et al. (2008) reported that in commercially grown hybrids panicles complete flowering in 8 days while whole fields take 13 days to flower. Maturation takes between 90 and 120 days depending on the variety.

2.3 Sorghum agronomy

Sorghum can grow anywhere from sea level to 2,500 meters above sea level and requires a minimum rainfall of 250mm per year and a minimum temperature of 10°C (Conley et al., 2005). Sorghum does best in sandy-loam soils of pH range of between 5 and 8.5. Sorghum requires fine seedbed for better seed establishment. When a tractor or oxen plough is used to open the ground it is advisable to harrow it after ploughing. Planting is by drilling or hole planting where early and dry planting is highly recommended. The recommended seed rate is 7-10 kg/ha with an optimum spacing of 75 cm x 20 cm giving a plant population of 6600 plants. The planting depth should be 2.5 cm to 4 cm at the onset of rains and 5 cm when dry planting (Conley et al., 2005). It can be planted as a monocrop or as an intercrop with beans, pigeon pea cowpea and green gram. Weeding is carried out 3 weeks after emergence and thinning should be done after the first weeding to leave one seedling per hole.
2.4 Sorghum production and economic importance

Sorghum yields in Africa range between 500-850 kg per hectare compared with yield levels of up to 7,000 kg per hectare in the developed world. Eighty percent of the area devoted to sorghum is located within Africa and Asia with average yields of 810 and 1150 kg/ha respectively. In Kenya the average yield from 1990 to 2011 remained low at 800 kg per hectare despite the development of new seed varieties with the potential to yield 2000 to 5000 kg per hectare (Atera et al., 2013).

Sorghum is a staple food crop for many low income households in Kenya. It is typically grown by small scale, resource poor farmers and is mainly used for home consumption (MacOpiyo et al., 2010). Most sorghum grain in Kenya is consumed by rural households, who typically grind it into flour to make porridge and “ugali”. Some sorghum grain is also processed into flour by commercial mills and sold in urban markets, some 10 percent goes to the animal feed industry and 2 percent is used as seed for planting (FAO, 2004). In recent years there has been growing demand for sorghum within the brewing industry for use in beer production. As such KARI (Kenya) in collaboration with EABL (East African Breweries Limited) has been promoting the use of high quality sorghum varieties such as Gadam, to supplement barley (MacOpiyo et al., 2010) in beer production. This development has spurred renewed interest in the commercial production of sorghum as it offers farmers prospects for higher returns.
2.5 Constraints to sorghum production

Under optimal field conditions grain yield can reach 15000 kg/ha when rain is not a limiting factor (FAO, 1995). In marginal environments the average sorghum yields are low ranging from 800 kg/ha in Africa to 3400 kg/ha in America. In Eastern Africa more than 70% of sorghum is cultivated in dry and lowlands with low production yields per unit area (Gethi et al., 2005). Despite the importance of sorghum, its production is characterized by low on-farm yields ranging from 800 to 1500 kg per hectare (Omanyà et al., 2004). The realized yields are far below the potential yield of 5000 kg ha\(^{-1}\) in improved varieties under high levels of management in the absence of pests and diseases. Notable factors limiting production of sorghum in tropical Africa include: poor climatic conditions, low soil nutrients, weeds and insect pests (Voortman et al., 2003). Insect pests particularly stem borers and shoot flies cause the greatest yield loss, *Striga* and other weeds are the second most important constraints that cause yield reduction in sorghum (Patrick et al., 2004).

Sorghum grain yield is reduced due to poor soil fertility (in terms of nitrogen and phosphorous deficiency) and bird damage especially Quelea. While they are not among the first eleven constraints listed, sorghum diseases like grain mold, anthracnose and head smut also cause considerable yield reductions in sorghum productivity in East Africa. Some of these limitations have been solved through farm management and development of suitable plant varieties (Ejeta et al., 2007). The most economically important weed is the root parasite *Striga hermonthica* which causes estimated crop yield losses ranging from 40-100% (Gressel et al., 2004).
2.5.1 Striga as a constraint to sorghum production

2.5.1.1 Striga origin and distribution in Kenya

It is believed that *Striga hermonthica* and *S. asiatica* originated in the Nubian hills of Sudan and Semien mountains of Ethiopia. These areas are also known to be the origin of sorghum and pearl millet which are readily infected by the witch weed (Ejeta, 2007). *S. gesnerioides* may have originated in West Africa. Over the years, *Striga* has spread to other parts of sub-Saharan Africa through the activities of man. There are nine (9) *Striga* species found in Kenya (Table 2.1). Among them, *S. hermonthica* is considered to be the most dangerous and common particularly in the densely populated regions of Nyanza and Western Provinces of Kenya (Dogget, 1965; MacOpiyo *et al.*, 2010). *S. asiatica* is predominantly found in the Coastal region infecting upland rice (Gethi *et al.*, 2005) and exists sporadically in Isiolo, Busia and Naivasha (Mohamed *et al.*, 2001). The species that is adapted as a pest of legume crops, *S. gesnerioides*, has a wide geographical distribution in Kenya compared to the other species. It occurs as far as Kilifi (Coastal province of Kenya) spreading to Homa hills (Nyanza province, Western Kenya) infecting cow pea.
<table>
<thead>
<tr>
<th>Striga species</th>
<th>Host Plants</th>
<th>Occurrence Area</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. asiatica</em></td>
<td>Maize, rice, sorghum, pearl millet, finger millet, sugar cane, wild grasses</td>
<td>Kilifi, Isiolo, Mathews range, Alupe, Daka Chom, Kiunga</td>
</tr>
<tr>
<td><em>S. bilabiata</em></td>
<td>Wild grasses</td>
<td>Naivasha, Chyulu hills, Rumbia, Kahawa, Mathews range</td>
</tr>
<tr>
<td><em>S. elegans</em></td>
<td>Wild grasses</td>
<td>Nairobi, Loitokitok, Laikipia, Rumuruti</td>
</tr>
<tr>
<td><em>S. forbesii</em></td>
<td>Sorghum, rice, maize, sugar cane</td>
<td>Narok, Mara plains, Kipini, Chyulu hills, Uasin Gishu</td>
</tr>
<tr>
<td><em>S. gesneroides</em></td>
<td>Cowpea</td>
<td>Gishu plateau, Trans Nzoia, Kilifi, Buna, Homa hills, Rongo, Nairobi, Naivasha</td>
</tr>
<tr>
<td><em>S. hermonthica</em></td>
<td>Maize, rice, sorghum, pearl millet, finger millet, sugar cane, wild grasses</td>
<td>Alupe, Churaimbo, Miwani, Bungoma, Kendu, Migori, Kuria, Nyamira, Siaya, Homabay</td>
</tr>
<tr>
<td><em>S. latericea</em></td>
<td>Sugar cane, wild grasses</td>
<td>Samburu, Mariakani, Kwale, Voi, Machakos, Sultan Hamud, Kilifi, Mwea</td>
</tr>
<tr>
<td><em>S. lutea</em></td>
<td>Wild grasses</td>
<td>Kwale, Shimba hills, Embu, Chyulu hills</td>
</tr>
<tr>
<td><em>S. pubiflora</em></td>
<td>Sugar cane, wild grasses</td>
<td>Kwale, Shimba hills, Voi</td>
</tr>
</tbody>
</table>

Source: Atera et al., 2013
2.5.1.2 Economic importance of *Striga*

*Striga* infestation causes a loss of 30-50% to Africa’s agricultural economy on 40% of its arable land (Amudavi *et al.*, 2007; Hearne, 2009). A survey conducted in 30 communities in Borno state, Northern Nigeria, indicated that farmers’ rated *Striga* infestation as the leading priority constraint together with low soil fertility to crop production (Dugje *et al.*, 2006). Similar earlier surveys (Weber *et al.*, 1995; Kim *et al.*, 1997) showed *S. hermonthica* as a serious problem in Guinea savanna of Nigeria and yield losses ranged from 10 to 100%.

In Western Kenya, a survey of 83 farms revealed that 73% of the farms are infested with *S. hermonthica* (Woomer and Savala, 2009). The average yield loss due to *Striga* is 1.15, 1.10 and 0.99 tons per hectare for maize, sorghum and millet, respectively (MacOpiyo *et al.*, 2010). However, the damage can reach as high as 2.8 tons per ha in maize and sorghum in some locations with high *Striga* densities (Andersson and Halvarsson, 2011). The loss represents 12.3% of the 2.4 million metric tonnes of maize that Kenya produces annually. This translates to about 39.6 kg of maize loss per capita, amounting to about 20% of a typical person’s annual food requirement.

2.5.1.3 Biology and infection mechanism of *Striga*

*Striga hermonthica* (Del.) Benth commonly known as witch weed or *Striga* belongs to the genus *Striga* that comprise obligate root hemi parasites that can live either parasitically or free (Lagoke *et al.*, 1986). The genus is now classified in the family of Orobanchaceae although earlier authors placed it in Scrophulariaceae (Gethi *et al.*, 2005).
The genus consists of 30 species of parasitic plants that occur naturally in parts of Africa, Asia and Australia. Only five are presently of economic importance in Africa, these are *S. hermonthica* (Del.) Benth, *S. asiatica* (L.) Kuntze, *S. gesnerioides* (Willd) Vatke, *S. aspera* (Willd) Benth and *S. forbesii* Benth. Except *S. gesnerioides* which affects cowpea and tobacco all the others are parasites of cereal crops namely sorghum, maize, millet and rice in Africa. (Ramaiah *et al.*, 1983). Crops such as wheat (Ejeta, 2007) and napier grass (Atera *et al.*, 2013) previously unaffected by *Striga* are now showing serious infestation in Sahel. Among the *Striga* species *S. hermonthica* poses the greatest risk in agricultural production. According to Watt (1936) *Striga* has been in existence in farmer’s fields in the Lake Victoria Basin, Western Kenya. *S. hermonthica* is an erect plant whose height does not exceed a meter. The flowers are purple in colour arranged in spikes 15-45 cm long. The seeds develop in small capsules that split open to release their seeds. One capsule can contain 250-500 seeds with a *Striga* plant producing up to 50000 seeds (Oswald and Odhiambo, 1999).

Seeds are the sole source of inoculum in soil. In the soil the seeds require moisture for one to two weeks before they can be ready to germinate (Scholes, 2008). This period is referred to as preconditioning. The seeds germinate in the presence of chemical stimulants called strigolactones exuded by the root of the host plant (Bouwmeester *et al.*, 2003; Yoneyama *et al.*, 2010). The radical of the germinated seed grows towards the root of the host plant, the radical tip of the *Striga* seedling then makes contact with the host root and enlarges giving rise to a structure known as haustorium (Fig 2.1). The haustorium has three functions, attachment, penetration and nutrient acquisition from the host (Stewart and Press, 1990; Patrick *et al.*, 2004; Patrick and Ejeta, 2008;). On contact to the host root the haustorium attaches itself by sticky root hairs. It then
forms a wedge shaped group of cells that penetrate through the host root cortex and endodermis. The xylem cells then establish xylem continuity with the host and the haustorium undergoes further development (Scholes, 2008). After attachment the *Striga* plant derives water, carbohydrates and nutrition from the host (Parker and Riches, 1993; Press and Graves, 1995). After six weeks the *Striga* plant emerges above the ground, however not all seedlings will emerge and many remain underground. The *Striga* plant flowers 3 to 4 weeks after emergence and within fourteen days produce the first viable seeds.

Figure 2.1: The *Striga* life-cycle (Rich and Ejeta, 2007).
2.6 *Striga* control

Manipulation of *Striga* seed number in the soil is central to *Striga* control strategies, either through reduction of *Striga* seed bank in the soil, or through limitation of *Striga* seed production (Haussmann *et al.*, 2000). It is also important to prevent dissemination of *Striga* seed to un-infested fields through movement of grain contaminated with *Striga* seed. Some of the available control options for *S. hermonthica* include: fallowing, hand pulling, hoe weeding, use of trap and catch-crops, seed treatment, application of appropriate rate of nitrogen fertilizer, herbicide spray and use of biological control agents (Lagoke *et al.*, 1988; Stroud 1993). These methods have their advantages and disadvantages, and in general are not able to effect total control.

Fallowing and crop rotation have to be continued for many years because *Striga* seeds remain viable for 20 years in the soil (Dogget, 1988). In addition, any wild hosts and volunteer plants have to be weeded out for these two methods to be effective. Hand pulling is the most widely practiced control method for *Striga* in most African countries (Esilaba, 2006). It is labour intensive and it is recommended to begin 2-3 weeks after *Striga* begins to flower to prevent seeding (Parker and Riches, 1993). Trap crops stimulate *Striga* seed germination but are not parasitized by *Striga*. They cause suicidal germination of *Striga* when used because the parasite is not able to attach to non-host crops. When grown several times they lead to gradual depletion of *Striga* seed bank. The most widely used trap crops are leguminous crops such as cowpea, soy bean, pigeon pea, chick pea, kenef and ground nut (Esilaba, 2006). The limitation of this method is the heavy demand for land to produce the preferred food crops that are mainly cereals. Catch crops stimulate *Striga* seed germination and are parasitized but the crop should be destroyed before *Striga* sets seed. This poses problems because
the season length is very short such that it is not possible to plant another crop after
the catch crop has been removed. The other limitation to this method is the more than
tree years required for rotation to reduce the parasitic weed (Dogget, 1988; Esilaba
and Ransom, 1997).

Chemicals such as imazapyr have been shown to provide early season Striga control
in specific maize varieties. Dicamba applied during pre-emergent of Striga can con-
trol early Striga attachment and increase yield under restricted circumstances (Kan-
ampiu et al., 2002). Herbicides can be used but are not normally affordable to re-
source poor farmers. Sorghum is mostly grown as a subsistence food crop, thus under
such circumstances cannot be economical to smallholder farmers even if they afford-
ed them. Moreover continued use of chemicals causes environmental pollution posing
potential danger to farmers who apply the herbicides and harm to other non-targeted
organisms.

The application of fertilizer or manure raises soil fertility and promotes plant growth,
resulting in suppression of Striga (Stroud, 1993). The practice requires that the whole
field to be treated. Resource poor farmers cannot afford fertilizer and have too few or
no livestock to provide sufficient manure. Biological control is also being explored to
provide control to Striga. Few systematic studies of individual natural enemies of
Striga and their influence on the population of host have been conducted; Genus Smi-
cronyx has been identified as of greatest interest for biological control (Lendzemo et
al., 2006). Fungal pathogens of the Fusarium Genus have also been isolated from
emerged plants and have been found to reduce or kill S. hermonthica (Zahran et al.,
2008) However further studies under field conditions need to be carried out.
None of the available control measures has been found to provide complete *Striga* eradication in isolation which has led to the development of several integrated techniques for the control of *Striga*. These involve the combination of various strategies for example, Ransom and Odhiambo (1994), found that incorporating stover combined with fertilisers and hand weeding had the highest yield in maize after several on farm trials conducted in Western Kenya. At present, efforts are being made to alleviate the parasitic weed problem in the badly infested soils through host plant resistance and improvement of soil fertility (Esilaba, 2006). A number of basic resistance mechanisms to *Striga* including low stimulant production, mechanical barriers to *Striga* ingress, antibiosis and hypersensitivity, have been suggested in sorghum (Mohamed et al., 2003).

2.7 Host resistance

Resistance is the ability of a plant, whether host or non-host, to evade parasite attack or following attack to prevent establishment and growth of the parasite (Rich et al., 2004). Among the control methods, host-plant resistance is the most economic control measure since it is affordable to farmers and resistant cultivars can be grown without additional inputs (Hess and Ejeta, 1992). *Striga* has co-evolved with sorghum whose origin is Africa. The crop has therefore developed various complex mechanisms of resistance to the parasite as a survival strategy (Hess et al., 1992; Mohamed et al., 2003; Mutengwa, 2004; Ejeta, 2007). For example, the mechanism of resistance of SRN39 to *Striga* species was observed to be a combination of low stimulant production, and restricted parasite development following attachment to host roots (Patrick and Ejeta, 2008).
Earlier research has identified four specific mechanisms of resistance to *Striga* in a series of cultivated sorghums and some wild accessions (Mohamed 2003; Mohamed *et al.*, 2003; Rich *et al.*, 2004; Ejeta, 2007). These are low production of germination stimulant (LGS), low production of the haustoria initiation factor (LHF), hypersensitive response (HR) and incompatible response (IR). Initial genetic studies have also hinted on the inheritance of some of these resistance mechanisms in sorghum. For example, the low germination stimulant production was said to be inherited as a single recessive gene. Sorghum genotypes that produce very low levels of the germination stimulants have been found to be resistant to *Striga* in field tests (Ramaiah, 1987; Hess *et al.*, 1992). All highly susceptible sorghum genotypes appear to be high producers of the germination stimulant (Ejeta, 2007). The sorghum cultivars SRN39, Framida, 555, IS9830, ICSV1006 and a wild accession *S. bicolor* subspecies *drummondii* have been reported to exhibit the LGS character as their mechanism of resistance to *Striga* (Ejeta *et al.*, 2007). It was earlier reported that the LGS character is controlled by a single recessive gene in sorghum (Vogler *et al.*, 1996). However, Haussmann *et al.* (2000) reported that one major gene and several minor genes were involved in the stimulation of *S. hermonthica* seed germination.

In other studies on general combining ability (GCA) effects for germination distance in agar-gel assay, it was found that different sets of alleles were responsible for stimulation of *S. hermonthica* seed germination in some sorghum cultivars such as Framida, IS9830 and 555 (Haussmann *et al.*, 2001). These findings suggest that there is still need for additional studies to fully understand the inheritance of the LGS character as a mechanism of *Striga* resistance in sorghum.
In resistance based on LHF, germinated *Striga* near the roots of sorghum possessing this trait normally do not form haustoria and therefore die because they are unable to attach to their potential host. Mohamed (2003) employed an extended agar-gel assay to study the inheritance of LHF. He concluded that a single dominant gene conditioned the LHF character. Most of the studies on the LHF have been based on wild sorghum accessions (Patrick *et al.*, 2004). Few or no studies have been carried out on cultivated sorghum genotypes. There is need to explore this trait and understand its genetic behaviour in cultivated sorghum genotypes that may exhibit resistance to *Striga*.

Resistance based on the HR involves localised necrosis of host tissues surrounding the site of attempted parasite attachment, presumably coupled with a release of phytoalexins that kill the attached *Striga*. Hypersensitive response has been extensively studied in a wide range of host-parasite associations, where it is generally characterized by the appearance of a necrotic zone around the site of attempted infection. In this case, host cell death results in unsuccessful establishment of the parasite and leads to its ultimate demise. Hypersensitive response has been observed in sorghum cultivars Dobbs, Framida, Serena and wild accessions *S. bicolor* subspecies *drumondii*, *S. hewisonni* and *S. b. verticilliflorum* (Patrick *et al.*, 2004).

In the IR mechanism parasite development beyond attachment is discouraged. In host genotypes whose *Striga* resistance is based on IR, *Striga* seedlings that succeed in penetrating host tissue may not develop beyond emergence of the first leaves (Mo-
hamed, 2003). Some *Striga* will be observed to develop normally at first but later show signs of stunted growth (Ejeta, 2007). The reaction is similar to that observed when *Striga* unsuccessfully infests non-host plants. According to Patrick *et al.* (2004), sorghum cultivars SRN39, ICSV761 and the wild accession *S. b.verticilliflorum* possess this trait. However, its inheritance is so far not clear. This study investigated the existence of post-attachment stage resistance (host root penetration) on selected field resistant sorghum lines.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

Sorghum seeds used in this study comprised of four lines (SRS1208/2, SRS3308/5, SRS2408 and SRS2208). These four were picked from nine sorghum lines that were developed and selected for field resistance to *S. hermonthica* in Uganda (Olupot, 2011). They were developed by crossing four *Striga* resistant sorghum lines (Brhan, N13, SRN39 and Framida) with four locally adapted and high yielding sorghum lines (Sekedo, Hakika, Dobbs and Karimtama) according to the North Carolina II mating design (Comstock *et al.*, 1952). The parent lines were crossed as follows: Brhan x Dobbs (SRS3108), Brhan x Karimtama (SRS4609), N13 x Sekedo (SRS609), N13 x Dobbs (SRS1708), N13 x Karimtama (SR3108), SRN39 x Sekedo (SRS3408), SRN39 x Hakika (SRS2408), Framida x Hakika (SRS1208) and Brhan x Hakika (SRS2208). The seeds were obtained from the National Semi-Arid Resources Research Institute, Serere, Uganda courtesy of Dr. Robert Olupot.

The highly resistant parental line Brhan and the commercially available cultivar CSH-1 previously shown to be susceptible to infection by *Striga* species (Press *et al.*, 1987; Cechin and Press, 1993; Frost *et al.*, 1997) were used as controls. The CSH-1 was obtained from the animal and plant sciences laboratory of University of Sheffield, UK courtesy of Prof. Julie Scholes. The *Striga* seeds used in this study consisted of four *S. hermonthica* ecotypes, three *Striga* ecotypes were harvested in sorghum and maize infested fields in Kenya, courtesy of Dr. Steven Runo. The Tanga *Striga* eco-
type was harvested from sorghum in Tanga, Tanzania and cold preserved in the animal and plant sciences laboratory of University of Sheffield, UK courtesy of Prof. Julie Scholes (Table 3.1).

Table 3.1: *Striga hermonthica* ecotypes used to infect the SRS sorghum lines

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Details</th>
<th>Latitude : longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Striga hermon-thica</em>- Kibos</td>
<td>Collected from maize grown at the Kenya Agricultural Research institute (KARI), Kibos, Kisumu, Kenya in 2009</td>
<td>0°02'20&quot;S : 34°47'57&quot;E</td>
</tr>
<tr>
<td><em>Striga hermon-thica</em>- Alupe</td>
<td>Collected from sorghum grown in a farmer's field in Alupe near Busia, Kenya in 2009</td>
<td>0°28'N : 34°05'E</td>
</tr>
<tr>
<td><em>Striga hermon-thica</em>- Tanga</td>
<td>Collected from sorghum grown in farmer's field in Tanga, Tanzania in 1992</td>
<td>5°04'08&quot;S : 39°05'55&quot;E</td>
</tr>
<tr>
<td><em>Striga hermon-thica</em>- Mbita</td>
<td>Collected from sorghum grown in farmer's field in Mbita, Kenya in 2011</td>
<td>0°26'20&quot;S : 34°13'08&quot;E</td>
</tr>
</tbody>
</table>

3.2 Methods

3.2.1 Seed sterilization

Sorghum seeds were surface sterilized using 20 ml of 5 % (v/v) Virkon in falcon tubes for 5 min twice then rinsed thoroughly with sterile distilled water to remove traces of the disinfectant.

*Striga* seeds were placed in sterilin tubes then surface sterilized in 10% (v/v) commercial bleach (sodium hypochlorite) for 7 min and then rinsed thoroughly with sterile distilled water. The seeds were then transferred onto 9 cm diameter discs of glass fiber filter paper (GF/A Whatman, BDH, Poole, UK) on a filter funnel placed on a conical flask and rinsed with about 150 cm³ of distilled water. The seeds on the filter
paper discs were then transferred onto a sterile Petri dish and sprayed gently with distilled water to spread them evenly on the paper.

3.2.2 Preconditioning and germination of *Striga* seeds

Petri dishes containing surface sterilized *Striga* seeds were sealed with parafilm and wrapped with aluminium foil then incubated in a controlled environment room (CER) maintained at a temperature of 30 °C for 14 days. On the thirteenth day, eighteen hours before infection 2 ml of the artificial stimulant GR24 (0.1 ppm) solution was added into each petri dish containing *Striga* seeds as recommended by Gurney *et al.* (2006), the plates were then resealed and returned to the CER. The GR24 was added to ensure synchronous attachment of *Striga* to sorghum roots.

3.2.3 Germination tests

The pre-germinated *Striga* seeds were detached from the GFA paper by gently washing them with distilled water, the suspended *Striga* seeds were then poured in a beaker and about 1 ml of the suspension (containing approximately 50 seeds) was sucked using a Pasteur pipette and placed in each well of microtitre plate. The wells were then examined under dissecting microscope and the numbers of germinated seeds were counted to determine the germination percentage.

3.2.4 Growth and infection of sorghum plants

Sorghum seeds were germinated between two sheets of moistened glass fibre filter paper (GF/A Whatman, BDH, Poole, UK) supported by a block of moistened horticultural Rockwool (Aquaculture, Sheffield, UK) these were placed on potting trays
and kept in the dark in a temperature controlled chamber, at 30 °C for two days to stimulate even germination. The seedlings were then transferred in the controlled environment growth room for another four days. The growth rooms operated with a 12 hr photo period and a photon flux density of 800 μmol m⁻² s⁻¹. Day: night temperatures were maintained at 27:20 °C and day: night humidity was maintained at 50:70 %. After 7 days, a single sorghum seedling was transferred to a root observation chamber (rhizotron). A rhizotron consisted of a 22 cm x 22 cm x 2 cm³ petri dish filled with vermiculite onto which a mesh was placed (100 μm polyester, Plastic Group, Birkenhead UK). The roots of the sorghum grew down the mesh and openings at the top and bottom allowed for shoot growth and root growth respectively while a block of rock wool at the base aided in drainage.

A diagram of a rhizotron and photos of the experimental set-up are given in Figures 3.1 and 3.2 respectively. The rhizotrons were wrapped in aluminium foil to prevent light from reaching the roots. The rhizotrons were drip fed with 40 % (v/v) Long Ashton solution containing 1 mol m⁻³ ammonium nitrate (Hewitt, 1966) at four hour intervals during each photoperiod to give a total volume of 200 ml d⁻¹. Five replicate plants for each sorghum line were established for each treatment in four independent experiments. Rhizotrons were placed in completely randomized design.

Fifteen days after planting (DAP) each sorghum plant was infected with 15 mg preconditioned, pre-germinated Striga seeds by carefully aligning the seeds along the roots using a fine paint brush. The Striga seeds were first rinsed twice with distilled water to remove traces of the artificial germination stimulant, GR24 and suspended in about 20 ml of distilled water. They were germinated prior to infection to ensure
synchronous attachment of the parasite to the sorghum roots and to overcome any resistance at the level of germination. The rhizotrons were returned into the growth room in a completely randomized design.

Fig 3.1: Rhizotron setup used in the post-attachment sorghum resistance assays. Once the chamber lid was affixed, the rhizotron was wrapped in aluminium foil to keep the root system in the dark.

Fig 3.2: A typical rhizotron set-up in a growth room.
3.2.5 Post attachment resistance assays

To screen cultivars for post attachment resistance, *Striga* plants were harvested using forceps from the roots of infected sorghum plants 21 days after infection (DAI). Harvested *Striga* plants from each host plant were placed in a 90 mm petri dish and photographed using a CCD camera (Diagnostic Instruments Inc.) mounted on a Leica MZFIII stereomicroscope (Leica instruments GmbH). The number and length of *Striga* plants in each host plant was calculated from the photographs using image analysis software- ImageJ, v. 1.45 (http://rsb.info.nih.gov/ij/). The *Striga* was then incubated at 60°C for one week and thereafter the dry biomass was measured using a digital balance.

3.2.6 Mechanisms of sorghum resistance to *Striga* (microscopy)

To determine the extent of parasite growth within the sorghum root cortex small sections of parasite root at the point of haustoria attachment were dissected from host plants at 3 and 10 DAI. Sections were fixed using Carnoy’s fixative 1 (3:1 v/v, 100% ethanol: glacial acetic acid) and vacuum infiltrated for 20 min. The samples were then washed in 2 x 100% ethanol for 30 min each and then embedded using Technovit 7100 kit (TAAB, UK). They were transferred to ethanol and Technovit solution, 1:1 for 1 hr and then into 100% Technovit solution for 1 hr. The samples were then transferred into fresh Technovit solution for 3 days and refrigerated at 4°C. After 3 days samples were transferred into Eppendorf lid moulds and Technovit solution and hardener 1 (1:15 v/v) were added. As the Technovit resin became viscous, samples were positioned in a vertical position for sectioning. The blocks were left overnight for the resin to harden. The resin blocks were then mounted on histoblocs. They were then
trimmed and sectioned into five micron thick sections using a Leica R12145 microtome (Leica instruments GmbH). The sections were then transferred to adhesive treated microscope slides (poly-lysine slides; SLS, Nottingham, UK). Sections were stained with 0.1 % azure 11 stain in 100 mM phosphate buffer PH 7 for 20 seconds, washed in distilled water and dried at 65°C for 30 min on a hot plate. Sections were mounted on glass slides with DePex (BDH, Poole, UK) and observed using an Olympus BX51 microscope (Olympus Optical Ltd, London, UK), then photographed using a digital camera (Olympus DP71; Olympus Optical Limited).

3.2.7 Statistical analyses

*Striga* dry biomass, number and length supported by each host were analysed using analysis of variance (ANOVA) procedures for a complete randomized design at 95% confidence interval using the software IBM SPSS Statistics (V21). Tukey’s honestly significant difference (HSD) test was then performed to separate the means at 5% probability level.
CHAPTER FOUR

RESULTS

4.1 Germination studies

*Striga hermonthica* seed from the four regions under study: Kibos; Mbita; Alupe and Tanga germinated in the presence of the artificial germination stimulant GR 24 (0.1 ppm). The seeds exhibited a narrow range in percentage germination varying from 44 percent in Alupe ecotype to 63 percent in Mbita (Fig 4.1).

![Figure 4.1: Percentage germination of *Striga hermonthica* seeds in the presence of the artificial stimulant GR 24.](image-url)
4.2 Resistance of the SRS sorghum cultivars to *Striga hermonthica* ecotypes

4.2.1 Resistance to *Striga hermonthica* Mbita ecotype

The SRS lines exhibited a range of susceptibility to *Striga hermonthica* from Mbita (Sh-Mbita). Table 4.1 shows the mean biomass, number of *Striga* seedlings and the average length of the *Striga* seedlings measured after harvesting 21 DAI. The sorghum line SRS 1208/2 exhibited the greatest resistance with very few attachments (27.2) which were comparatively small in terms of weight and length (Table 4.1). However the mean biomass of the *Striga* attached to the SRS 1208/2 was not significantly different to that attached on the resistant control (Brhan). The susceptible control (CSH-1) recorded the highest biomass and average length of attached *Striga* seedlings of 29.5 mg and 7.76 mm respectively (Table 4.1). The SRS lines 2208, 2408 and 3308/5 exhibited intermediate resistance to this particular *Striga* ecotype. There was no significant difference in the number of attached parasites amongst all the sorghum cultivars evaluated including the controls (Table 4.1).
Table 4.1: Biomass, number and mean lengths of *S. hermonthica* (Mbita) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21 DAI

<table>
<thead>
<tr>
<th>VARIETY</th>
<th>Striga biomass (mg)</th>
<th>Number of attachments</th>
<th>Length of <em>S. hermonthica</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brhan</td>
<td>$5.24 \pm 0.9042_a$</td>
<td>$34.8 \pm 10.077_a$</td>
<td>$4.032 \pm 0.9082_{ab}$</td>
</tr>
<tr>
<td>1208/2</td>
<td>$3.86 \pm 0.8165_a$</td>
<td>$27.2 \pm 5.398_a$</td>
<td>$2.1328 \pm 0.3439_a$</td>
</tr>
<tr>
<td>2408</td>
<td>$16.2 \pm 2.5374_b$</td>
<td>$62.25 \pm 19.512_a$</td>
<td>$5.1765 \pm 0.5572_b$</td>
</tr>
<tr>
<td>2208</td>
<td>$8.85 \pm 2.5287_{ab}$</td>
<td>$57.75 \pm 19.019_a$</td>
<td>$3.7438 \pm 0.4625_{ab}$</td>
</tr>
<tr>
<td>3308/5</td>
<td>$8.84 \pm 1.8114_{ab}$</td>
<td>$42.2 \pm 11.711_{a}$</td>
<td>$3.8888 \pm 0.2599_{ab}$</td>
</tr>
<tr>
<td>CSH-1</td>
<td>$29.5 \pm 3.8301_c$</td>
<td>$80.33 \pm 25.757_{a}$</td>
<td>$7.7633 \pm 0.3890_c$</td>
</tr>
<tr>
<td>MEAN</td>
<td>$10.71$</td>
<td>$47.77$</td>
<td>$4.20$</td>
</tr>
<tr>
<td>P VALUE</td>
<td>0.000</td>
<td>0.193</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data presented are means ± SE. Means within a column not followed by the same lower case letter show significant difference (Tukey’s HSD) at 5% probability level.

### 4.2.2 Resistance to *Striga hermonthica* Kibos ecotype

When infected with *Striga hermonthica* Kibos ecotype—similar to infection with Sh-Mbita ecotype—there was no significant difference in the number of attached *Striga* seedlings in the roots of all the sorghum cultivars including the controls ($P = 0.173$) (Table 4.2). There was significant difference in the biomass ($P = 0.000$) and the average length ($P = 0.001$) of the attached *Striga* seedlings. Brhan had the lowest *Striga* biomass of 7.32 mg while CSH-1 had the highest *Striga* biomass of 21.14 mg (Table 4.2). Whereas the *Striga* biomass of SRS 2408 was not significantly different from that of Brhan, the average length of the *Striga* seedlings attached to it was highly significant from the other sorghum cultivars recording the highest length of 6.89 mm.
Table 4.2: Biomass, number and mean lengths of *S. hermonthica* (Kibos) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21DAI

<table>
<thead>
<tr>
<th>VARIETY</th>
<th><em>Striga</em> biomass (mg)</th>
<th>Number of attachments</th>
<th>Length of <em>S. hermonthica</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brhan</td>
<td>7.32 ± 1.2520&lt;sub&gt;a&lt;/sub&gt;</td>
<td>43.4 ± 8.992&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.418 ± 0.1921&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>1208/2</td>
<td>11.04 ± 1.9049&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>53.4 ± 12.007&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.7668 ± 0.6854&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>2408</td>
<td>12.14 ± 2.9415&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>58.8 ± 15.230&lt;sub&gt;a&lt;/sub&gt;</td>
<td>6.8918 ± 0.3807&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>2208</td>
<td>11.74 ± 1.5190&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>53.8 ± 12.118&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.598 ± 0.4314&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
<tr>
<td>3308/5</td>
<td>15.62 ± 1.2196&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>60.4 ± 6.554&lt;sub&gt;a&lt;/sub&gt;</td>
<td>3.875 ± 0.6854&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>CSH-1</td>
<td>21.14 ± 1.5292&lt;sub&gt;c&lt;/sub&gt;</td>
<td>85.2 ± 7.1172&lt;sub&gt;a&lt;/sub&gt;</td>
<td>5.911 ± 0.3009&lt;sub&gt;bc&lt;/sub&gt;</td>
</tr>
<tr>
<td>MEAN</td>
<td>13.16</td>
<td>61.71</td>
<td>4.99</td>
</tr>
<tr>
<td>P VALUE</td>
<td>0.000</td>
<td>0.173</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data presented are means ± SE. Means within a column not followed by the same lower case letter show significant difference (Tukey’s HSD) at 5% probability level.

4.2.3 Resistance to *Striga hermonthica* Alupe ecotype

The interaction between the sorghum cultivars and the *S. hermonthica* collected in Alupe showed similar trend to that observed with the *S. hermonthica* ecotypes Sh Mbita and Sh Kibos. There was no significant difference in the number of attached *Striga* seedlings (*P* = 0.074) (Table 4.3), but the average size of the parasites was lower. SRS1208/2 remained very resistant to this ecotype recording the lowest *Striga* biomass (2.14 mg) and average *Striga* length (2.829mm). There was a significant difference in the mean biomass and average lengths of attached *Striga* (*P* = 0.000) (Table 4.3).
Table 4.3: Biomass, number and mean lengths of *S. hermonthica* (Alupe) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21DAI

<table>
<thead>
<tr>
<th>VARIETY</th>
<th><em>Striga</em> biomass (mg)</th>
<th>Number of attachments</th>
<th>Length of <em>S. hermonthica</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brhan</td>
<td>2.525 ± 0.5764 &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>14.75 ± 4.990 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.600 ± 0.2329 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1208/2</td>
<td>2.14 ± 0.3172 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.6 ± 9.309 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.829 ± 0.6320 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2408</td>
<td>2.8 ± 1.3976 &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.5 ± 7.858 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8605 ± 0.6567 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2208</td>
<td>11.45 ± 2.4415 &lt;sup&gt;abc&lt;/sup&gt;</td>
<td>46.5 ± 3.227 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.451 ± 0.7513 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3308/5</td>
<td>14.15 ± 1.6555 &lt;sup&gt;bc&lt;/sup&gt;</td>
<td>41.0 ± 12.942 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9895 ± 0.8097 &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CSH-1</td>
<td>18.76 ± 4.8143 &lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.2 ± 13.796 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.329 ± 0.6782 &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MEAN</td>
<td>8.78</td>
<td>29.08</td>
<td>4.28</td>
</tr>
<tr>
<td>P VALUE</td>
<td>0.000</td>
<td>0.074</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data presented are means ± SE. Means within a column not followed by the same lower case letter show significant difference (Tukey’s HSD) at 5% probability level.

4.2.4 Resistance to *Striga hermonthica* Tanga ecotype

In contrast with what was observed in the number of attached *Striga* on sorghum with all the other *Striga* ecotypes there was a significant difference in the number of attached parasites in the roots of the sorghum cultivars (P=0.005), however the average length of the attached seedlings was not significantly different (P=0.246) (Table 4.4). Similar to all the other ecotypes there was a significant difference in the biomass of the attached *Striga* seedlings. The biomass, number and average lengths of the attached *Striga* seedlings recorded for Sh Tanga across the sorghum cultivars were lowest.
Table 4.4: Biomass, number and mean lengths of *S. hermonthica* (Tanga) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21 DAI

<table>
<thead>
<tr>
<th>VARIETY</th>
<th><em>Striga</em> biomass (mg)</th>
<th>Number of attachments</th>
<th>Length of <em>S. hermonthica</em> (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brhan</td>
<td>$1.10 \pm 0.601_a$</td>
<td>$5.6 \pm 2.943_a$</td>
<td>$2.4634 \pm 1.0205_a$</td>
</tr>
<tr>
<td>1208/2</td>
<td>$0.975 \pm 0.2955_a$</td>
<td>$5.75 \pm 1.109_a$</td>
<td>$2.3425 \pm 0.6472_a$</td>
</tr>
<tr>
<td>2408</td>
<td>$3.18 \pm 1.0929_a$</td>
<td>$13.6 \pm 5.955_{ab}$</td>
<td>$6.2162 \pm 2.7112_a$</td>
</tr>
<tr>
<td>2208</td>
<td>$1.467 \pm 0.4372_a$</td>
<td>$10.0 \pm 2.887_{ab}$</td>
<td>$2.5313 \pm 0.8468_a$</td>
</tr>
<tr>
<td>3308/5</td>
<td>$8.3671 \pm 0.9563_b$</td>
<td>$36.0 \pm 9.866_{ab}$</td>
<td>$3.8477 \pm 0.2084_a$</td>
</tr>
<tr>
<td>CSH-I</td>
<td>$12.26 \pm 0.8363_b$</td>
<td>$43.4 \pm 12.311_{b}$</td>
<td>$6.0142 \pm 0.83305_a$</td>
</tr>
<tr>
<td>MEAN</td>
<td>$4.64$</td>
<td>$18.96$</td>
<td>$4.08$</td>
</tr>
<tr>
<td>P VALUE</td>
<td>$0.000$</td>
<td>$0.005$</td>
<td>$0.246$</td>
</tr>
</tbody>
</table>

Data presented are means ± SE. Means within a column not followed by the same lower case letter show significant difference (Tukey’s HSD) at 5 % probability level.

4.4 Multiple resistance

SRS 1208/2 had the lowest biomass of attached *Striga* at 21 DAI in three ecotypes of *Striga* i.e Mbita (3.9mg), Alupe (2.1mg) and Tanga (1.0mg). Brhan however had lower biomass of attached Striga from Kibos. There was significant difference ($P=0.000$) in the biomass of attached striga in the roots of all sorghum lines. There was no significant difference ($P \geq 0.05$) in the number of attached *Striga* seedlings in the roots of all the sorghum lines.SRS1208/2 had the lowest average length of attached *Striga* (2.83mm). There was a significant difference ($P<0.05$) in the average lengths of attached *Striga* from Kenya.(Table 4.5)
Table 4.5: Biomass, number and mean lengths of *S. hermonthica* (four ecotypes) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21 DAI

<table>
<thead>
<tr>
<th>Striga eco type</th>
<th>SH Mbita</th>
<th>SH Kibos</th>
<th>SH Alupe</th>
<th>SH Tanga</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomass (mg)</td>
<td>Number of attachments</td>
<td>Length of <em>S. hermonthica</em> (mm)</td>
<td>Biomass (mg)</td>
</tr>
<tr>
<td>SRS Sorghum line</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brhan</td>
<td>5.24</td>
<td>34.8</td>
<td>4.03</td>
<td>7.3</td>
</tr>
<tr>
<td>1208/2</td>
<td>3.9</td>
<td>27.2</td>
<td>2.13</td>
<td>11.0</td>
</tr>
<tr>
<td>2408</td>
<td>16.2</td>
<td>62.3</td>
<td>5.18</td>
<td>12.1</td>
</tr>
<tr>
<td>2208</td>
<td>8.9</td>
<td>57.8</td>
<td>3.7</td>
<td>11.7</td>
</tr>
<tr>
<td>3308/5</td>
<td>8.8</td>
<td>42.2</td>
<td>3.9</td>
<td>15.6</td>
</tr>
<tr>
<td>CSH-1</td>
<td>29.5</td>
<td>80.3</td>
<td>7.8</td>
<td>21.1</td>
</tr>
<tr>
<td>MEAN</td>
<td>10.7</td>
<td>47.8</td>
<td>4.2</td>
<td>13.2</td>
</tr>
<tr>
<td>P VALUE</td>
<td>0.000</td>
<td>0.193</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data presented are means and significant differences (Tukey’s HSD) at 5% probability level.
At 21 DAI Striga attached on the sorghum cultivars were either brown, few and small as observed in SRS1208/2 and the resistant control Brhan or were green, more and elongated as observed in the susceptible control CSH-1 (Fig 4.2).

Fig 4.2 *Striga hermonthica* (Sh-Mbita) growing on the roots of sorghum cultivars (in rhizotrons) 21DAI.

4.5 Phenotype of resistance to *Striga hermonthica* by the sorghum cultivars

Transverse sections through the sorghum roots at the site of haustorial attachment on the 3rd day showed that the parasite had penetrated through the cortex in all the studied sorghum cultivars (Fig 4.3) however at 10 DAI the phenotype of resistance varied between the SRS lines and Brhan. The SRS lines exhibited a compatible interaction whereby the parasite had penetrated the endodermis and begun to form connections
with the host xylem (Fig 4.3). Eventually the parasite haustorium formed few xylem-
xylem connections with the host. Haustorium growing on the SRS lines showed poor
tissue differentiation and the resulting parasites grew slowly and small except in the
interaction between *S. hermonthica* Mbita ecotype and SRS 3308/5 (Fig 4.3). In the
compatible interaction (3308/5 ), *S. hermonthica* parasites penetrated through the cor-
tex and endodermis and by day ten the haustorium was well developed showing three
defined regions, a densely stained hyaline body (Hb), the vascular core (Vc) consist-
ing of the xylem vessels and the endophyte that penetrated the host root cortex and
endodermis (En) (Fig 4.3).

In the incompatible interaction (Brhan) the parasite penetrated the host cortex but was
unable to traverse the endodermis and form a connection with the xylem vessels of the
host (Fig 4.3). The parasite grew around the host endodermis and the haustorium
failed to differentiate, the parasite vessel cells were not observed in the haustorium
and hyaline body was reduced in size at 10 DAI (Fig 4.3).
Fig 4.3: Light micrographs of transverse sections of *Striga hermonthica* haustoria penetrating sorghum roots. Technovit embedded tissues were cross sectioned at 3DAI (a) and 10 DAI (b, c, d, e and f). Structures (b, c, e, and f) represent compatible interactions with SRS 1208/2, 3308/5 SRS CSH-1 and SRS 2208, respectively while (d) represent incompatible interaction (Brhan). En, endodermis; Hb, hyaline body; Px, parasite xylem; Hx, host xylem Vc, vascular core. Bar, 200 μm
Another phenotype of resistance observed was in the interaction between the SRS lines and Sh-Tanga, the parasites that had elicited a resistant response were turning brown, dying and the host root showed intense necrosis at the point of attachment (Fig 4.4).

4.4: Sorghum roots with attached *Striga* seedlings (arrow) showing incompatible response of withering *Striga* seedlings growing on SRS 3308/5.
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Germination studies

The germination percentage of each *Striga* ecotype was determined before infection. Infection of sorghum roots with pre-germinated *Striga* seeds is essential when quantifying post-attachment resistance, as it ensures synchronous attachment of the parasites to the roots and eliminates any differences that may occur as a result of variation in the production of germination stimulants by the different sorghum cultivars (Jamil *et al.*, 2011). There was a narrow percentage range in baseline germination between the populations, indicating that any genotype by genotype interactions between the different sorghum cultivars could be directly compared between the four *Striga* populations without normalization to the amount of germinable seed per population.

5.1.2 Variability of post-attachment resistance of SRS sorghum lines to *S. hermonthica* ecotypes

The SRS sorghum cultivars were developed in a study aimed to develop *Striga* resistant sorghum varieties that have farmer preferred characteristics and are (Olupot, 2011). SRS 2408 was found to be resistant to *Striga* in the field; SRS 1208/2 exhibited pre-attachment *Striga* resistance mechanisms of low germination stimulant production and low haustoria initiation characters; the lines SRS 3308/5 and SRS 2208 did not express *Striga* resistance but were preferred by farmers for their high grain production. These lines have not had their post-attachment resistance determined before
this study. Therefore the SRS lines were infected with different Striga ecotypes to determine whether they exhibited post-attachment resistance.

The four SRS sorghum lines under study exhibited different levels of post attachment resistance against different Striga ecotypes. The resistance ranged from low resistance in 3308/5, intermediate resistance (SRS 2408 and SRS 2208) to highly resistant (SRS 1208/2). The sorghum cultivars showed the same pattern of resistance to the three Striga ecotypes from Kenya (Sh-Kibos, Sh-Alupe and Sh-Mbita). SRS 1208/2 showed similar levels of resistance with Brhan against the ecotypes of Striga used except with Sh-Alupe where it exhibited higher resistance. All the sorghum cultivars used showed strong resistance to Sh-Tanga except for the susceptible control CSH-1 which was susceptible to all the Striga ecotypes.

None of the SRS lines exhibited complete resistance to the ecotypes of Striga used in this study, however the strongest resistance was characterized by very few and small Striga seedlings. The most resistant cultivar-SRS 1208/2 was more resistant than Brhan which is known to exhibit very strong resistance to S. hermonthica (Pescott, 2013). The strong resistance in SRS 1208/2 is probably controlled by a few loci. The SRS cultivars share a large part of their genome between them because of their breeding history (Olupot, 2011), thus it might be expected that only a few genetic regions would explain the difference in the levels of resistance observed between SRS 1208/2 (highly resistant) and the other SRS lines that exhibited intermediate resistance. There were also clear differences in the rate of growth of the parasites, those on Brhan and the SRS lines developed slowly than those on the susceptible control that developed more quickly and were eventually larger (Fig 4.2). The difference in growth rate and
size of the parasite may be due to differences in the ability of the host to supply nutrients to the parasites (Gurney et al., 2003), but it may be more likely to reflect genotypic differences in the susceptibility/resistance of the different cultivars.

The three different post-attachment parameters (biomass, length and number) used in this study may reflect different underlying aspects of the host-parasite interaction. Rhizotron studies of Striga-host interactions have used a variety of scoring methods that have either categorized attachments into different parasite developmental stages, and have interpreted these directly and/or analysed derivatives of these (Gurney et al., 2003, 2006; Huang et al. 2012), or have used parasite length and/or dry weight (Gurney et al. 2003; Cissoko et al., 2011). Different cellular and molecular interactions may lie behind different phenotypic measures of a parasite's success on its host. For example, in rhizotrons, the number of attachments on a host may be high, but individual parasites may not develop to any great size (Cissoko et al., 2011). Furthermore these studies found that using Striga total dry weight as the response metric in a quantitative genetic study of rice resistance led to clearer identification of QTL than when using the number of Striga attachments. Similarly the results reported here indicate that parasite length and total dry weight provide a greater separation in parasite resistance between the different hosts than the number of attached parasites, although all three responses differentiated hosts to some extent; this suggests the choice of a response metric is important when reaching conclusions about host-parasite interactions.
5.1.3 Multiple resistance against *Striga* ecotypes

Interactions between species may often be dependent on the genetic composition of the populations or individuals involved (Whitham *et al.* 2006). Understanding when this is important can provide insights into the dynamics of particular communities, which may be of fundamental interest for ecology and its applications (Hersch-Green *et al.*, 2011). Sorghum and *S. hermonthica* have a long co-evolutionary history (Welsh and Mohamed, 2011), and some research has already been performed on the variable interactions between these species (Omanyana *et al.*, 2000). Given the numerous varieties of sorghum, and the fact that *S. hermonthica* is geographically widespread and genetically diverse (Mohamed *et al.*, 2007), these two species provide many opportunities to investigate the importance of G × G interactions for plant-plant parasitism. Their agricultural importance increases the likelihood that large, multi-site experiments can be run, providing valuable insights for crop breeding, as well as more general insights into host parasite ecology. This study has shown that G × G interactions can be highly significant in controlled host-parasite studies, and may depend on the virulence/resistance metric used, indicating the complexities of these species' interactions.

5.1.4 Phenotypes of attachment resistance in *S. hermonthica*

The rhizotron studies of sorghum post-attachment resistance indicated the presence of significant host-parasite interactions for all three metrics; this was supported by different resistance rankings and *post hoc* results, between the four *Striga* populations. The biomass and length of *Striga* attachments showed that all the sorghum cultivars with the exception of CSH-1 exhibited strong resistance to the *Striga* from Tanzania than they did to the three nearby populations of *S. hermonthica* from Kenya, suggesting a geographic component to host resistance. The *Striga* population from Tanga
used in this study was associated with sorghum for many years and may have led to
development of resistance in this host species. In contrast the Striga ecotypes from
Kenya were collected from regions that cultivate maize, sorghum and millet either by
intercropping or rotational growing. These populations are unlikely to have exclusively
adapted to any one cereal. Thus these may show greater virulence when infecting
the sorghum cultivars in this study compared with that of Striga from Tanzania. It
may also suggest that the high genetic diversity of the Striga seed bank led to the rap-
id evolution/buildup of virulent genotypes from a subset of the natural seed bank pop-
ulation over a period of time (Huang et al., 2012).

The parental line Brhan which was used in this study as a resistant control has been
investigated in the field and in other lab experiments where it has been shown to ex-
hibit very high levels of Striga resistance both at the pre-attachment and the post-
attachment stages of development (Olupot, 2011; Pescott, 2013) in this study it exhib-
ited multiple resistance to all the Striga ecotypes used, as such it can be used as a
source of Striga resistance genes when breeding for new Striga resistant sorghum va-
rieties.

5.1.4 Phenotype of resistance to Striga hermonthica by the sorghum cultivars

Transverse sections of the haustorium-host interface of the SRS lines revealed that the
parasite endophyte had penetrated the host root cortex and the endodermis and had
formed successful xylem-xylem connections however the haustorium failed to differ-
entiate and developed poorly (Fig 4.3).
Consistent with this observation is the resistance to *S. hermonthica* identified in *Tripsacum dactyloides*, a wild relative of maize (Gurney *et al.*, 2003). In this case the haustorium failed to differentiate either because the host lacked signals required for haustorial development or because *T. dactyloides* produced a signal that inhibited haustorial development. The inhibitory compound(s) may be mobile within the *S. hermonthica*-cereal root system or may be released by host root exudate. A mature haustorium is crucial for successful parasitism because it allows the movement of nutrients from host to parasite and the organelle rich cells of the hyaline body may be involved in active nutrient synthesis and starch storage (Maiti *et al.*, 1984). The tissues of the hyaline body demonstrate intense metabolic activity and many of the enzymes involved in nitrogen metabolism are located in this region (Taylor, 2001). The poor differentiation of the hyaline body as observed in the SRS lines is likely to have serious implications for parasite nutrition and the *Striga* supported by the SRS lines may be nutrient starved. This may partially explain the reduced size of the attached parasites. From the observation it is clear that in the SRS lines resistance was established after penetration and establishment of host-parasite xylem-xylem connections.

These results indicate that the cortex is not the last barrier for *S. hermonthica* in sorghum roots, rather the parasitisation cannot be considered successful even after the connection of the vasculatures. This resistance in the SRS lines involves factors that act after establishment of vascular connections and delay development of the parasite. This characteristic incompatible phenotype is similar to that observed in cow pea - *S. hermonthica* interaction by Yoshida and Shirasu (2009). Another resistance mechanism observed was browning and the eventual death of the attached parasite in incompatible reactions on some SRS lines (Fig 4.4). A similar resistance mechanism
was observed in some sorghum cultivars following infection by *S. asiatica* (Mohamed *et al.*, 2003) and in resistant cowpea cultivars infected by *S. gesnerioides* (Li and Timko, 2009). In the case of cowpea resistance, this phenotype was associated with a gene for gene resistance mechanism. In the SRS lines infected with *S. hermonthica* it is not clear whether the underlying resistance mechanism involve gene for gene interactions, as resistance is considered to be controlled by several quantitative trait loci, although there are indications that a proportion of this quantitative resistance is controlled by a few genes of major effect (Gurney *et al.*, 2006).

The early stages of parasite development on Brhan were similar to those on the SRS sorghum cultivars and the susceptible control, CSH-1; parasites attached to the host root system within a day of inoculation and by the third day, the parasite endophyte had successfully penetrated the host root cortex, this demonstrates that host specific factors necessary for early haustorial formation and successful penetration of the cortex were present in Brhan. However in contrast to the SRS lines and CSH-1, the parasite did not breach the endodermis in Brhan (Fig 4.3 d). The reason for this is unclear as there was no visible difference between the endodermis of Brhan and that of the SRS lines. By day ten the parasite was unable to form parasite-host xylem-xylem connections instead it encircled the vascular core of the host within the root cortex, this response is similar to the observation made in the resistant rice cultivar Nipponbare (Gurney *et al.*, 2006). Vascular continuity between host and parasite may be involved in providing factors required for further differentiation of the haustorium. The haustorium attached to Brhan did not mature and differentiate (Fig 4.3). Thus by day 21 most of the parasites were dead and they did not elongate substantially.
Brhan is resistant to *S. hermonthica*, partly because the parasite failed to form vascular continuity with the host. This type of resistance differed from that observed in the non-host interaction between *S. asiatica* and marigold where the penetration of the cortex was terminated early and the endophyte rarely reached the endodermis (Hood *et al.*, 1998). Using differential display techniques Gowda *et al.* (1999) identified genes expressed in marigold roots in response to penetration by *S. asiatica*. They isolated and cloned a novel gene (NRSA-1) that was homologous to known plant disease resistance genes, but which differed structurally to those previously described. The resistance observed in Brhan is most similar to that observed in the resistance interaction between vetch *Vicia atropupurea* cultivar Popany and the parasite, *Orobanche aegiaptica* (Goldwasser *et al.*, 2000). In this interaction the parasite haustorium penetrated the root, but was blocked at the root endodermis layer, the blockage was accompanied by a large secretion of unknown composition which prevented the parasite from establishing vascular continuity. It is unclear whether Brhan exhibited a similar mechanism because this was not established in this study.

Gowda *et al.* (1999) examined *Striga* resistance by the non-host marigold and reported necrosis of host cortical cells around the penetrating endophyte and cell wall thickening at the *Striga*-marigold interface. Other host resistance mechanisms that have been illustrated include physical barriers to infection, for example in the association between *Orobanche cumana* and sunflower the production of an encapsulation layer prevents further invasion of the endophyte (Labrousse *et al.*, 2001). Chemical barriers to infection have also been associated with post attachment resistance includ-
ing phenolic compounds (Goldwasser et al., 1999) and induced phytoalexins (Wegmann et al., 1991).

5.2 Conclusions

The data presented here shows that SRS 1208/2 had very high post-attachment resistance to the *S. hermonthica* ecotypes used in this study, SRS 2408 and SRS 2208 exhibited intermediate resistance while SRS 3308/5 had low resistance. The difference in biomass, number and length of attached *Striga* seedlings upon infection with the different *Striga* ecotypes clearly indicate genetic variability for *Striga* resistance in the selected lines.

This study was also undertaken to determine the phenotype of resistance mechanism present in the selected SRS sorghum lines and the resistant control genotype Brhan. The phenotype of resistance in Brhan was characterized by an inability of the parasite endophyte to breach the endodermis thereby failing to form vascular continuity with the host.

In the SRS sorghum however the phenotype of resistance was characterized by any of the two mechanisms; turning brown and eventual death of the attached parasites (as observed in SRS 3308/5 infected by the Tanga *Striga* ecotype) and poor differentiation and development of the haustorium. Thus in these sorghum lines the root cortex is not the last barrier for *S. hermonthica* and parasitisation can be considered successful even after formation of vascular continuity between host and parasite.
5.3 Recommendations

1. Future research efforts should also be directed towards understanding genetic basis of host resistance mechanisms and the adaptation of *Striga* populations (parasite virulence) to new host resistance genotypes. Such insights would facilitate the stacking of appropriate resistance loci in farmer preferred and *Striga* tolerant cultivars to enhance the durability and stability of defense in the long term.

2. Among the four sorghum lines studied, cultivar SRS 1208/2 was the most promising source of resistance to obligate root parasite *S. hermonthica* and can be recommended for future use in sorghum breeding programs in East Africa.
REFERENCES


