

Genetic diversity and virulence of *Striga hermonthica* from Kenya and Uganda on selected sorghum varieties

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Abstract. Joel KA, Runo S, Muchugi A. 2018. Genetic diversity and virulence study of *Striga hermonthica* from Kenya and Uganda on selected sorghum varieties. *Nusantara Bioscience* 10: 111-120. Parasitic weeds pose a severe problem in agricultural production, causing massive crop losses in many regions of the world and especially Africa. One example to be considered the most significant biological constraint to food production in sub-Saharan Africa (SSA) is *Striga hermonthica*, the most widespread among the *Striga* 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 the quick breakdown of resistance in crops hence making control of the weed difficult. Efficient and effective control of *S. hermonthica* demands 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 aimed at evaluating the 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 genetic diversity was moderate as shown by the Nei's genetic distance values. AMOVA measured low genetic differentiation among the populations. This study also investigated twelve sorghum varieties for their response to *S. hermonthica* infection. The result demonstrated that the varieties resistance responses to *S. hermonthica* varied widely. The phenotype of resistant interaction was characterized by the inability of the weeds haustoria to penetrate the sorghums root endodermis due to severe 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, while the most susceptible varieties were Sap 027, Epurpur which had the highest mean number of *S. hermonthica* plantlets growing on their roots. There was a highly significant difference in the means of the number of *Striga* growing on the roots of sorghum varieties, *Striga* dry biomass and *S. hermonthica* length between the susceptible and resistant ones. This knowledge holds unique potentiality since resistant sorghum germplasm tested will be sourced and targeted to the seven specific geographical areas where virulence of the particular *S. hermonthica* populations was characterized.

Keywords: Ecotypes, genetic diversity, Kenya, *Striga hermonthica*, Uganda, sorghum, virulence

INTRODUCTION

Striga weed is commonly known as witchweed and a noxious root hemi-parasite that has devastated cereal production in Sub-Saharan Africa (SSA) (Runo et al. 2012). The genus comprises 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 dominant agricultural *Striga* species are; *S. hermonthica*, *S. asiatica*, which infects cereals like maize (*Zea mays*), millet (*Sorghum bicolor*) and rice (*Oryza sativa*) and *S. gesnerioides* which infect 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 severe infection.

Depending on species, *Striga* parasites reproductive

schemes vary from autogamy to obligate allogamy (Musselman 1987). *S. hermonthica* and *S. aspera* are the only two species known to be obligately allogamous and require insect pollinators. These two species can hybridize and produce viable and virulent offspring; a phenomenon that produces broader genetic variability within the species, creating even much more challenging to develop resistant crop varieties (Parker 2009).

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

Striga parasite infests 40 % of the cereal producing areas of SSA, resulting in crop losses estimated at US\$ 7 billion annually affecting the 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 many factors; *Striga* density, land use system, host species, soil nutrient status and rainfall patterns (Atera et al. 2012b). A survey done in Western Kenya in 2009 reported that 73 % of the fields used for growing crops were infected with *S. hermonthica* (Woomer and Savala 2009); this is a fair indication that *Striga* is the most damaging pest experienced by farmers in those areas. *Striga* weeds, in essence, impairs the struggle to attain food security in Africa and consequently its control must be addressed by all efforts (Atera et al. 2012a).

Cultural management methods for *Striga* have not achieved much under its control (Atera et al. 2012b). Practices such as weeding, pulling and slashing are futile because the devastating effects of the parasite are accomplished before its emergence. Many research has been done to identify cultivars of several species that are resistant to *Striga* parasitization (Mohamed et al. 2003; Ejeta 2007b). The utilization of resistant varieties has however been ineffective 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 (Rispaal et al. 2007). The use of herbicides such as 2,4 D, Oxyfluorfen, and Imazapyr is the available application for use. Nevertheless, it is a control measure that is unfortunately beyond the investment abilities of most small-scale farmers (AATF 2011). Therefore, it calls for an urgent need for the establishment of better policies to promote, implement and ensure long-term sustainable *Striga* control programs.

Recent molecular advance has provided the necessary tools that can be used in *Striga* diversity studies. Molecular markers are DNA sequences associated with certain parts of the genome and are presumed to be the most critical applications in the study of population genetic structures and genetic variability of crop pathogens (Koyama 2000). Examples of molecular markers include; Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), 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 full range of distribution of *Striga* species, few studies on the genetic diversity have been performed relative to their total current distribution (Runo et al. 2012). In general, there is lack of knowledge by farmers on the most resistant sorghum varieties which have contributed to low sorghum production. Screening of sorghum varieties to determine the most resistant ones is critical for farmers in Kenya and Uganda since the use of the resistant varieties is considered the most appropriate means of combating the weed.

The objectives of this research were (i) to determine if there exist 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.

MATERIALS AND METHODS

Genetic diversity of *Striga hermonthica* ecotypes

Plant materials

Striga hermonthica seeds were collected from sorghum fields from seven locations in Kenya and Uganda in 2013 (Table 1).

Seed pre-conditioning and culturing

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

Following conditioning, the *Striga* seeds were treated with a sterile germination stimulant (GR 24) to induce germination. The stimulant (five milliliters) was added to each petridish having the pre-conditioned seeds. The seeds were then transferred into the growth chambers (14 hours) to allow for germination before being transferred to solid MS media (Murashige and Skoog 1962). Germination is completed when the radicle protruded through the seed coat.

The seed germination was confirmed by viewing under a stereo microscope. The glass fiber filter papers having the sprouted *Striga* seeds were removed from the Petri dishes and dried for 20 minutes in the laminar flow as the surplus moisture was removed through evaporation. After drying, the seeds were placed to another petridish containing solid MS growth media (Murashige and Skoog 1962) by detaching them off using a soft sterile paint brush. Incubation of the seeds was carried out in darkness at 29 °C for 21 days after which the *Striga* seedlings had enough tissue for DNA extraction.

Table 1. Locations where *Striga* seeds for the study were collected in Kenya and Uganda

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

DNA extraction procedure

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

DNA estimation and quantification

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

Polymerase chain reaction (PCR)

Primers consisting of five pairs of oligonucleotide labeled with fluorescent dye (Table 2) (Applied Biosystems UK) were used to amplify SSRs in PCR reactions containing 5 µl of 5x reaction Buffer with 5 mM dNTPs, 15 mM MgCl₂, forward and reverse primer, 0.3 µl of MyTag DNA polymerase and DNA template in a final volume of 25 µl PCR reaction mixture.

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

Fragment analysis

One microliter of each reaction of the PCR amplification was mixed with 9 µl of HIDi formamide and Liz 500 size standard cocktail (1:12 v/v). In a 96 well sequencer plate, the mixture was vortexed and denatured at 95 °C for 3 minutes and immediately quenched on ice for 5 minutes. Analysis of fragment was carried out by capillary electrophoresis (ABI 3730[®] Applied Biosystems). GeneMapper software version 4.0 (Applied Biosystems) was used for genotypes scoring.

Data analyses

Genetic diversity parameters were calculated using GenAIEx 6.1 (Peakall and Smouse 2012) and PowerMarker 3.0 (Liu and Muse 2006) software. The genetic distance matrix was generated according to Nei (1972), using the PowerMarker software. A Principal Component Analysis (PCA) was run based on genetic distance with data standardization in GenAIEx software to visualize differences between populations. As a supplement to the PCA analysis, a dendrogram was constructed using the unweighted pair group method (UPGMA) for estimating

the genetic similarity based on Nei's genetic distance among the populations.

Comparisons of the pairwise population 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 calculate the genetic differentiation between the populations (F_{ST}) using stepwise mutation. The number of migrants per generation was measured from F_{ST} value using the equation $Nm = [(1-F_{ST})^{-1}] / 4$ to determine the gene flow among the populations.

Assessment of sorghum for their response to *Striga hermonthica* infection

Plant materials

Sorghum seeds were collected from USA, Sudan, Eritrea, Kenya and Uganda (Table 3).

Sorghum seed germination

Sorghum seeds were germinated between blocks of moistened horticultural rockwool (Growdan[®] Vital Growdan, 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.

Table 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')		
SSR 26	Forward	CAACAAAACAATGCGTGGA
	Reverse	GGGTTGATCACTCTGTGCAGT
SSR 43	Forward	CCACTGAAATCACAGGACGA
	Reverse	GTGACCGCCGTTACCATTAG
SSR 53	Forward	GCAACTGAATGGAAGGAAGC
	Reverse	CTCGGCTGATCCATCTGTTT
SSR 58	Forward	ACCGAGTGGTTCAAGATTGC
	Reverse	TGCTTCCAATACCGATCACA
SSR 63	Forward	TTTTGTTGGGGTTTATGTGGA
	Reverse	TGGGAGAAAATGGAATTAA

Table 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 034	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°25'N, 30°20'E

Striga seeds conditioning

The same day sorghum seedlings were transferred to the rhizotrons 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 before 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).

Infection of sorghum seedlings with Striga hermonthica

Ten days after the transfer of sorghum plants to the rhizotrons, Sorghum roots were inoculated with pregerminated *S. hermonthica* seeds (40 mg) from Malava population. The seeds were aligned along the host roots using a fine paint brush. The rhizotrons were 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) twice a day. One plant was utilized for each treatment in three independent experiments.

Determination of attachment of Striga hermonthica radicle on roots of sorghum seedlings

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

Phenotype of resistance

Striga seedlings growing on the roots of each of the infected sorghum plants were harvested 21 days after infection. The harvested *Striga* plants from each host plant were photographed using Sony Cyber-shot (Sony Corporation Minato-ku Tokyo, Japan). The length and number of *Striga* plants on each host plant were calculated from the photographs using image analysis software (ImageJ, Media Cybernetics). Next, the *Striga* seedlings were dried at 48 °C in the oven for two days, and the weigh of dry mass per host plant determined.

Statistical analysis

Statistical analysis of variance (ANOVA) was performed using the version 3 of Statistical Package for the Social Sciences (SPSS) (IBM Corporation). Data for dry biomass, number of *Striga* seedlings growing on the sorghum roots and the length were log transformed to achieve the assumptions of ANOVA. Tukey's Honestly Significant Difference (HSD) test was tested to calculate

the HSD and to establish the different groups. All values ≤ 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Genetic diversity of *Striga hermonthica* ecotypes

Striga seed germination frequency

Striga seeds germinated 18 hours post-introduction of the germination stimulant (GR 24). They showed a radicle protruding from the imbibed seed-coat (Figure 1). Germination frequency of the *S. hermonthica* seeds among the populations ranged from 38.2 % to 42.2 % (Table 4).

Fragment analysis

GeneMapper^(R) software (V 4.0) scoring produced electropherograms showing the number of amplified fragments and their sizes (Figure 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 5). The total number of bands visualized across all the primer combinations after correcting for repeatedness was 38 out of 39 bands. Thus, 97 % of polymorphic bands were observed among the *S. hermonthica*.



Figure 1. In vitro germinated *Striga* seeds 18 hours after introduction of the artificial germination stimulant (GR 24).

Table 4. Germination frequency of *Striga hermonthica* seeds

<i>Striga</i> 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

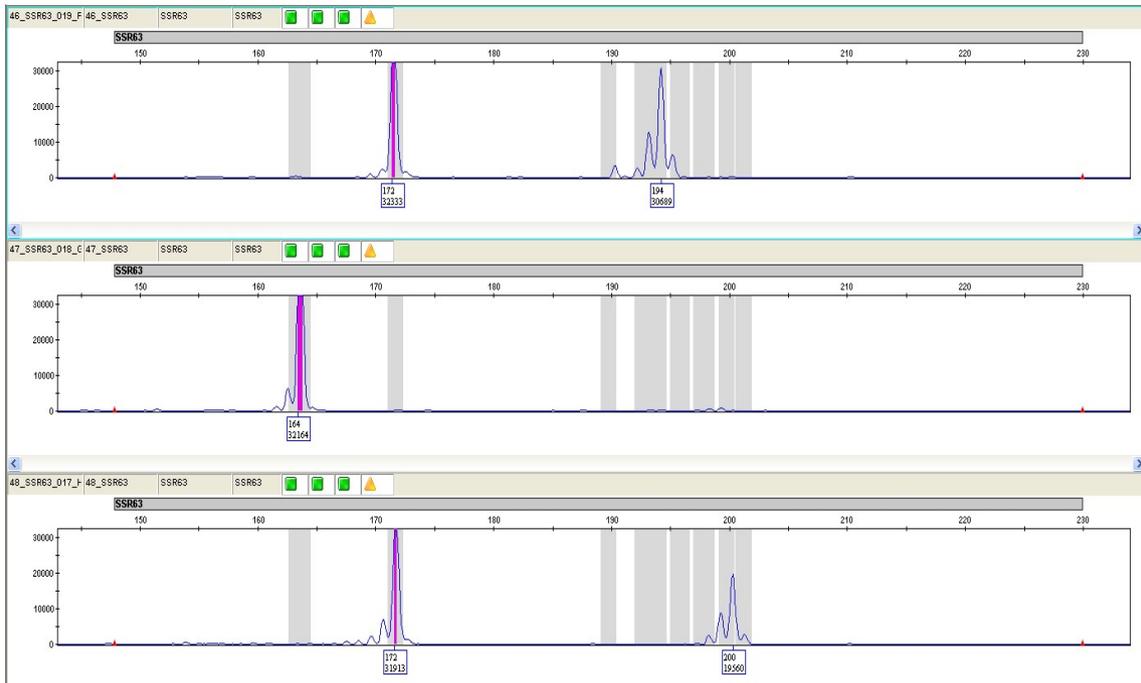


Figure 2. Representative electropherogram generated by the GeneMapper V 4.0 software for samples 17, 18, 19 using marker SSRs 63

Table 5. 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

Note: Ho-observed heterozygosity, He-expected heterozygosity, PIC-polymorphic information content

The average polymorphic information content (PIC) was 0.6445. The lowest PIC value was 0.354 for primer SSR 58. Meanwhile, the highest was 0.890 for primer SSR 26. The markers SSR 26, SSR 63 and SSR 53 gave the highest diversity values of 0.899, 0.757 and 0.752, respectively (Table 5). Average observed heterozygosity (Ho) was 0.529 ranging from 0.376 to 0.729 while average expected heterozygosity (He) was 0.585 (Table 5).

Nei's genetic distance value measured the genetic relationship of seven *Striga* populations that ranged from 0.122 to 0.710, in which, the smaller values indicating a closer relationship (Table 6). The highest similarity (0.122) was observed between the Bugiri and Iganga *Striga* populations. The lowest similarity (most diversified) was found between *Striga* populations Malava from Western Kenya and Mbale from Eastern Uganda revealing high genetic diversity between the two communities.

Table 6. Pairwise population matrix of Nei's genetic distance (1972) for the seven *Striga 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 indicated that the first and second axis explained 28 % and 14 % of the observed variations, respectively (Figure 3). Still, the efficiency of the PCA was low based on the low percentage of the 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 grouped. However, the PCA is not highly supported because axis 1, and 2 are explaining only 42 % of the observed pattern. Meaning that the data should be interpreted with caution since the PCA is not well supported.

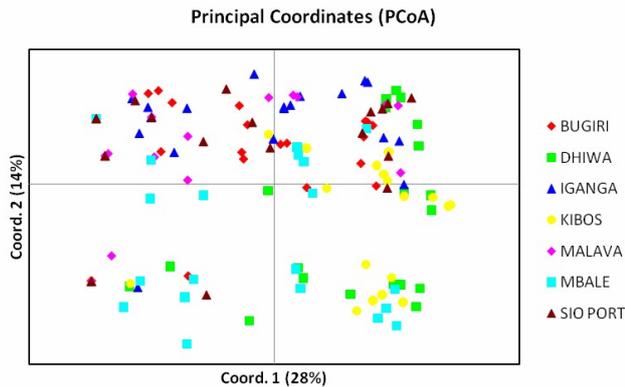


Figure 3. PCA of the genetic distance at the population level illustrating the spreading of the *Striga hermonthica* populations. The axes 1 and 2 clarified 42 % of the variance in the distribution of the populations.

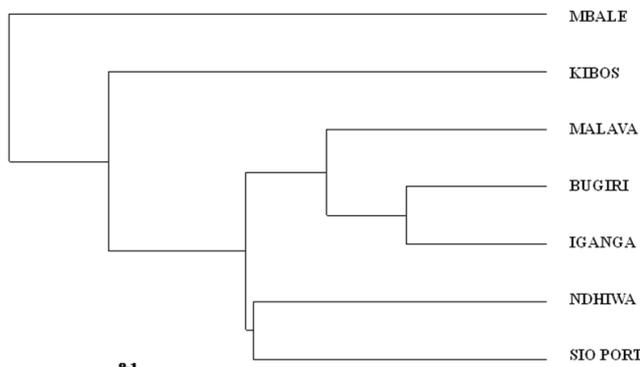


Figure 4. Phylogenetic relationship among the population of *Striga hermonthica*. Shown is a UPGMA dendrogram constructed based on Nei's (1972) genetic distance with PowerMarker 3.25 software.

A dendrogram constructed by the PowerMarker software using Unweighted Pair Group Method of Arithmetic Averages (UPGMA) resulted to 4 distinct clades (Figure 4) with Mbale population forming its 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, suggesting 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 strictly associated with Malava population from Western Kenya. Kibos population from Kenyan formed the 3rd clade which showed a distant relationship with the 1st and 2nd clades (Figure 4).

Analysis of Molecular Variance (GenAlEx 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 rather low (13 %) (Table 7). Genetic differentiation among the populations was shown by the (F_{ST}) value = 0.100. The number of migrants (Nm) determined the gene flow among the populations (Nm = 2.239).

Table 7. Analysis of molecular variance for *Striga 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

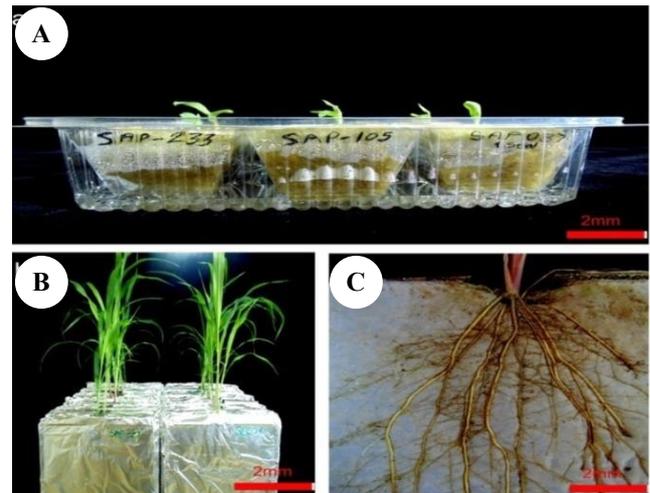


Figure 5. A. Four days old sorghum seedlings germinated on moist rockwool ready for transfer to the rhizotrons. B. Sorghum plantlets growing on the rhizotrons 10 days after transfer. C. Roots of a sorghum plant well spread on the rhizotrons and ready for infection with the *Striga* seeds.

Assessment of sorghum for the response to *Striga hermonthica* infection

Transfer of sorghum seedlings to rhizotrons

Four days old sorghum seedlings were transferred to the rhizotrons after germination of sorghum seeds (Figure 5.A). Ten days after transfer, the sorghum plants had well-developed root system ready for infection with the *Striga* seeds (Figure 5.B-C).

Striga hermonthica attachment and phenotype of resistance

Striga hermonthica adheres to the roots of the susceptible sorghum varieties by the 4th day after inoculation. The strong 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 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 6.D).

Evaluation of susceptibility and resistance

Twenty-one days post-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 7.A-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 7.D-F), an indication that they were not well supported.

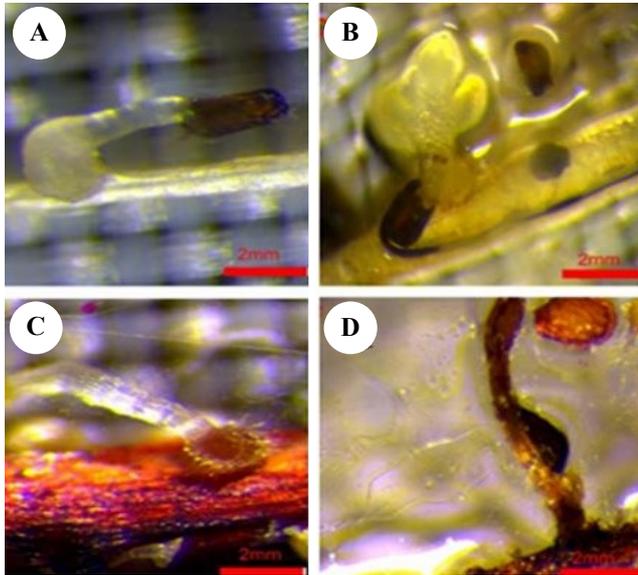


Figure 6. Sorghum varieties being infected by *Striga hermonthica*. A. Radicle attached to the host root and swollen into a tubercle. B. Leaf pre-mordia is emerging. C. The host root is exhibiting severe necrosis at the site of radicle attachment leading to the death of haustoria cells (darkening at the site of attachment). D. Successful attachment of *S. hermonthica* radicle but the haustoria failed to increase in size, and the parasite dies.

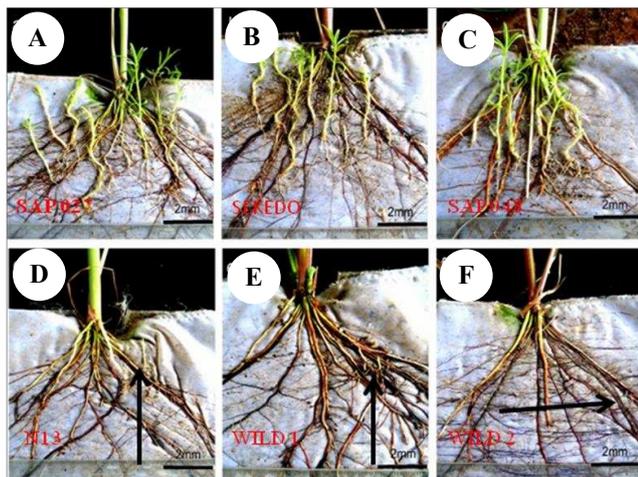


Figure 7. *Striga hermonthica* was growing on roots of selected Sorghum varieties in rhizotrons 21 days post-infection. A. Sap 027, B. Sekedo, C. Sap 048 are sorghum varieties that were significantly susceptible to *S. hermonthica* with the most significant number of *Striga* weeds attachment on their roots. D. N13, E. Wild 1, F. Wild 2 sorghum varieties that were least susceptible to *S. hermonthica* with the smallest number of *Striga* weeds attached that are small in size (indicated by the arrow)

Table 8. Mean number of *Striga hermonthica* plants, dry biomass and length of parasites on each host plant

Variety	No. of <i>Striga</i> plants	<i>Striga</i> dry biomass (g)	<i>Striga</i> 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

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

Evaluation of Striga hermonthica dry biomass, length, and number

Twenty-one days post-infection, average *Striga* length on each of the sorghum plant ranged from 0.267 cm (Wild 1) to 7.967 cm (Sap 027), *Striga* dry biomass varied 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 8).

Generally, Sap 027, Sap 034, Sap 048, Epurpur, SRS 208/1, Sekedo and Ochuti varieties promoted the highest number of the *Striga* plants growing on their roots; the highest *Striga* means dry biomass and the most upper mean length (Table 8). 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 highly significant difference in the means of some *Striga*, *Striga* dry biomass and *Striga* length growing on the roots of sorghum varieties between the susceptible and resistant varieties (P < 0.05) (Table 8).

Discussion

Genetic diversity of Striga 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. *S. 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). Also, the low Fst value (Fst = 0.100) indicates a low level of genetic differentiation among the seven *S. hermonthica* populations, and thus the first hypothesis is accepted.

The aforementioned low levels of differentiation among the *S. hermonthica* populations perhaps due to a recent colonization effect East into Kenya and West into Uganda

and the high level of gene flow ($N_m = 2.239$) among the people. A low number of migrants per generation is considered enough gene flow to obscure or prevent the process of drift that causes populations to differentiate over time (Matt et al. 2011). This high gene flow 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 frequently occurs 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 the 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) associates with *Striga* from Iganga and Bugiri populations (Uganda). The two areas are however most geographically separated within the study area. Therefore, it suggests substantial gene flow between the regions. In the early 1970s and 1980s, there is evidence of active cereal trade between Kakamega and Ugandan people at an infamous market Chebukube. There are high chances that the cereals may have been contaminated with *S. hermonthica* seeds considering that most cereals are threshed on the ground within the contaminated field.

The EST-SSR sequences are excellent choices for genetic markers, which can be applied for molecular diagnosis and for investigating the genetic diversity as well as population structure of *S. hermonthica* (Yoshida et al. 2010). The SSR markers have been successfully employed in studying 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 is resulting in 38 unique bands ranging from 3 to 14 fragments per primer combination with a mean of 7.8 bands per primer. These values demonstrate the effectiveness of the SSR markers to elucidate diversity within the *S. hermonthica* populations. These findings are in line with work reported by Yoshida et al. (2010) where the SSR showed 27 alleles with an average of 2.7 alleles per locus. Genetic divergence analysis revealed an average gene diversity of 0.33 which is considered a moderate level of genetic diversity. Matt et al. (2011) while working on the genetic diversity of *S. hermonthica* in Mali using SSR described genetic diversity ranging from 0.687 to 0.748 whereas Yoshida et al. (2010) described genetic diversity range of 0.375 to 0.625 when working on the genetic diversity of *S. hermonthica* populations in Sudan. Using AFLP markers, Gethi et al. (2005) got shallow genetic distance values falling from 0.007 to 0.025 on the analysis of *S. hermonthica* populations from Kenya. Homogeneity among the Kenyan *S. hermonthica* populations was credited to recent colonization from the Lake Victoria and the allogamous

breeding system of *S. hermonthica*. However, the small genetic diversity in the Kenyan communities (Gethi et al. 2005) may have been due to less sensitive markers used (AFLP markers) as compared to the current study.

Assessment of sorghum for the response to Striga infection

This study demonstrated that there is varied resistance response to *S. hermonthica* by the different sorghum varieties studied and therefore the second hypothesis was rejected. Some varieties showed high resistance responses while others were highly susceptible to the parasite which may be attributed to the existence of genetic variations in the different sorghum lines in response to *S. hermonthica* parasitism (Haussmann et al. 2000a). Nevertheless, complete resistance to *S. hermonthica* has not been identified in cultivated sorghum varieties (Gurney et al. 2002). All the resistant sorghum varieties tested showed some strong parasite attachment on the roots.

Among the sorghum varieties tested, there were those that were readily susceptible to *S. hermonthica* infection and others that showed resistance to the weed. The susceptible sorghum varieties had the highest number of *S. hermonthica* parasites attached on their roots, and the parasites were growing fast and healthy, suggesting that they were getting enough nutrient support from the host. On the contrary, 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 obtain adequate nutrients from their host as is the case with those growing on the susceptible sorghum varieties. The average number, the mean length, and the dry biomass of *S. hermonthica* parasites growing on the roots of each of the sorghum variety were 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, suggests 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 gene pool and other sorghum cultivars showing resistance phenotype to develop elite cultivars. Mapping the resistance alleles will lead to the development of markers that can be used in the marker-assisted selection (MAS). Among the sorghum varieties tested, N13, Asareca AG3, and Asareca W2 also exhibited resistance phenotype to *S. hermonthica*. The result was not surprising since the genomic regions (QTL) associated with stable *S. hermonthica* resistance from the N13 variety were determined (AATF 2011) and selection of the QTL done and introgressed into sorghum varieties Asareca AG3 and Asareca W2 varieties at ICRISAT. The varieties N13, Asareca AG3, and Asareca W2 are known resistant genotypes with superior resistance to *S. hermonthica* as reported in the current study.

Wild 1, Wild 2, N13, Asareca AG3 and Asareca W2 sorghum tested in this study exhibited some resistance responses which included acute necrosis at the site of

attachment, secure attachment but failure in growth and growth of *Striga* radicle away from the host root. Intense disease at the site of attachment resulted in the death of the parasites haustoria cells (marked with darkening at the tip of parasite radicle). The necrotic regions appeared at the site of parasite attachment because of localized cell death of the host tissue. Lane et al. (1993) observed acute necrosis at the site of parasite attachment to cowpea when infected with *S. gesnerioides* parasite. Scholes et al. (2011) showed similar findings in rice genotypes. The growth of *Striga* radicle away from the host root suggests the existence of substances that could inactivate chemical signals that attract parasite radicle to the host root (Fasil et al. 2010).

Susceptible sorghum 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 plants 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 the genetics of resistance to *S. hermonthica* in sorghum (Ahonsi et al. 2004).

The introduction and rapid diffusion of *Striga* resistant varieties is the most appropriate survival strategy in resource-poor, *Striga* prone rural economies where small-scale farmers can't afford the other high-cost control measures against the *Striga* weed. However, as much as the distribution of resistant sorghum cultivars is a cost-effective method of *Striga* management, its use *per se* probably have little advantages if other control strategies are not taken. Those involving the 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 infected 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).

In conclusion, the *S. hermonthica* populations from Western Kenya and Eastern Uganda have low genetic differentiation, and therefore the first hypothesis is accepted. 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 significantly resistant to the weed and therefore the second hypothesis is rejected.

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