GENETIC DIVERSITY AND VIRULENCE STUDY OF SEVEN *Striga hermonthica* ECOTYPES FROM KENYA AND UGANDA ON SELECTED SORGHUM VARIETIES

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DECLARATION

Declaration by the candidate

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

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Declaration by the supervisors

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

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DEDICATION

This thesis is dedicated to my son Marques Atanda Kataka.

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First, I thank God the almighty for the good health he granted me during this period. I appreciate the support and encouragement from my supervisors, family members, friends and colleagues. May God bless you all.

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ABBREVIATIONS AND ACRONYMS

AATF	African Agricultural Technology Foundation
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
APS	Agricultural Production System
Bp	Base Pairs
CDFA	California Department of Food and Agriculture
cDNA	Complementary Deoxyribonucleic Acid
CIMMYT	International Maize and Wheat Improvement Centre
EA	East Africa
EPPO	European and Mediterranean Plant Protection Organization
EST	Expressed Sequence Tags
FAO	Food and Agriculture Organization
GAPs	Good Agricultural Practices
He	Expected Heterozygosity
Ho	Observed Heterozygosity
ICIPE	International Centre of Insect Physiology and Ecology
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IITA	International Institute of Tropical Agriculture
ISC	Integrated Striga Control
ISM	Integrated Striga Management
LTC	Life Technologies Cooperation
MAS	Marker Assisted Selection
NCBI	National Center for Biotechnology Information
Nm	Number of migrants
OISAT	Online Information Service for Non-Chemical Pest
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
PPM	Parts per million
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
$sddH_2O$	Sterile double distilled water
Spp	Species
SSA	Sub-Saharan Africa
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
VNTRs	Variable Number Tandem Repeats

ABSTRACT

Parasitic weeds are a serious problem in agricultural production, causing large crop losses in many parts of the world and particularly Africa. One of the most economically important parasitic weed is Striga. Striga weed is considered to be the greatest biological constraint to food production in sub-Saharan Africa (SSA). This genus includes races; *Striga hermonthica, S. asiatica, S. gesnerioides, S. aspera and S. forbesii* that are considered to be most harmful to crops. *Striga hermonthica* is the most widespread among the species in the semi-arid tropical African zones. The wide geographical distribution set conditions for genetically structured populations. The genetic variations among the weed populations allow for quick breakdown of resistance in crops hence making control of the weed difficult. Efficient and effective control of *S. hermonthica* requires knowledge on inherent genetic variability within local and regional races of the weed. However, the genetic diversity and virulence of *S. hermonthica* ecotypes in Kenya and Uganda on selected sorghum varieties remain unknown. This study evaluated genetic diversity among seven *S. hermonthica* populations from locations in Kenya and Uganda using 5 primer sets of Expressed Sequence Tags – Simple Sequence Repeats (EST-SSR). The size of the amplified fragments ranged from 87 to 202 base-pairs. The total number of bands detected across all the primer combinations after correcting for repeatedness was 38. The genetic diversity among the seven populations was moderate as revealed by the Nei's genetic distance values which ranged from 0.122 to 0.710 with an average of 0.33. AMOVA revealed low genetic differentiation among the populations (Fst = 0.100). This study also evaluated twelve sorghum varieties for their response to *S. hermonthica* infection. It was established that the varieties resistance responses to *S. hermonthica* varied widely. The phenotype of resistant interaction was characterized by inability of the weeds haustoria to penetrate the sorghums root endodermis due to intense necrosis and in rare cases the parasites radicle growing away from the host root. The resistant sorghum varieties were the Asareca W2, Asareca AG3, N13 and the Wild type which had low mean number of *S. hermonthica* plantlets growing on their roots (< 1.0) while the most susceptible varieties were Sap 027, Epurpur which had the highest mean number of *S. hermonthica* plantlets growing on their roots (>8.0). According to Tukey's Honest Significant Difference test, there was high significant difference in the means of number of Striga growing on the roots of sorghum varieties, Striga dry biomass and *S. hermonthica* length between the susceptible and resistant ones ($P < 0.05$). This knowledge holds great potentiality since resistant sorghum germplasm tested will be sourced and targeted to the seven specific geographical areas where virulence of the specific *S. hermonthica* populations was characterized.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Striga weed is commonly known as witchweed or witches weed. It is a noxious root hemi-parasite that has devastated cereal production in Sub-Saharan Africa (SSA) (Runo *et al.,* 2012). The genus is composed of 30 to 35 species and now classified in the family of Orobanchaceae although earlier authors placed it in Scrophulariaceae family (Gethi *et al.,* 2005). Over 80 % of Striga species are found in Africa, while the rest occur in Asia (Westwood, 2009).

The major agricultural Striga species are; *Striga hermonthica, S. asiatica*, which infects cereals like maize (*Zea mays*), millet (*Sorghum bicolor*) and rice (*Oryza sativa)* and *S. gesnerioides* which infects Vatke legumes like cowpea (*Vigna unguiculata*). Other species such as *S. forbesii* and *S. aspera* have been reported to have sporadic effects on cereal crops in their locations (Parker, 2009). Crops such as wheat (Ejeta, 2007a) and Napier grass (Atera *et al*., 2012a) previously unaffected by Striga are now showing serious infection.

Striga parasites reproductive schemes vary from autogamy to obligate allogamy depending on species (Musselman, 1987). *Striga hermonthica* and *S. aspera* are the only two species known to be obligately allogamous and require insect pollinators. The two species can hybridize and produce viable and virulent offspring; a

phenomenon that generates broader genetic variability within the species, making it even much more difficult to develop resistant crop varieties (Parker, 2009).

Striga hermonthica is the most widely spread root parasitic weed among all species (Rasha *et al.,* 2009). The geographical distribution and the infestation level of the weed are steadily increasing particularly in Sub-Saharan Africa (Emechebe, 2004; Ejeta, 2007a). This is favored by convenient climate, reduced soil fertility (Sauerborn *et al.,* 2003), increased land use (on depleted soils) and expansion of the area cropped with susceptible host crops (Gressel *et al.,* 2004).

Striga parasite infests 40 % of the cereal producing areas of SSA, resulting to crop losses estimated at US$ 7 billion annually affecting livelihood of about 300 million people (Ejeta, 2007a). The most affected are subsistence farmers losing about 20 % to 80 % of their yield (Gethi *et al.,* 2005). However, the percentage yield loss depends on a number of factors; Striga density, host species, land use system, soil nutrient status and rainfall patterns (Atera *et al.,* 2012b). A survey done in Western Kenya in 2009 revealed that 73 % of the fields used for growing crops are infected with *S. hermonthica* (Woomer and Savala, 2009); this is a clear indication that Striga is the most damaging pest encountered by farmers in those areas. Striga weed in essence, undermines the struggle to attain food security in Africa and therefore its control must be addressed by all efforts (Atera *et al.,* 2012a).

Cultural management practices for Striga have not achieved much in its control (Atera *et al.,* 2012b). Practices like weeding, pulling and slashing are futile because

the devastating effects of the parasite are accomplished prior to its emergence. A lot of research has been done to identify cultivars of several species that are resistant to Striga parasitization (Mohamed *et al.,* 2003; Ejeta, 2007b). The use of resistant varieties has however been challenged by the high genetic diversity within the Striga species; the resistance is often weak and tends to break down with the infestation of new races of the Striga species (Rispail *et al.,* 2007). The use of herbicides such as 2 - 4 D, Oxyfluorfeen and Imazapyr is available application for use, however, it is a control measure that is unfortunately beyond the investment abilities of most small scale farmers (AATF, 2011). This therefore calls for urgent need for the establishment of better policies to promote, implement and ensure long term sustainable Striga control programs.

Recent molecular advancements have provided the necessary tools that can be used in Striga diversity studies. Molecular markers are DNA sequences associated with certain parts of the genome (Koyama, 2000). Molecular markers are presumed to be the most important applications in the study of population genetic structures and genetic variability of crop pathogens (Koyama, 2000). Examples of molecular markers include; Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR). These markers offer better characterization due to their high level of polymorphism as compared to the morphological markers.

Considering the wide range of distribution of Striga species, limited studies on the genetic diversity have been conducted relative to their total current distribution (Runo *et al,* 2012). Generally there is lack of knowledge by farmers on the most resistant sorghum varieties which has contributed to low sorghum production. Screening of sorghum varieties to determine the most resistant ones is crucial for farmers in Uganda and Kenya since the use of the resistant varieties is considered the most appropriate means of combating the weed.

1.2 Problem statement

Striga parasite infests cereal crops that are depended on in Africa as staple food (Gethi *et al.,* 2005). The parasite infests about 40 % of cereal producing areas of SSA resulting to losses estimated at US$ 7 billion annually, affecting the livelihood of about 300 million people (Ejeta, 2007b). Striga genus includes the following species which are considered to be most harmful to crops; *Striga hermonthica, S. asiatica, S. gesnerioides, S. aspera and S. forbesii*. *Striga hermonthica* is the most widespread among species affecting cereals in East Africa (EA). This wide geographical distribution and host specificity, set conditions for genetically structured populations which are diverse, breaking host range, attacking non-traditional host crops and colonizing new areas and therefore making its control very difficult hence continuing to devastate farming in EA (Ejeta, 2007b).

Sorghum is the second most important cereal grain after maize in Africa. It is an important staple food crop that is grown by subsistence farmers in SSA (Berner *et al.,* 1995). However, sorghum is widely distributed in *S. hermonthica* prone regions

in Africa hence its productivity is constrained by the obligate parasitic weed which causes an estimated 40 % yield loss in the crop (Ayongwa *et al.,* 2010). Lack of knowledge by farmers on the most resistant sorghum varieties has contributed to low sorghum production in Western Kenya and Eastern Uganda. The use of resistant sorghum varieties however, is the most appropriate means of combating the weed. As such the current study was designed to investigate genetic diversity of *S. hermonthica* ecotypes collected from Kenya and Uganda and their corresponding virulence on selected sorghum varieties.

1.3 Justification

Determination of the genetic variability of *S. hermonthica* populations in Africa is crucial for the control of the weed. The virulence variability within these species makes breeding programs for resistant crop varieties more complicated. This therefore calls for a much detailed determination of the level of genetic variability within *S. hermonthica* populations for effective breeding programs. The use of such resistant cultivars is considered an effective and affordable component of integrated Striga control strategy (Yoder and Scholes, 2010). Considering the wide range of distribution of Striga species, not enough studies on the genetic diversity have been conducted relative to their total current distribution (Gethi *et al.,* 2012).

Sorghum is a major crop in SSA and especially in the marginal areas where other crops do not do well (AATF, 2011). *Striga hermonthica* infestation has remained a major constraint to Sorghum production in SSA. It is estimated that 21.9 million hectares of sorghum and millet fields in Africa are affected by Striga, compared to an

overall 26.43 million hectares of all cereal crops, making sorghum the most affected cereal crop by Striga in Africa. Screening of sorghum cultivars to determine the most resistant varieties is crucial for farmers in EA since the use of resistant sorghum varieties is considered the most appropriate means of combating the weed.

1.4 Hypotheses

i. *Striga hermonthica* populations in Western Kenya and Eastern Uganda are genetically similar.
ii. The selected sorghum varieties are susceptible to infection by the seven *S. hermonthica* populations.

1.5 Objectives

1.5.1 General objective

To determine the genetic diversity of seven *S. hermonthica* populations sourced from Western Kenya and Eastern Uganda and their virulence on selected sorghum varieties.

1.5.2 Specific objectives

i. To determine if there exists genetic diversity and variations among the seven *S. hermonthica* populations infesting sorghum in Western Kenya and Eastern Uganda using EST-SSR.
ii. To screen selected sorghum varieties for their response to *S. hermonthica* infestation.

1.6 Significance of the study

Knowledge from this study is particularly important towards advising farmers on preferred sorghum varieties for specific agro-ecological zones based on Striga tolerance and coupled with other techniques including molecular breeding and genetic engineering, this study will find great application in enhancing food security.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin, distribution and infestation of Striga to crops

Striga weeds are widely distributed all over the world in more than 40 countries; however, they are generally native to the tropical and semi-arid areas of Africa (Vasey *et al.,* 2005; Ejeta, 2007b). Only three species are native to Australia; *S. curviflora, S. Multiflora a*nd *S. parviflora*. *Striga asiatica* is native to tropical parts of Asia and Africa including the Philippines, Cambodia, Indonesia, china , Malaysia, Thailand, Vietnam, Mauritius, India and the Arabian peninsula and later on introduced into the United States of America particularly Carolina state (Mohamed *et al.,* 2007) .

Striga gesnerioides is native to Africa, Arabia and Asia and later introduced to the United States (Mohamed *et al.,* 2007). *Striga hermonthica* is native to semi-arid areas of Northern Tropical Africa, from Senegal to Ethiopia and the Democratic Republic of Congo and into the South West Arabia and Southern tropical Africa, including Angola, Namibia, Madagascar and Tanzania (Parker and Riches, 1993).

Crops that play host to Striga parasite are cereals which are depended on as food by the small scale farmers in Africa (Table 2.1), this makes it a major constraint to agriculture of the semi-arid and regions in the sub-Saharan Africa. Despite the increasing demand for food in Africa due to exponential population growth, there is

a decline in productivity as a result of Striga infestation among other factors (Alakonya, 2004).

Table 2.1: Crops infested with Striga weed in SSA and the degree of infestation

Species	**Crops**						
	Maize	**Sorghum**	**Rice**	**Perl millet**	**Finger millet**	**Cowpea**	**Sugar cane**
S. hermonthica	XXX	XXX	XX	XX	XXX	-	XX
S. angustifolia	-	XX	-	-	-	-	XX
S. asiatica	XXX	XXX	XX	XX	XX	-	X
S. forbesii	X	X	X	-	-	-	X
S. aspera	XX	X	XX	-	X	-	X
S. gesnerioides	-	-	-	-	-	XXX	-
S. latericea	-	-	-	-	-	-	X
S. pubiflora	-	-	-	-	-	-	X

SOURCE: Parker and Riches, 1993.
xxx – Serious infection
xx - Moderate infection
x – Less infection
- No infection

2.2 Striga biology

Striga weeds are characterized by bright green stems and leaves with small brightly colored and attractive flowers (Sand *et al.,* 1990). Morphology of Striga however varies with the species (Figure 2.1). *S. hermonthica* for example, has large bright purple or pink flowers, grows up to 60 cm tall and the stems are hard and quadrangle-shaped (OISAT, 2005). *Striga gesnerioides* has dull pink, purple or creamy white flowers, the stems are 30 cm tall and purplish or brown in color, branched, with leaves reduced to scales and the root swollen to a tuber up to 3 cm in diameter while *S. asiatica* has bright red flowers (Musselman and Hepper, 1986; Parker and Riches, 1993).

Most Striga species are 15 to 20 cm tall when mature while other species grow up to 60 cm tall. Striga stems are square in cross-section and 1 to 25 mm in diameter. The stems are sparsely covered with coarse short white bulbous based hairs. Leaves are nearly opposite; narrowly lanceolate and about 1 to 3 cm long with successive leaf pair's perpendicular to one another (Rich *et al.*, 2004).

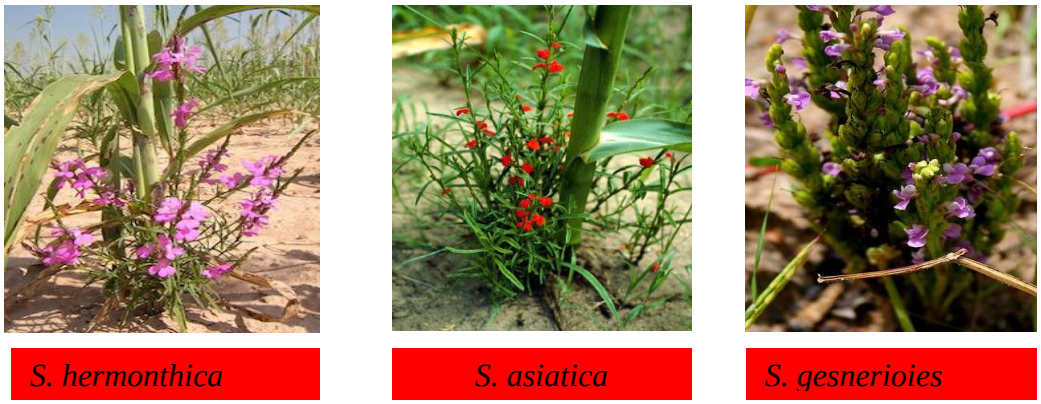

Figure 2.1: Pictures of *S. hermonthica*, *S. asiatica* and *S. gesnerioides* showing their variations in morphology. Photo: Marco Schmidt 2009.

The life cycle of Striga is synchronized to that of the host (Figure 2.2) and a number of mechanisms ensure the co-ordination of the parasites life cycle and that of its host (Bouwmeester *et al*., 2003). Germination of the seeds, attachment of the roots of the weed to those of the host, haustoria formation and penetration into the plants vascular system, establishment of the vascular connections, absorption and accumulation of the host's nutrients, flowering and seed production; marks the most important stages of development of the Striga weed (Parker and Riches, 1993).

The interaction between the host and the parasite begins with the secretion of secondary metabolites (Strigolactone) from the roots of the host (and some false non-

host), that induce the germination of the parasite seeds (Radoslava *et al.*, 2005). However, the seeds will only germinate after exposure to environmental cues for some time, for example, exposure of the seeds to warm and moist environments so that the imbibed seeds may respond to the hosts' stimulation. Strigolactone induces Striga seeds to germinate by growing of the radicle in the direction of increasing chemical volatile concentration (Ejeta, 2007b). The germinated seedlings form haustoria which are round shaped organs specialized in host attachment and penetration (Yoder, 2001). The formation of haustoria also requires host derived signals compounds. The haustoria penetrate the host's roots and finally the parasite establishes host xylem connections within a period of five days (Albrecht *et al.*, 1999). At this stage they form direct contact with the xylem vessels leading to nutrient siphoning until maturity when the plant produces seeds completing its life cycle.

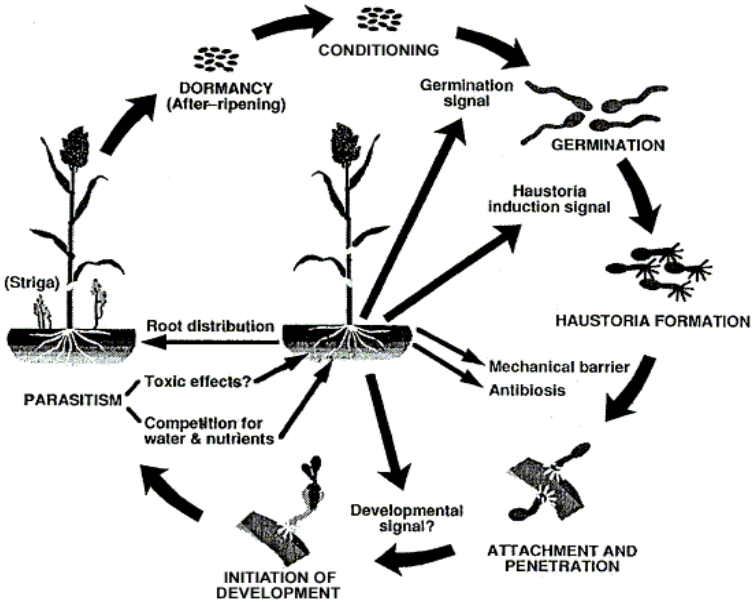

Figure 2.1: Life cycle of Striga showing the major stages of development. Source: Bouwmeester *et al*. (2003).

2. 3 Economic importance of Striga weed

Food security in Sub-Saharan Africa is deteriorating significantly and this is due to infestation of Striga in areas considered the "food baskets" for the continent. Agricultural production sector in sub Saharan Africa is the major source of food, foreign exchange and most importantly the main source of family income through direct and indirect employment (AATF, 2006). In Kenya for example, about 80 % of the workforce is engaged in agriculture and food processing. However, Striga threatens the same source of livelihood (AATF, 2006).

Striga infestation has contributed to low food production in Africa (FAO, 2006). Food production per person is said to be the same as it was in 1960 despite the fact that population has increased exponentially (FAO, 2011). Striga is perceived to be

the major biological constraint to crop production in Sub Saharan Africa and the most damaging pest encountered by farmers growing sorghum, millet and maize in sub Saharan Africa (Rasha *et al.,* 2009). The economic loss due to Striga is enormous (Runo *et al.,* 2012). Striga infests two-thirds of the arable land of Africa and constitutes the biggest single biological cause of crop damage in Africa (Ejeta, 2007b) The prize for controlling Striga is progress in food security, economic development and well being of millions of people living in Africa (Atera *et al.*, 2013). It is important to note that Striga weed is pervasive and therefore time is of essence in its control.

It is estimated that Striga seeds have infested about 100 m hectares of land in Africa (Scholes and Press, 2008) and the infestation causes 30 % to 50 % loss of Africa's agricultural economy in 40 % of its arable land (Amudavi *et al.,* 2007; Hearne, 2009). The weed is responsible for about 26 % loss of sorghum and millet in African countries (Gressel *et al.,* 2004). In Kenya, the parasite is said to be infesting about 217,000 hectares causing annual crop losses of US $ 53 million (Woomer and Savala, 2009). The average loss due to Striga is 1.15, 1.10 and 0.99 tons per hectare for maize, sorghum and millet respectively in Kenya (MacOpiyo and Snders, 2010). This loss represents 12.3 % of the 2.4 million metric tons of maize that Kenya produces annually. This translates to about 39.6 kg of maize loss per capita amounting to 20 % of typical person's annual food requirement (MacOpiyo and Sanders, 2010).

Preliminary forecast by FAO (2011) showed that Kenya needed to import 2.3 million tons of cereals to bridge a production deficit (Table 2.2) over 2011/2012 cropping season due to the presence of Striga weed. Survey done in 30 communities in Nigeria, indicated that farmers rated Striga weed as the leading priority constraint to crop production (Dugse *et al.,* 2006). In Western Kenya, a survey of 83 farms revealed that 73 % of the farms are infested with *S. hermonthica* (Woomer and Savala, 2009). A survey conducted in the Sudan savannah zone of Ghana showed that an average number of 9,384 seeds m^{-2} was found in the land that had been put to cultivation after fallow (Abunyewa and Padi, 2003).

FAO (2006) estimated that cereal yields in SSA increased by a paltry 29 % between 1961 and 2005 against a population increase by 216 % within the same period. This implies that much effort should be put in cereal production in SSA to feed the exponentially growing human population. The consequences of Striga infestation are severe rendering small scale farmers helpless and often bewildered.

Table 2.1: Cereal production and consumption in Kenya in 2009

Crop	**Area under crop cover (ha)**	**Production (tons)**	**consumption**
Maize	1,888,075	2,442,823	3,240,000
Wheat	131,594	219,301	96,480
Rice	21,829	42,202	410,000
Sorghum	173,172	94,555	81,000
Finger millet	104,576	56,417	40,000

Source: Ministry of Agriculture, Kenya, 2010.

2.4 Importance of sorghum and the extent of infestation by Striga in SSA

Sorghum is the fifth major cereal crop in the world in terms of production after maize (*Zea mays*), wheat (*Triticum aestiuum*), rice (*Oryza sativa*) and barley (*Hordeum vulgare L.*) (FAO, 1998). Sorghum remains a viable food grain for many of the world's most food insecure people who live in marginal areas with erratic rains and poor soils. Sorghum is a major crop in SSA and especially in the marginal areas where other crops do not do well (AATF, 2011).

Striga hermonthica infestation has remained a major constraint to Sorghum production in SSA. It is estimated that 21.9 million hectares of sorghum and millet fields in Africa are affected by Striga, compared to an overall 26.43 million hectares of all cereal crops, making sorghum and millet the most affected cereals by Striga in Africa. There are two Striga species that attack sorghum, *S. hermonthica* and *S. aspera*. Of the two, *S. hermonthica* is the most wide spread in SSA and most damaging to sorghum (AATF, 2011). Typical yield losses due to Striga range between 5 % to 70 % depending on the infestation level and susceptibility of the cultivar (AATF, 2011).

In terms of tonnage, sorghum is Africa's second most important cereal. Sorghum production in SSA is estimated at 26 million metric tons (MT); a steady increase over the past 40 years where production was estimated at 10 million tons (FAO, 1998). The growth in sorghum production could be attributed to increased industrial demand from the malting and brewing companies. Nigeria is the leading sorghum producer in Africa and the second in the world after USA, while Mali ranks the

second in Africa and sixth in the world (AATF, 2011). Sorghum in Nigeria is gaining commercial significance with the Nigerian malting and brewing industries consuming about 152,000 MT per annum. Similar efforts are being replicated in Ghana, Uganda and Kenya where the brewing industries are contracting sorghum farmers to grow sorghum on agreed prices.

However, sorghum yields in SSA remain low as compared to potential yields mainly due to Striga infestation among other factors (AATF, 2011). Research work on the control of Striga in sorghum and maize has been undertaken for several years (AATF, 2011). The methods used for control of Striga in sorghum range from, cultural methods such as soil and water management, intercropping, use of cover crops, use of trap crops (Ekere *et al.,* 2002) and recently use of herbicides, Striga resistant and tolerant varieties where some degree of success has been reported in countries like Kenya and Ethiopia (AATF, 2011).

2.5 Striga control and management

Appropriate agricultural technologies such as replenishing soil fertility, use of certified seeds, utilizing Good Agricultural Practices (GAPs), reducing weed soil seed banks and reducing disease and pest pressure on the crops have been recommended for the management of the weed (Bruce, 2010).

Several institutions both private and government have been involved or have supported in conducting research on Striga. These institutions include but not limited to; International Maize and Wheat Improvement Centre (CIMMYT), International

Centre of Insect Physiology and Ecology (ICIPE), International Crop Research Institute for the Semi Arid Tropics (ICRISAT), African Agricultural Technology Foundation (AATF) and International Institute of Tropical Agriculture (IITA). These institutes have recommended control options to farmers in Africa geared towards reducing infestation and damage (AATF, 2011). These options include; intercropping of cereals and legumes, crop rotation, the use of trap crops that stimulate suicidal germinations, application of manure and nitrogen fertilizers and the use of resistant crop varieties (AATF, 2011).

Trap cropping can also be used as control method. This involves planting a species in an infested field that will reduce the Striga seeds to germinate but will not support attachment of the parasite. This method has been used in sorghum fields by planting *Celosia argentea* and in maize fields by planting *Desmodium uncinatum* (Radford, 2003).

Imposed ‘’suicidal germination’’ in the fields not yet planted with crops can be used as a control measure. This method involves inducing seeds present in the soil to germinate by injecting ethylene gas in the soil. Ethylene gas mimics the natural physiological response tied to host recognition. Since no host roots will be available, the seedlings will fail to attach and therefore dies. However, this method is relatively expensive and not affordable to the subsistence farmers in developing countries (Olupot *et al*., 2003).

Control of Striga can also be achieved through planting of resistance improved cultivars. The use of such resistant cultivars is considered an effective and affordable component of integrated Striga control strategy (Yoder and Scholes, 2010). Resistant cultivars have the ability to prevent or limit Striga attachment and growth, hence able to grow and produce in areas that are infested with Striga.

Integrated Striga Control (ISC) is a control option where a wide range of technologies are combined into a program for the control of Striga as opposed to the application of a single method in the control. ISC is recommended for effective management of Striga. Franke *et al.* (2006) observed that ISC approach reduced the soil Striga seed bank by 46 % and improved crop productivity by 80 %. The major objective of ISC is to reduce Striga densities in the soil to avoid new plants emerging in the subsequent seasons.

2.6 Genetic diversity within and between *S. hermonthica* populations

The genetic diversity inherent in *S. hermonthica* is thought to be as a result of persistent seed bank of several generations of populations, broad geographical distribution, increased use of cultivars with improved resistance, hybridization, long distance dispersal and local host adaptation (Mohamed *et al.,* 2007).

Striga hermonthica is well known for its impressive abilities to adapt to different habitats and agro-ecosystems by developing host specific strains and ecotypes across their ranges (Cochrane and Press, 1997). This makes *S. hermonthica* to be the most wide spread among the species affecting cereals in the semi arid and tropical African

zones, setting conditions for genetically structured populations based on geographical clines.

The development and increased use of cultivars with improved resistance to Striga has concomitantly increased the diversity inherent in Striga. As the resistance mechanism appear in host populations, new forms of the parasite largely resistant to these mechanism will most likely get selected for, which will reproduce and form distinct species. Force of evolutionary direction exerted on major pathogens of cereals by growing of highly resistant varieties has frustrated many attempts to breed for stable resistance. This is because virulent resistance breaking forms of the pathogens can multiply freely without competition on the varieties which alone can attack (Russell, 1978).

Geographical distribution appears to play a role in genetic diversity of *S. hermonthica* (Aigbokhan *et al.*, 2000). The virulence variability within these species makes breeding programs for resistant crop varieties more complicated. This therefore calls for a much detailed determination of the level of genetic variability within *S. hermonthica* populations for effective breeding programs. It is important to note that the overall efficiency and effectiveness of Striga weed control programs will be enhanced by the knowledge on genetic relatedness or diversity available within local and regional germplasm collections.

However, considering the wide range of distribution of Striga species, not enough studies on the genetic diversity have been conducted relative to their total current

distribution (Gethi *et al.,* 2005). As such, the current study is meant to determine the genetic diversity of *S. hermonthica* ecotypes from Western Kenya and Eastern Uganda and their virulence effect on selected sorghum varieties.

2.7 Research achievements on *S. hermonthica* diversity

Diversity studies in Striga have been performed using a number of approaches including; morphological, physiological and genetic traits analysis. The advent of techniques to estimate genetic diversity has enabled scientists to focus their work on describing morphotypes, hybrids, local races and general genetic diversity inherent in Striga genus. As much as some work has been done on genetic diversity of *S. hermonthica*, much has to be done considering its relatively high total current distributions and its great invasive potential (Mohamed *et al.,* 2007).

The first study on genetic diversity in *S. hermonthica* used allozyme electrophoresis. Allozyme electrophoresis of nine loci in two populations from Burkina Faso, one adapted to pearl millet, one adapted to sorghum and one population adapted to sorghum from Sudan (Bharathalakshmi *et al.,* 1990; Musselman *et al.,* 1991) showed that geographic distance played a greater role in genetic differentiation of *S. hermonthica* populations than host specialization (Welsh and Mohamed, 2011). Gel electrophoresis was used to study genetic diversity and host specificity in 14 populations of *S. hermonthica* parasitizing sorghum, pearl millet, maize and wild grasses in Burkina Faso, Mali and Niger (Olivier *et al.,* 1996; Olivier *et al.,* 1998). This study showed a low allelic divergence within populations. There was a slight

geographic distance effects and little or no host specificity effect on genetic variability, indicating low selectivity for host may be the trend in *S. hermonthica*.

Welsh and Mohamed (2011) determined the evolutionary force between host specificity and geography which played the greatest role in shaping genetic diversity within *S. hermonthica* in Ethiopia. They carried out an assessment of the level of genetic differentiation between different geographic locations and between witchweed infecting different hosts. A sufficient amount of genetic diversity was detected using AFLP primer combinations. Geography played a significant role in shaping the genetic diversity of *S. hermonthica*.

Koyama (2000), combined allozyme electrophoresis and RAPD markers to investigate genetic diversity in *S. hermonthica*, she sampled two sites in Mali and one site each from Nigeria and Kenya. Using cluster analyses, both methods showed high levels of genetic diversity existing between and within the Striga populations.

Gethi *et al.* (2005) carried out an AFLP analysis of genetic diversity of 24 *S. hermonthica* populations from Kenya. He reported very low genetic diversity (90 % similarity) between and within the populations studied. This was attributed to the homogeneity of the Kenyan populations of *S. hermonthica* to substantial gene flow between the populations and noted that seed dispersal basically could have been through contaminated seeds. This was in agreement with studies on *S. asiatica* and *S. gesnerioides* colonization events in the United States where both showed genetic uniformity in introduced populations suggesting a single successful colonization event (Gethi *et al.,* 2005).

Welsh and Mohamed (2011) showed that *S. hermonthica* samples collected from Ethiopia were genetically distinct and all populations were significantly different from each other and attributed the Kenyan population homogeneity as determined by (Gethi *et al.,* 2005) to a small area sampled.

However, a more detailed analysis of genetic diversity in *S. hermonthica* population is required for understanding the parasite well for effective management. The recent advancement in molecular markers has provided the necessary tools that can be used in *S. hermonthica* diversity studies (Koyama, 2000). As such, the current study has utilized Expressed Sequence Tags – Simple Sequence Repeats (EST-SSR) markers to determine the genetic diversity in *S. hermonthica* populations in Western Kenya and Eastern Uganda.

2.8 Molecular markers used in diversity studies

Molecular markers are DNA sequences associated with certain parts of the genome. They have been used to assess genetic diversity as well as to establish taxonomical and phylogenetic relationship in living organisms (Rasha *et al.,* 2009). Molecular markers are presumed to be one of the most important applications in the study of population genetic and variability of crop pathogens (Koyama, 2000). There are three major classes of molecular markers, these are; morphological markers, biochemical markers and the DNA markers.

2.8.1 Morphological markers

These are classical or visible markers. Variations between individuals or between populations in a species are evaluated using visible traits such as flower color, seed shape, growth habit or pigmentation (Semagn *et al.,* 2006).

Morphological markers have been used in the evaluation of variability in *S. aspera*, *S. hermonthica* and their hybrids. The major drawbacks when using morphological markers are; they are disguised by the environmental factors, morphological traits that exhibit continuous variations between individuals in a population often obscure the evaluation of genetic diversity, the paucity of discernible morphological traits. Morphological markers are limited in numbers and late onset of some morphological markers during plant development also render unequivocal assessment difficult (Thomas, 1960).

2.8.2 Biochemical markers

These are allelic variants of enzymes called isozyme. The discovery that genes encoded proteins and enzymes, led to the utilization of isozyme and other proteins as marker system for genetic analysis of population (Scandalios, 1969; Hamrick *et al.*, 1979) The differences in the enzymes are detected by electrophoresis and specific staining that produces specific banding pattern (Linda *et al.,* 2009). Although protein markers circumvent the effect of environment, just like the morphological markers, the biochemical markers are limited in terms of the number of detectable isozyme, are influenced by the environmental factors and the stage of growth of the plant.

2.8.3 DNA markers

These are markers that reveal sites of variations in DNA, which arise from different classes of DNA mutations such as substitution, insertion and deletion. Molecular genetic techniques using DNA polymorphism have been increasingly used to characterize and identify novel germplasm (genetic diversity) within the available germplasm collections for uses in crop breeding process (O' Neil *et al.,* 2003). These markers were introduced to genetic analysis in 1980s.

DNA markers are abundant in nature, their profiles are not affected by the environment (Tevena, 2009), they are not constrained by tissue or developmental stage specificity as the case with isozyme markers, and lastly, they are selectively neutral because they are usually located in the non-coding regions of DNA, hence considered the most suitable and reliable markers. Moreover, DNA markers are more efficient and reliable as compared to morphological and biochemical markers.

DNA markers can be grouped in two generations. The first generation is that of DNA markers that employ Southern Blot Technology. Examples of such markers include the Restriction Fragment Length Polymorphism (RFLP) and Variable Number Tandem Repeats (VNTRs) (Yong-Jin *et al.,* 2009). The second generation of DNA markers includes those that employ Polymerase Chain Reaction Technology (PCR) (Yong-Jin *et al.,* 2009).

PCR revolutionized genetic and ecological analyses of populations (Yong-Jin *et al.,* 2009). The use of PCR technology in diversity studies is advantageous in that: first it

requires only a small amount of DNA to allow analysis and secondly, it is inexpensive. A variety of PCR based techniques have been applied in investigations of genetic diversity in Striga: of these, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR) are the major ones with the rest just being modifications of the three mentioned above.

Issues to do with correlation between different PCR based finger printing techniques have been raised, particularly for reproducibility across time and space; comparisons of RAPD, RFLP, AFLP and SSR results correlate for estimates of genetic distance and variability within and among populations (Nybom *et al.,* 1991).

2.8.3.1 Random Amplified Polymorphic DNA

Random amplified polymorphic DNA (Williams *et al.,* 1991) are DNA fragments generated in PCR reactions that use a single short primer (in a normal PCR, a primer pair is used). The primer must be complementary to sequences that are on opposite strands within a small number of base pairs. The DNA strand between these two sites is amplified in a PCR. Polymorphism is determined by individuals who have mutations at those sites, and therefore will not show a product on the gel.

RAPD has allowed rapid generation of reliable, reproducible DNA fragments or fingerprints in a wide variety of species, including those in the Striga (Aigbokhan *et al.,* 1998). This technique is based on PCR and instead of two specific primers, short single primers of arbitrary or random base sequences are used to amplify genomic

DNA under low stringency annealing conditions. Only the sequences that have proximal priming sites in the correct orientation will be amplified

Advantages of RAPD include, prior knowledge of the DNA sequences of the species being studied is not necessary as the case with other DNA makers, the primer has a certain chance of randomly generating a PCR product making RAPDs cheap to make, it is reported that RAPD technique offers advantages in speed and technical simplicity (Weising *et al.,*1995).

2.8.3.2 Restriction Fragment Length Polymorphism

Restriction Fragment Length Polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences (Fatokun *et al.,* 1997). It refers to a difference between samples of homologous DNA molecules that come from different locations of restriction enzyme sites. The DNA sample is broken into pieces (digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis and transferred to a membrane via the southern blot procedure (Linda *et al*., 2009). Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complimentary to the probe. RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.

RFLP has several advantages which include; high reproducibility, relatively easy to score, co-dominant inheritance, any source of DNA can be used for the analysis,

locus specificity, good transferability and no sequence information needed prior to usage (Semagn *et al*., 2006; Agrwal *et al*., 2008).

2.8.3.3 Amplified Fragment Length Polymorphism

Amplified Fragment Length Polymorphism (AFLP) is a molecular marker generated by a combination of restriction digestion and PCR amplification. AFLP is emerging as a powerful addition to the molecular toolkit of ecologists and evolutionary biologists (Muller and Wolenbarger, 1999). It is a genomic DNA fingerprinting technique that approaches an ideal situation. AFLP has been applied in many uses including, use in genetic mapping and in cloned DNA sequences in a variety of cultivated species as well as the study of genetic variations within populations (Yan and Aaron, 2003). However, AFLP cannot be able to identify homologous markers (alleles).

AFLP has been used widely in plant species, especially in crop plants (Akesson and Bensch, 2005). Gethi *et al.* (2005) demonstrated the utility of AFLP analysis in *S. hermonthica* and identified four primer combinations that generated a large number of diverse fragments in the species.

2.8.3.4 Simple Sequence Repeats

Simple Sequence Repeats (SSR) are stretches of 1 to 6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes (Weber and May, 1989). The SSR are very polymorphic due to the high mutation rates affecting the number of repeat units. The repeating sequence is small, consisting of 2, 3 or 4 nucleotides and

can be repeated 3 to 100 times with the longer loci generally having more alleles due to greater potential for slippage.

Length variations in SSRs are easy to trace in the laboratory and allows tracking genotype variations in breeding programs. High resolution gels are used to detect the length polymorphism in SSRs by running PCR amplified fragments obtained using a unique pair of primers flanking the repeats (Weber and May, 1989). The PCR reaction includes a small amount of one labeled nucleotide primer to allow visualization of amplification products via autoradiography after electrophoresis on a standard sequencing gel. Variations in PCR product length is a function of the number of SSR units.

The DNA sequences flanking SSRs are conserved, this makes it possible for selection of PCR primers that will amplify the intervening SSR in all genotypes of the target species (Cregan, 1992) and with the abundance of the PCR technology, primers that flank microsatellites loci are simple and quick to use, however, the development of correctly functioning primers is often a tedious and costly procedure

The use of SSRs has proven extremely useful (Wang *et al.,* 2008). Advantages of SSRs include; high reproducibility of its profile as the experimental procedures are simple, the hyper variable nature of SSRs produces very high allelic variations even among closely related varieties, SSR polymorphism is co-dominant which is suitable for genetic analysis in segregating F2 populations or parentage analysis in hybrids,

SSR markers are abundantly distributed in genomes and they are preferentially associated with non- repetitive DNA.

2.8.3.4.1 Development of SSR

Simple Sequence Repeats are developed using two main protocols; first, classical method which involves isolating SSRs from partial genomic libraries containing small size inserts by colony hybridization with probes that contain SSR sequence motifs (Condit and Hubbel, 1991; Kelly and Willis, 1998). This technique however, is inefficient and a laborious task (Varshney *et al.,* 2009) in cases where species have large genomes with the frequencies of colonies containing SSR motifs being relatively low to circumvent this. More recent techniques involve using oligonucleotide sequences consisting of repeats complimentary to repeats in the microsatellite to 'enrich' the DNA extracted.

Secondly, SSRs can be developed by mining data stored in the databank library. In the databank, genome scale molecular resources are deposited after sequencing the genome of plants or animals. The sequences can be accessed and used by anyone for molecular diagnosis, for biotyping and for investigating genetic diversity and population structure of subject organisms. It begins by construction of a full length enriched complimentary DNA (cDNA) library and generation of a large scale Expressed Sequence Tags (EST) dataset by reading the sequences of individual clones. The SSRs markers developed from the cDNA clones stored in the databank are referred to as ESTs. ESTs are a less expensive alternative for gaining information about the expressed genes of an organism (Rudd, 2003).

Expressed Sequence Tag (EST) is a short sub-sequence of a cDNA sequence. EST results from one short sequence of a cloned cDNA. The cDNA used for EST generation are typically individual clones from a cDNA library. The ESTs are instrumental in identifying gene transcripts, for gene discovery, gene sequence determination and in phylogenetics. EST from a full length enriched cDNA library provide the complete sequences of functional proteins (Sarukai *et al.,* 2007). Primer sets to be used in Polymerase Chain Reaction (PCR) are designed using sequences that are flanking the EST-SSR markers. The PCR process is used to test for polymorphism between individuals in a population or between populations in a species.

Yoshida *et al.* (2010) constructed a full length enriched cDNA library from *S. hermonthica* weed and generated a large scale EST dataset. They found 1,445 putative SSRs that could be used as markers. They then designed primers using the sequences flanking the putative SSRs. In total, 64 primer sets were designed of which only 44 amplified bands that were clear. Of the 44 primer sets, 26 (59 %) produced smears or multiple bands that could not be counted and only 18 primer sets (41 %) amplified clear bands. The 18 primer sets were used to investigate relationship between different *S. hermonthica* populations from 6 different fields. Only 10 primers sets (Table 2.3), from the 18 sets used showed clear polymorphism for the *S. hermonthica* populations. The rest did not show clear polymorphism.

As such, EST-SSR markers seem to be effective in determining genetic diversity among Striga species. Not much work has been done on the genetic diversity of *S.*

hermonthica using EST-SSR. The current study used 5 sets of the 10 sets of primers (Table 2.3) designed and tested by (Yoshida *et al.,* 2010), to evaluate genetic diversity in *S. hermonthica* populations from Western Kenya and Eastern Uganda.

Table 2.2: Primer sets designed and tested for use in genetic diversity studies of *S. hermonthica*

Primer name	Repeat unit	No of repeats
SSR 17	AC	18
SSR 26	AG	15
SSR 33	AG	13
SSR 43	CCG	10
SSR 50	AAG	9
SSR 53	ACC	8
SSR 57	AACT	6
SSR 58	AAAC	7
SSR 59	AAAC	6
SSR 63	AAAG	5

Source: Yoshida *et al.,* 2010.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Genetic diversity of *S. hermonthica* ecotypes

3.1.1 Plant materials

Striga hermonthica seeds were collected from sorghum fields from seven locations in Kenya and Uganda in 2013 (Table 3.1).

Table 3.1: Locations where Striga seeds for the study were collected in Kenya and Uganda

Country	Population number	Region	Locality
Kenya	1	Malava	0^{o}26'N, 34^{o}51'E
	2	Sio port	0^{o}13'N, 34^{o}01'E
	3	Ndhiwa	0^{o}44'S, 34^{o}21'E
	4	Kibos	0^{o}04'S, 34^{o}49'E
Uganda	5	Mbale	1^{o}04'N,34^{o}10'E
	6	Bugiri	0^{o}34'N, 33^{o}45'E
	7	Iganga	0^{o}39'N, 33^{o}25'E

3.1.2 Seed pre-conditioning and culturing

The seeds of *Striga* spp. require preconditioning (or warm stratification) for a certain period of time at a suitable temperature before they can become responsive to germination stimulants (Matusova *et al.,* 2004). Preconditioning was performed under sterile conditions in a Laminar flow hood. Striga seeds (20 mg) were surface sterilized in 10 % (v/v) commercial bleach (sodium hypochlorite) solution for 30 minutes in a test tube, with gentle agitation. The seeds were then rinsed thoroughly with 200 ml of sterile distilled water, then spread on a glass fiber filter paper (Whatman GFA), put into sterile petridishes and wet with 5 ml of sterile distilled water. The petridishes were then sealed with parafilm and wrapped with aluminium foil and placed in an incubator for 11 days at 29 ^{0}C for conditioning.

After conditioning, the Striga seeds were treated with a sterile germination stimulant (GR 24) to induce germination. Five milliliters of the stimulant was added to each petridish having the pre-conditioned seeds. The seeds were then placed in the growth chambers for 14 hours to allow for germination before being transferred to solid MS media (Murashige and Skoog, 1962). Seeds were considered germinated when the radicle protruded through the seed coat.

Striga seed germination was confirmed by viewing under a stereo microscope. The glass fiber filter papers having the germinated Striga seeds were removed from the petridishes and dried for 20 minutes in the laminaflow as the surplus moisture was removed through evaporation. After drying, the seeds were transferred to another

petridish containing solid MS growth media (Murashige and Skoog, 1962) by brushing them off using a soft sterile paint brush. The seeds were incubated in darkness at 29 ^{0}C for 21 days after which the Striga seedlings had enough tissue for DNA extraction.

3.1.3 DNA extraction procedure

DNA was extracted from 30 randomly selected individual seedlings from each of the sampled populations using CTAB method (Doyle and Doyle, 1987) (Appendix 1).

3.1.4 DNA estimation and quantification

The quality and quantity of the Striga genomic DNA was estimated by running it on a 1 % (w/v) agarose gel. Two microliters of extracted genomic DNA was mixed with 5µl of loading dye (New England Bio Labs Company) (NEB) and 1 µl of SYBR$^{(R)}$ green (LTC). The DNA was elecrophoresed alongside 5 µl of 1 kb ladder (NEB).The gel was run at 100 volts for 30 minutes and then visualized using an ultra-violet transilluminator (Bio view) and photo documented using a digital camera. DNA concentration of the samples was estimated by measuring the absorbance of a subsample at 260 nm with a dilution factor of 50 using nanodrop spectrophotometer ND-100 (Thermo Fisher Scientific Inc.). The quality of the DNA was determined using the 260/280 ratio. Only samples of high DNA quality were used for downstream analysis.

3.1.5 Polymerase chain reaction (PCR)

Five pairs of oligonucleotide primers labeled with fluorescent dye (Table 3.2) (Applied Biosystems UK) were used to amplify SSRs in PCR reactions containing 5 µl of 5x reaction Buffer containing 5 mM dNTPs and 15 Mm $MgCl_2$, 1 µl each of forward and reverse primer, 0.3 µl of MyTag DNA polymerase and 2 µl of template DNA in a final volume of 25 µl PCR reaction mixture.

The PCR amplification reactions were performed in an Eppendorf master cycler. Parameters for a single thermocycle were, initial denaturation at 94 ^{0}C for 3 min, first cycle first step; 94 ^{0}C for 1 minutes, annealing 40 ^{0}C for 1 minute, extension 72 ^{0}C for 1 min. The PCR consisted of 40 cycles with a final elongation of 10 min at 72 ^{0}C. The PCR product was stored at -20 ^{0}C until analyzed.

Table 3.2: Sequences of oligonucleotide fluorescent dye labeled primers used to PCR amplify SSRs. The primers are given in their 5' to 3' orientation

	Primer sequence (5' to 3')	
	Forward	Reverse
SSR 26	CAACAAAACAATGCGTGGA	GGGTTGATCACTCTGTGCAGT
SSR 43	CCACTGAAATCACAGGACGA	GTGACCGCCGTTACCATTAG
SSR 53	GCAACTGAATGGAAGGAAGC	CTCGGCTGATCCATCTGTTT
SSR 58	ACCGAGTGGTTCAAGATTGC	TGCTTCCAATACCGATCACA
SSR 63	TTTTGTTGGGGTTTATGTGGA	TGGGAGAAAAATGGAATTAA

3.1.6 Fragment analysis

One microliter of each reaction of the PCR amplifications was mixed with 9 µl of HIDI formimide and Liz 500 size standard cocktail (1:12 v/v) and put into each well of the 96 well sequencer plate. The mixture was vortexed and denatured at 95 ^{0}C for 3 minutes and quickly quenched on ice for 5 minutes. Fragment analysis was carried out by capillary electrophoresis (ABI 3730® Applied Biosystems). Genotypes were scored using GeneMapper software version 4.0 (Applied Biosystems).

3.1.7 Data analyses

Genetic diversity parameters were calculated using GenAIEx 6.1 (Peakall and Smouse, 2006) and PowerMaker 3.0 (Liu and Muse, 2005) softwares. Genetic distance matrix was generated according to Nei (1972), using PowerMarker software. To visualize differences between populations, a Principal Component Analysis (PCA) was run based on genetic distance with data standardization in GenAIEx software. As a supplement to the PCA analysis, the genetic distance matrix generated in PowerMarker software was also used to develop a dendrogram. The dendrogram was constructed using the unweighted pair group method (UPGMA) for estimating the genetic similarity based on Nei's genetic distance among the populations.

Pairwise population comparisons were analyzed with an Analysis of Molecular Variance (AMOVA) using GenAIEx 6.1 software based on 999 permutations. The AMOVA estimates and partitions total molecular variance within and between

populations and then test the significance of partitioned variance components using permutational testing procedures. The AMOVA was also conducted to estimate the genetic differentiation between the populations (F_{ST}) using stepwise mutation. The number of migrants per generation was calculated from F_{ST} value using the equation Nm = [(1-Fst)-1] /4 to determine the geneflow among the populations.

3.2 Assessment of sorghum for their response to *S. hermonthica* infection

3.2.1 Plant materials

Sorghum seeds were collected from USA, Sudan, Eritrea, Kenya and Uganda (Table 3.3).

Table 3.3: The country of origin of the sorghum genotypes used in this project

Sorghum	Country	Locality
SAP 027	USA	37°39'N, 88°19'W
SAP O34	USA	37°39'N, 88°19'W
SAP 048	USA	37°39'N, 88°19'W
N13	ICRISAT KENYA	0°00'S, 37°53'E
SRS 208/1	USA	37°39'N, 88°19'W
ASARECA AG3	SUDAN	12°50'N, 30°20'E
ASARECA W2	SUDAN	12°50'N, 30°20'E
EPURPUR	UGANDA	1 °25'N, 30°20'E
WILD 1	SUDAN	12°50'N, 30°20'E
WILD 2	SUDAN	12°50'N, 30°20'E
OCHUTI	KENYA	0°00'S, 37°53'E
SEKEDO	UGANDA	1°25N, 30°20'E

3.2.2 Sorghum seed germination

Sorghum seeds were germinated between blocks of moistened horticultural rockwool (Growdan®VitalGrowdan, Roermond, Netherlands). Four days old sorghum

seedlings were transferred to a root observation chamber (Rhizotron) as earlier discussed by Gurney *et al.* (2006). A single sorghum seedling was transferred to each rhizotron.

3.2.3 Striga seeds conditioning

Striga seeds were conditioned (as earlier mentioned in chapter 3.1.2), the same day sorghum seedlings were transferred to the rhizotrons. This was meant to ensure that by the time the sorghum plant is ready for infection with the Striga seeds, they have already been conditioned. The germination of the Striga seeds was induced by adding 5 ml of artificial germination stimulant GR24 (0.1 ppm) in each petridish containing the seeds, 18 hours prior to infection (Gurney *et al.,* 2006). Striga Seeds were triggered to germinate before being inoculated on sorghum roots to ensure their synchronous attachment to the roots thereby eliminating any differences that may occur as a result of variations in production of germination stimulant by different sorghum plants (Jamil *et al.,* 2011).

3.2.4 Infection of sorghum seedlings with *S. hermonthica*

Ten days after transfer of sorghum plants to the rhizotrons, Sorghum roots were inoculated with 40 mg of pregerminated *S. hermonthica* seeds from Malava population. The seeds were aligned along the host roots using a fine paint brush. The rhizotrons were then covered with foil to prevent light from reaching the roots. Rhizotrons were fed with 25 ml of 40 % (v/v) Long Ashton nutrient solution (Hewitt,

1966) two times a day. One plant was used for each treatment in three independent experiments.

3.2.5 Determination of attachment of *S. hermonthica* radicle on roots of sorghum seedlings

To determine if the Striga radicles had attached to the sorghum roots, pictures were taken using a charge coupled device camera (CCD) (Diagnostic instruments Inc.) mounted on a Leica MZFL stereomicroscope (Leica Instruments GmbH) 6 days after infection. Normally it takes between 4 to 7 days for attachment to be observed. Twenty one days after infection of sorghum with the Striga, the root system of sorghum on each rhizotron was photographed using Sony digital camera (Sony Corporation Minato-ku Tokyo, Japan) for quantification of post-attachment resistance.

3.2.6 Phenotype of resistance

Striga seedlings growing on roots of each of the infected sorghum plants were harvested 21 days after infection. The harvested Striga plants from each host plant were placed in a petri plate and photographed using Sony cyber-shot (Sony Corporation Minato-ku Tokyo, Japan). The number and length of Striga plants on each host plant was calculated from the photographs using image analysis software (ImageJ, Media Cybernetics). The Striga seedlings were then dried at 48 ^{0}C in the oven for two days and the amount of dry mass per host plant determined.

3.2.7 Statistical analysis

Statistical analysis of variance (ANOVA) was performed using Statistical Package for the Social Sciences (SPSS version 3) (IBM Corporation). Data for Striga dry biomass, number of Striga seedlings growing on the sorghum roots and Striga length were log transformed to meet the assumptions of ANOVA. Tukey's Honestly Significant Difference (HSD) test was then performed to calculate the HSD and to establish the different groups. All values ≤ 0.05 were considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Genetic diversity of *S. hermonthica* ecotypes

4.1.1 Striga Seed germination frequency

Striga seeds germinated 18 hours after introduction of the germination stimulant (GR 24). The germinated Striga seeds had a radicle protruding from the imbibed seed-coat (Figure 4.1). Germination frequency of the *Striga hermonthica* seeds among the populations ranged from 38.2 % to 42.2 % (Table 4.1).

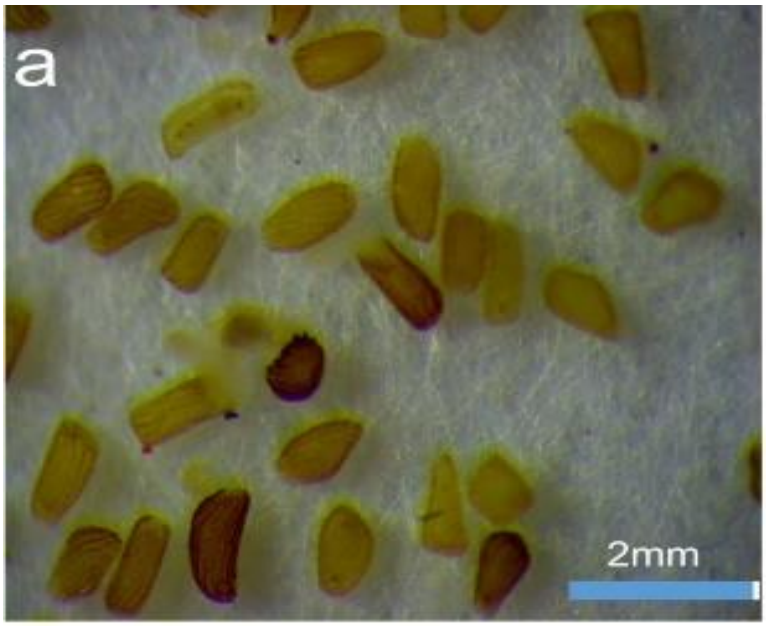

Figure 4.1: *In vitro* germinated Striga seeds 18 hours after introduction of the artificial germination stimulant (GR 24).

Table 4.1: Germination frequency of *S. hermonthica* seeds

Striga population	No. of seeds	Germinated seeds	% germination
Bugiri	200	81	40.5
Ndhiwa	180	76	42.2
Iganga	210	82	39.0
Kibos	199	78	38.2
Malava	210	84	41.0
Mbale	160	64	39.0
Sio port	300	120	40.0

4.1.2 Fragment analysis

GeneMapper(R) software (V 4.0) scoring produced electropherograms showing the number of amplified fragments and their sizes (Figure 4.2). Downstream fragment analysis by PowerMarker software showed that the number of fragments per each primer ranged from 3 (SSR 58) to 14 (SSR 26) with an average of 7.8 fragments (Table 4.2). The total number of bands detected across all the primer combinations after correcting for repeatedness was 38 out 39 bands. Hence 97 % polymorphic bands were observed among the *S. hermonthica.*

The average polymorphic information content (PIC) was 0.6445. The lowest PIC value was 0.354 for primer SSR 58 and the highest was 0.890 for primer SSR 26. Markers SSR 26, SSR 63 and SSR 53 gave the highest diversity values of 0.899, 0.757 and 0.752, respectively (Table 4.2).

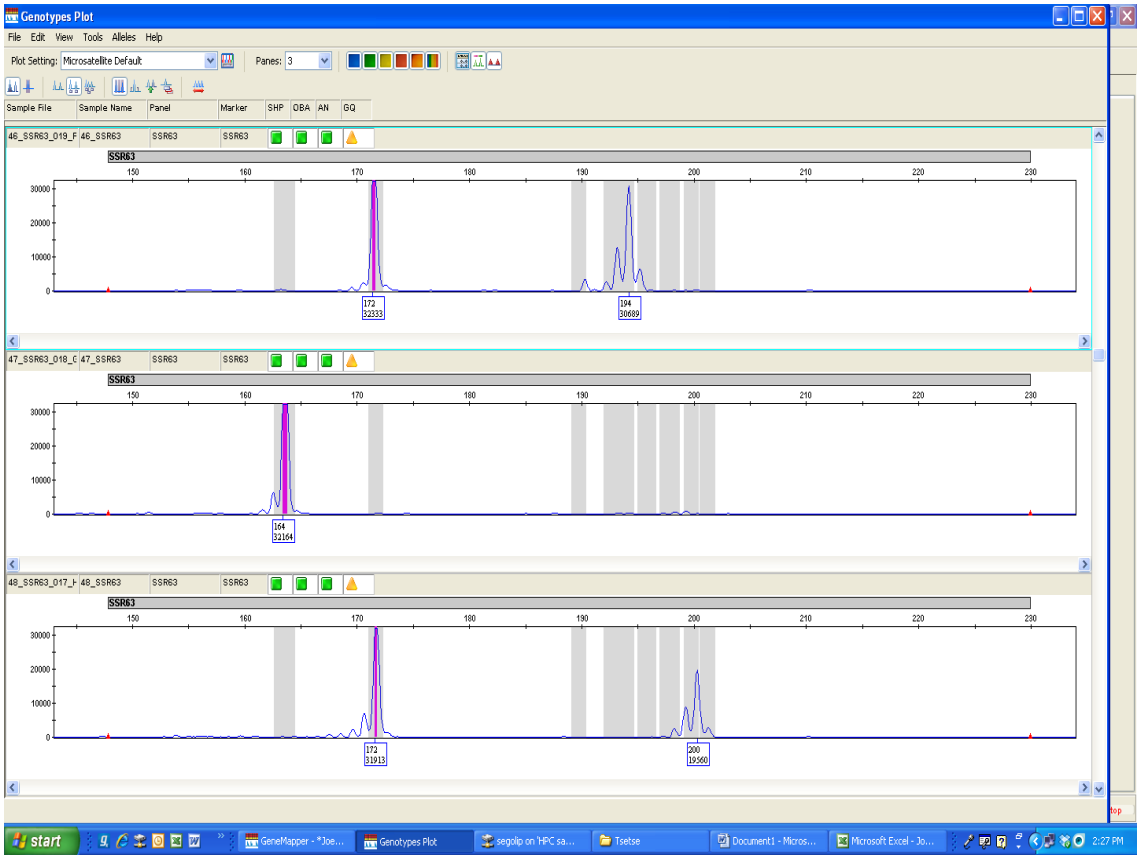

Figure 4.2: Representative electropherogram generated by the GeneMapper V 4.0 software for samples 17, 18, 19 using marker SSRs 63.

Table 4.2: Summary data for each primer

Marker	Major Allele Frequency	Allele No	Gene Diversity	Ho	He	PIC
SSR26	0.168	14	0.899	0.729	0.817	0.890
SSR43	0.629	7	0.565	0.416	0.544	0.532
SSR53	0.379	7	0.752	0.552	0.544	0.715
SSR58	0.665	3	0.450	0.571	0.366	0.354
SSR63	0.424	8	0.757	0.376	0.652	0.731
Mean	0.453	7.8	0.684	0.529	0.585	0.645

Ho – observed heterozygosity
He – expected heterozygosity
PIC – polymorphic information content

Average observed heterozygosity (Ho) was 0.529 ranging from 0.376 to 0.729 while average expected heterozygosity (He) was 0.585 ranging from 0.366 to 0.817 (Table 4.2).

The genetic relationship among the seven Striga populations was revealed by Nei's genetic distance values that ranged from 0.122 to 0.710, the smaller values indicating a closer relationship (Table 4.3). The highest similarity (0.122) was observed between Bugiri and Iganga Striga populations. The lowest similarity (most diversified) was observed between Striga populations Malava from Western Kenya and Mbale from Eastern Uganda revealing high genetic diversity between the two populations.

Table 4.3: Pairwise population matrix of Nei's genetic distance (1972) for the 7 *S. hermonthica* populations

	Bugiri	**Dhiwa**	**Iganga**	**Kibos**	**Malava**	**Mbale**	**Sio port**
Bugiri	0.000						
Dhiwa	0.219	0.000					
Iganga	0.122	0.352	0.000				
Kibos	0.340	0.258	0.507	0.000			
Malava	0.198	0.209	0.179	0.444	0.000		
Mbale	0.317	0.412	0.546	0.463	0.710	0.000	
Sio port	0.167	0.250	0.258	.307	0.333	0.283	0.0000

Principal component Analysis generated by GenAIEx software showed that the first and second axis explained 28 % and 14 % of the observed variations, respectively (Figure 4.3). However, the efficiency of the PCA was low based on the low percentage of observed pattern on the axis. On the 1st axis, which explains 28 % of

the observed pattern; Bugiri, Iganga and Sio port clustered together while on the 2nd axis, Ndhiwa and Kibos Striga populations seem clustered together. However, the PCA is not highly supported as axis 1 and 2 are explaining only 42 % of the observed pattern. Meaning that we the data should be interpreted with caution since the PCA is not well supported.

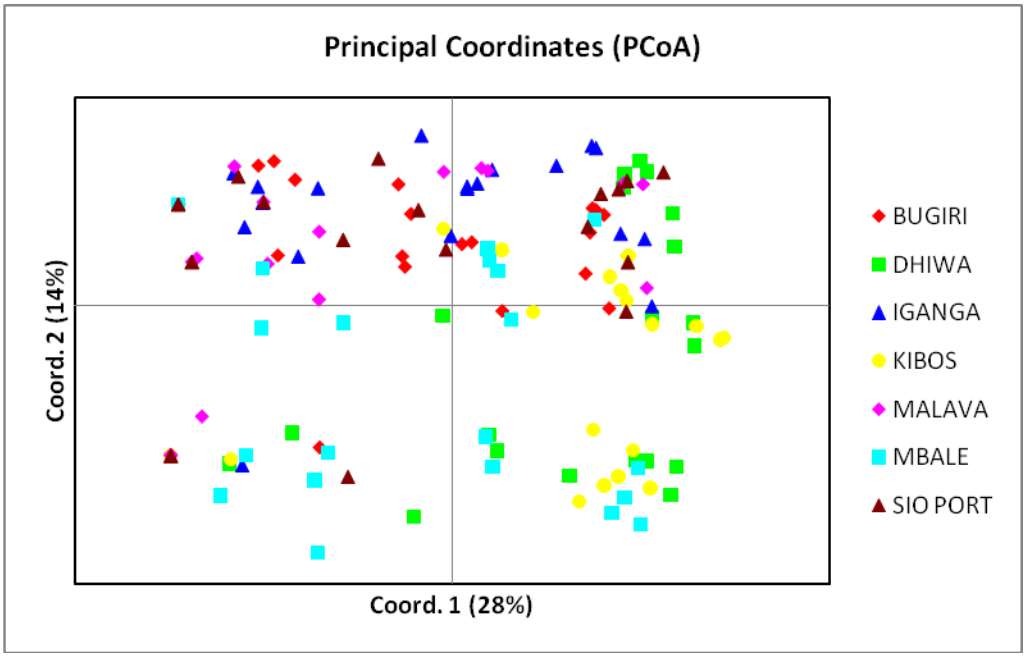

Figure 4.3: The Principal Component Analysis (PCA) of the genetic distance at the population level illustrating the distribution of the *S. hermonthica* populations. The axes 1 and 2 explained 42 % of the variance in distribution of the populations.

A dendrogram constructed by the PowerMarker software using Unweighted Pair Group Method of Arithmetic Averages (UPGMA) resulted to 4 distinct clades (Figure 4.4) with Mbale population forming its own clade (4th) and separating distinctively from the rest meaning that it is genetically distant from the rest. The 1st clade is made of Sio Port and Ndhiwa (Kenyan) populations, meaning that they are

related. The 1st clade is closely related to the 2nd clade which is made up of Iganga and Bugiri populations that are closely related to Malava population from Western Kenya. Kibos population from Kenyan formed the 3rd clade which showed distant relationship with the 1st and 2nd clades (Figure 4.4).

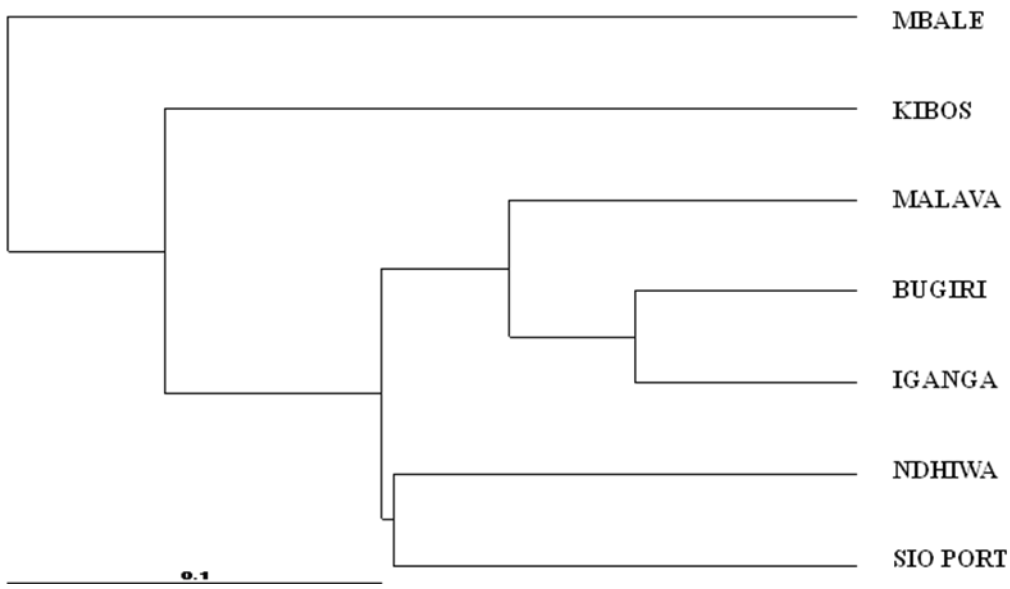

Figure 4.4: Phylogenetic relationship among the *S. hermonthica* populations. Shown is a UPGMA dendrogram constructed based on Nei's (1972) genetic distance with PowerMarker 3.25 software.

Analysis of Molecular Variance (GenAIEx 6.1) showed that most of the molecular variation in the *S. hermonthica* populations existed among individuals within populations (87 %) while the variation among populations was lower (13 %) (Table 4.4). Genetic differentiation among the populations was given by the (F_{ST}) value = 0.100. The number of migrants (Nm) determined the geneflow among the populations (Nm = 2.239).

Table 4.4: Analysis of molecular variance for *S. hermonthica* populations

Source	df	SS	MS	Est. Variance	%	P values
Among Population	6	121.336	26.223	0.759	13	0.001
Within Population	133	670.400	5.041	5.800	87	0.001
Total	139	791.736			100	0.001

4.2 Assessment of sorghum for the response to *S. hermonthica* infection

4.2.1 Transfer of sorghum seedlings to rhizotrons

Four days old sorghum seedlings were transferred to the rhizotrons after germination of sorghum seeds, (Figure 4.5 a) were ready for transfer to the rhizotrons. Ten days after transfer, the sorghum plants had well developed root system ready for infection with the Striga seeds (Figure 4.5 b; c).

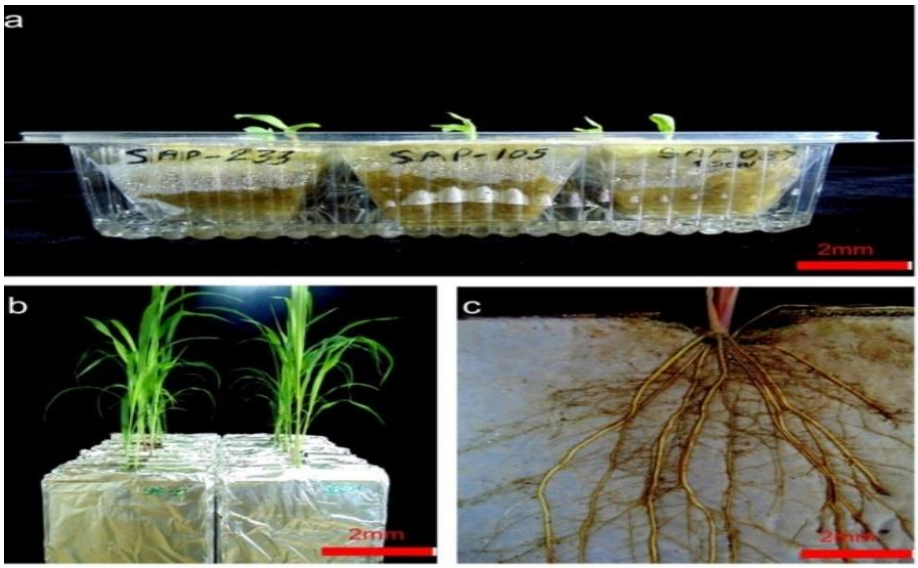

Figure 4.5: (a) Four days old sorghum seedlings germinated on moist rockwool ready for transfer to the rhizotrons. (b): Sorghum plantlets growing on the rhizotrons 10 days after transfer. (c): Roots of a sorghum plant well spread on the rhizotrons and ready for infection with the Striga seeds.

4.2.2 *Striga hermonthica* attachment and phenotype of resistance

Striga hermonthica attached to the roots of the susceptible sorghum varieties by the 4th day after inoculation. Successful attachment was characterized by swelling of the Striga radicle at the point of attachment. On the 7th day, in resistant interactions, parasites had elicited resistance response from the host. The most visible resistance response was intense necrosis at the site of radicle attachment leading to the death of haustoria cells (Figure 4.6 c) and in other cases, there was successful attachment but the haustoria failed to increase in size leading to the death of the parasite (Figure 4.6 d)

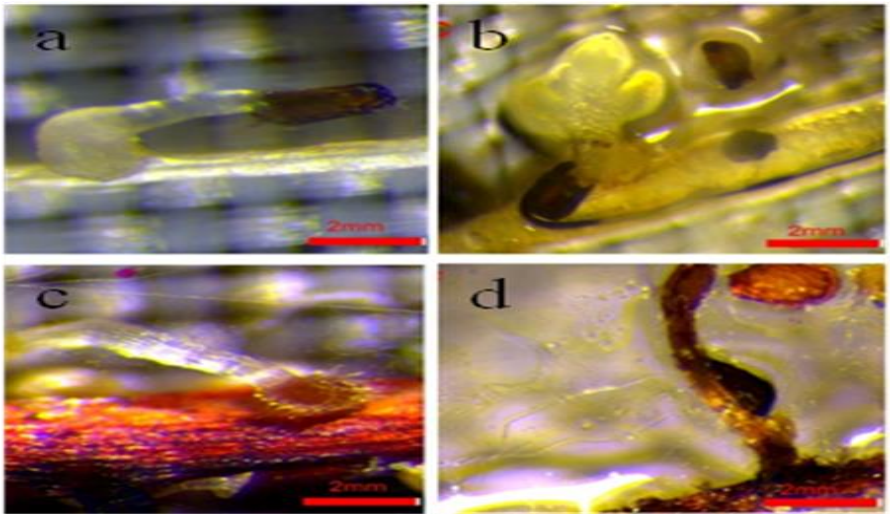

Figure 4.6: Sorghum varieties being infected by *S. hermonthica*. **(a)** Radicle attached to the host root and swollen into a turbercle. **(b)** Leaf pre-mordia emerging **(c)** The host root exhibiting intense necrosis at the site of radicle attachment leading to the death of haustoria cells (darkening at the site of attachment). **(d)** Successful attachment of *S. hermonthica* radicle but the haustoria failed to increase in size and the parasite clearly dying.

4.2.3 Evaluation of susceptibility and resistance

Twenty one days after infection of sorghum with the pre-germinated Striga seeds, the susceptible sorghum varieties had the highest number of the Striga weed-lets growing on their roots (Figure 4.7 a, b and c). The Striga weeds were growing rapidly and well developed while on the other hand the resistant varieties supported very few Striga weeds-lets which were growing slowly and were small in size (Figure 4.7 d, e and f), an indication that they were not being well supported.

Figure 4.7: *Striga hermonthica* growing on roots of selected Sorghum varieties in rhizotrons 21 days after infection. (a): Sap 027, (b): Sekedo, (c): Sap 048 are sorghum varieties that were highly susceptible to *S. hermonthica* with the highest number of Striga weeds attachment on their roots. (d): N13 (e): Wild 1 (f): Wild 2 sorghum varieties that were least susceptible to *S. hermonthica* with the least number of Striga weeds attached that are small in size (indicated by the arrow)

4.2.4 Evaluation of *S. hermonthica* dry biomass, length and number

Twenty one days after infection, average Striga length on each of the sorghum plant varied from 0.267 cm (Wild 1) to 7.967 cm (Sap 027), Striga dry biomass ranged from 0.003 g (Asareca AG3) to 0.323 (Sap 027) while Striga plant-let count on each sorghum plant ranged from 0.333 (Asareca AG3) to 10.667 (Sap 027) (Table 4.5).

Generally Sap 027, Sap 034, Sap 048, Epurpur, SRS 208/1, Sekedo and Ochuti varieties supported the highest number of the Striga plants growing on their roots,

the highest Striga mean dry biomass and the highest mean length (Table 4.5). Conversely, Wild 1, Wild 2, N13, Asareca AG3 and Asareca W2 varieties supported very few Striga plant-lets growing on their roots which had small mean length and low dry biomass (Table 4.5). According to Tukey's HSD test, there was a high significant difference in the means of number of Striga, Striga dry biomass and Striga length growing on the roots of sorghum varieties between the susceptible and resistant varieties ($P < 0.05$) (Table 4.5).

Table 4.5: Mean number of *S. hermonthica* plants, dry biomass and length of parasites on each host plant

Variety	No. of Striga plants	Striga Dry biomass (g)	Striga length (cm)
Sap 027	10.667 ± 1.201[a]	0.323 ± 0.020[a]	7.967 ± 0.033[a]
Epurpur	8.667 ± 1.201[a]	0.200 ± 0.012[b]	7.633 ± 0.318[a]
Sap 048	8.667 ± 1.452[a]	0.287 ± 0.024[a]	7.133 ± 0.186[a]
Sap 034	8.333 ± 0.881[a]	0.267 ± 0.018[a]	7.167 ± 0.376[a]
SRS 208/1	8.000 ± 2.000[a]	0.197 ± 0.032[b]	5.200 ± 0.416[a]
Sekedo	7.000 ± 1.527[a]	0.213 ± 0.041[b]	5.900 ± 1.137[a]
Ochuti	7.000 ± 0.577[a]	0.213 ± 0.018[b]	5.133 ± 1.184[a]
N13	0.667 ± 0.333[b]	0.017 ± 0.009[c]	0.633 ± 0.318[b]
Wild 1	0.667 ± 0.333[b]	0.007 ± 0.003[c]	0.267 ± 0.145[b]
Asareca W2	0.667 ± 0.333[b]	0.007 ± 0.003[c]	1.100 ± 0.666[b]
Wild 2	0.667 ± 0.666[b]	0.007 ± 0.007[c]	0.500 ± 0.500[b]
Asareca AG3	0.333 ± 0.333[b]	0.003 ± 0.003[c]	0.967 ± 0.967[b]

Data presented for Striga numbers, dry biomass and length are means ± SE. Means in the same column with the same letters are not significantly different according to Tukey,s HSD test ($P < 0.05$).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Genetic diversity of *S. hermonthica* ecotypes

Analysis of molecular variance (AMOVA) indicates that differences among individuals of *S. hermonthica* within the same population contributed 87 % (P = 0.001) of the genetic differences while 13 % of the variations were attributed to differences among *S. hermonthica* populations. *Striga hermonthica* is an obligate out-crossing species (Safa *et al.,* 1984) and therefore it is expected to show less differentiation between populations and greater diversity within populations than as seen in related autogamous species (Hamrick, 1982). In addition, the low Fst value (Fst = 0.100) indicates low level of genetic differentiation among the seven *S. hermonthica* populations and as such the first hypothesis is accepted.

Such low levels of differentiation among the *S. hermonthica* populations may be due to a recent colonization effect (a founder event) East into Kenya and West into Uganda and the high level of gene flow (Nm = 2.239) among the populations. A small number of migrants per generation is enough geneflow to obscure or prevent the process of drift that causes populations to differentiate over time (Matt *et al.,* 2011). This high geneflow among the *S. hermonthica* populations may have been caused by active grain trading activities in the region. Trading of cereals that are contaminated with Striga seeds has been shown to be frequent in the study region

(Berner *et al.,* 1994). Other forms of dispersal like wind, water and forage animals have also been shown to play a role in dispersal of *S. hermonthica* within the region hence reducing its diversity (Matt *et al.,* 2011). Other works have also shown high intra-population variability and no evidence of races (Olivier *et al.,* 1998; Koyama, 2000; Gethi *et al.,* 2005). The high genetic variability within *S. hermonthica* populations presents a challenge to develop reliable Striga resistant sorghum varieties (Koyama, 2000).

The dendrogram shows that Striga population from Malava (Kenya) groups with Striga from Iganga and Bugiri populations (Uganda). The two regions are however most geographically separated within the study area. This therefore suggests a substantial geneflow between the regions. There is evidence of active cereal trade between Kakamega and Ugandan people in the early 1970s and 1980s at an infamous market Chebukube. There are high possibilities that the cereals may have been contaminated with *S. hermonthica* seeds since most cereals are threshed on ground within the contaminated field.

The EST-SSR sequences are good candidates for genetic markers, which can be used for molecular diagnosis and for investigating the genetic diversity and population structure of *S. hermonthica* (Yoshida *et al.,* 2010). The SSR markers have been successfully used in investigating genetic diversity and phylogenetic relationships of Striga populations in Sudan (Yoshida *et al.,* 2010) and in Mali (Matt *et al.,* 2011) and also in leguminous plants (Sawadogo *et al.,* 2010). In this study, 5 informative SSR primer combinations were used to analyze diversity in seven *S. hermonthica*

populations collected from Kenya and Uganda. The SSR analysis yielded 38 unique bands ranging from 3 to 14 fragments per primer combination with an average of 7.8 bands per primer. These values demonstrate the effectiveness of the SSR markers to unravel diversity within the *Striga hermonthica* populations. These findings are in agreement with work reported by Yoshida *et al.* (2010) where the SSR revealed 27 alleles with an average of 2.7 alleles per locus. Genetic divergence analysis resulted in average gene diversity of 0.33 ranging from 0.122 to 0.710. This is a moderate level of genetic diversity among the *S. hermonthica* populations. Matt *et al.* (2011) while working on genetic diversity of *S. hermonthica* in Mali using SSR reported genetic diversity ranging from 0.687 to 0.748 while Yoshida *et al.* (2010) reported genetic diversity range of 0.375 to 0.625 while working on genetic diversity of *Striga hermonthica* populations in Sudan. Gethi *et al.* (2005) got very low genetic distance values ranging from 0.007 to 0.025 on the genetic diversity of *Striga hermonthica* populations from Kenya using AFLP markers. Homogeneity among the Kenyan *Striga hermonthica* populations was attributed to recent colonization from the Lake Victoria and the allogamous breeding system of *S. hermonthica*. However, the low genetic diversity in the Kenyan populations (Gethi *et al.*, 2005) may have been due to less sensitive markers used (AFLP markers) as compared to the current study.

5.1.2 Assessment of sorghum for the response to Striga infection

This study showed that there is varied resistance response to *Striga hermonthica* by the different sorghum varieties studied and therefore the second hypothesis was rejected. Some varieties exhibited high resistance responses while others were highly

susceptible to the parasite. This may be attributed to the presence of genetic variations in the different sorghum lines in response to *S. hermonthica* parasitism (Haussmann *et al.,* 2000a). However, complete resistance to *Striga hermonthica* has not been identified in cultivated sorghum varieties (Gurney *et al.,* 2002). All the resistant sorghum varieties tested showed some successful parasite attachment on the roots.

Among the sorghum varieties tested, there were those that were easily susceptible to *S. hermonthica* infection and those that showed resistance to the weed. The susceptible sorghum varieties had the highest number of *S. hermonthica* parasites attached on their roots, the parasites were growing fast and healthy, an indication that they were getting enough nutrient support from the host. On the other hand, the resistant sorghum varieties had very few *S. hermonthica* parasites growing on their roots and the parasites comparatively had stunted growth which indicated that they could not acquire enough nutrients from their host as is the case with those growing on the susceptible sorghum varieties. The mean number, the mean length (cm) and the dry biomass of *S. hermonthica* parasites growing on the roots of each of the sorghum variety was therefore used to group the sorghum varieties as susceptible or resistant (Berner *et al.,* 1995). In the current study, wild type sorghum was highly resistant to *S. hermonthica* as compared to the rest of sorghum varieties. This implies that a valuable source of resistance to sorghum may lie in the genetic potential of wild sorghum germplasm (Tanksley and McCouch, 1997). Breeding for *S. hermonthica* resistance should therefore take advantage of the natural resistance

available in the wild sorghum varieties genepool and other sorghum cultivars showing resistance phenotype to develop elite cultivars. Mapping the resistance alleles will lead to development of markers that can be employed in the marker assisted selection (MAS). Among the sorghum varieties tested, N13, Asareca AG3 and Asareca W2 also showed resistance phenotype to *S. hermonthica*. This was expected since the genomic regions (QTL) associated with stable *S. hermonthica* resistance from the N13 variety were identified (AATF, 2011) and selection of the QTL done and introgressed into sorghum varieties Asareca AG3 and Asareca W2 varieties at ICRISAT. These varieties N13, Asareca AG3 and Asareca W2 are known resistant genotypes with superior resistance to *S. hermonthica* as confirmed in the current study.

Wild 1, Wild 2, N13, Asareca AG3 and Asareca W2 sorghum tested in this study exhibited a number of resistance responses which included intense necrosis at the site of attachment, successful attachment but failure in growth and growth of Striga radicle away from the host root. Intense necrosis at the site of attachment resulted in the death of the parasites haustoria cells (darkening at the tip of parasite radicle). The necrotic areas appeared at the site of parasite attachment due to localized cell death of the host tissue. Lane *et al*. (1993) observed intense necrosis at the site of parasite attachment to cowpea when infected with *S. gesnorioides* parasite. Scholes *et al*. (2011) showed similar findings in rice genotypes. Growth of Striga radicle away from the host root suggests the existence of substances that could possibly inactivate chemical signals that attract parasite radicle to the host root (Fasil *et al*., 2010).

Susceptible soghurm varieties as tested in this project included; Sap 027, Sap 034, Sap 048, Epurpur, SRS 208/1, Sekedo and Ochuti. These sorghum varieties were characterized by many Striga parasites growing on their root system, the parasites were growing fast and healthy hence higher Striga mean dry biomass and mean length on each of the sorghum plant as opposed to the parasites growing on the resistant sorghum varieties, Wild 1, Wild 2, N13, Asareca AG3 and Asareca W2 varieties that had the lowest number of Striga parasites growing on their root system and the parasites had comparatively stunted growth leading to low mean Striga length and least Striga dry biomass. Similar findings have also been reported in genetics of resistance to *S. hermonthica* in sorghum (Ahonsi *et al.,* 2004).

The introduction and rapid diffusion of Striga resistant varieties is the most feasible survival strategy in resource poor, Striga prone rural economies where small scale farmers can't afford the other expensive control measures against the Striga weed. However, as much as the deployment of resistant sorghum cultivars is a cost effective method of Striga management, its use *per se* may have little beneficial effect if other control options (strategies) like use of clean planting materials that are not contaminated with Striga, crop rotation, proper crop management practices and quarantine measures that outlaw trading in cereals that are contaminated with the Striga weed seeds. Integrating genetic resistance with other control measures is the smartest option possible for effective control as well as for increasing durability of resistance genes (Ejeta *et al.*, 2007b).

5. 2 Conclusion

i. The *S. hermonthica* populations from Western Kenya and Eastern Uganda have low genetic differentiation and therefore the first hypothesis is accepted.

ii. The sorghum varieties Sap 027, Epurpur, Sap 048, Sap 034, SRS 208/1, Sekedo and Ochuti are highly susceptible to Striga infection while varieties N13, Asareca W2, Asareca AG3 and the Wild type 1 and 2 are highly resistant to the weed and therefore the second hypothesis is rejected.

5.3 Recommendations

i. There is need for identification of the genomic regions (QTL) associated with stable *S. hermonthica* resistance in the wild type sorghum studied and subsequent selection of the QTL and introgression into other susceptible sorghum varieties for breeding of resistant cultivars.

ii. There is need for further analysis of the constitutes released by resistant sorghum plants in response to Striga infection to find out what causes Striga radicle to grow in the opposite direction from the soghum root.

iii. There is need to further screen sorghum varieties for resistance to *S. hermonthica* on wider level and subsequent deployment of the resistant varieties as a component of Integrated Striga Management (ISM) program

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APPENDICES

Appendix 1: DNA extraction protocol

CTAB method, (Doyle and Doyle, 1989) - optimized for Striga spp.

1. Place tissue in 1.5 ml tube; and place a stainless steel ball inside each tube (to aid in lyophilisation of the frozen tissue) and close the tube.
2. Place the tubes in liquid nitrogen and vortex well until the tissue is converted to fine powder.
3. Pre-warm CTAB extraction buffer at 60^0C (32.5 ml dH_2O, 5.0 ml I M Tris 7.5, 5.0 ml 5 M EDTA 8.0) for 1 hour.
4. In each tube with the tissue powder, add 1 ml of the warm extraction buffer and mix gently by swirling to homogenize the tissue with the buffer.
5. Incubate the samples at 60^0C for 1 hour with continuous gentle rocking. Remove the tube from the water bath and allow cooling for 10 min.
6. Add 1 ml of chloroform (Isoamyl alcohol 24.1) to each tube and centrifuge samples for 10 min at 3500 rpm at room temperature. A yellow aqueous phase and a green organic phase are generated.
7. Transfer the supernatant aqueous phase to fresh labeled tubes and add 1 ml of chilled iso-propanol into the tubes.
8. Incubate the samples at -20^0C for 1 hour in order to precipitate.
9. Centrifuge at 3500 rpm for ten min and drain the supernatant (take care not to spill the pellet). Invert the tubes to dry the pellet.

10. Re-suspend the pellet in 100 µl of distilled water, add 10 µl of 3 M sodium acetate (NaoAC) pH 5.2 and then add 1 ml of chilled absolute ethanol.
11. Incubate at -20^{0}C for 1 hour, centrifuge for 10 min at 3500 rpm and drain the supernatant. Invert to dry the pellet.
12. Add 200 µl of ethanol to the pellet in each tube, centrifuge at 3500 rpm for 5 min, drain the supernatant and invert to dry the pellet for at least 1 hour until the pellet is completely dry.
13. Re-suspend the pellet in 50 µl of ddH_2O and store the DNA at -20^{0}C.