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# DETERMINATION OF POST *Striga* ATTACHMENT RESISTANCE IN SELECTED RESISTANT SORGHUM LINES IN KENYA

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**AUTHORS' CONTRIBUTIONS**
This work was carried out in collaboration between both authors. Author DWM designed the study, wrote the protocol and interpreted the data. Author FMN managed the literature searches and produced the initial draft. Both authors read and approved the final manuscript.

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## ABSTRACT

Breeding for *Striga* resistance in sorghum and other cereals is recognized as the most sustainable control measure, however there is a lack of cereal germplasm that exhibit post attachment to *Striga* that limits this noble approach. This study evaluated post attachment resistance levels of four *Striga* resistant sorghum (SRS) lines against four ecotypes of *Striga* from Kenya and Tanzania. Sorghum seeds were grown in rhizotrons (root observation chambers) and the seedlings were inoculated with pre-germinated *Striga* seeds and on emergence the attached parasites were harvested from the roots of sorghum and scored for the number of attachments, length and dry biomass. There was a significant difference in the biomass and average length of attached *Striga* seedlings among the three Kenyan ecotypes on all sorghum lines. The phenotype of a resistance mechanism was characterized by the inability of the parasite to penetrate host endodermis, necrosis and the browning and death of attached *Striga* seedlings. SRS 1208/2 had very high post- attachment resistance to the *S. hermonthica* ecotypes used in this study, SRS 2408 and SRS 2208 exhibited intermediate resistance while SRS 3308/5 had low resistance. The difference in biomass, number and length of attached *Striga* seedlings upon infection clearly indicated genetic variability for *Striga* resistance in the selected lines. Among the four sorghum lines studied, cultivar SRS 1208/2 was the most promising source of resistance to obligate root parasite *S. hermonthica* and can be recommended for future use in sorghum breeding programs in East Africa.

**Keywords:** *Striga*; ecotypes; resistance; selection; rhizotron.

## 1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most important cereal crop worldwide after wheat, rice, maize and barley with an annual production of 61 million tonnes over the past decade [1,2]. It is ranked second, after maize as the most important cereal crop in East Africa. The crop provides food security and is becoming a suitable alternative in many places where maize crop fails [1]. Sorghum is unique due to its tolerance to drought, waterlogging, saline or infertile soils and high temperature [3]. Sorghum is cultivated worldwide for fodder, grain and syrup production. Notable factors limiting production of sorghum in tropical Africa include poor climatic conditions, low soil nutrients, insect pests and

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weeds [3]. Important among the weeds is the parasitic weed *Striga* (*Striga hermonthica* Del. Benth) whose estimated yield losses in infested sorghum range from 40-100% [4]. According to Debrah [5], the parasitic weed purple witchweed is a major pest of cereals in African semiarid regions. Production of Sorghum (*Sorghum bicolor* L. Moench) has been greatly reduced by the parasitic weed *Striga hermonthica* (Del.) Benth. The intricate biological association between this hemi parasite and its sorghum host makes it difficult to control. To achieve sustainable sorghum production there is need to solve the *Striga* problem. Controlling *Striga* is an enormous task considering the high seed production rate of 10000-100000 seeds per plant which can remain viable for 14 years under field conditions. The dying-off process observed by Gbèhounou et al. [6] contradicts the common opinion on longevity of Striga seeds in their natural environment. "Wet dormancy" was not observed in the course of the study. Several methods have been recommended for the control of *Striga* including; cultural, biological, chemical and use of tolerant varieties.

Resistance based on the hypersensitive reaction (HR) involves localised necrosis of host tissues surrounding the site of attempted parasite attachment, presumably coupled with a release of phytoalexins that kill the attached *Striga*. Hypersensitive response has been observed in sorghum cultivars Dobbs, Framida, Serena and wild accessions *S. bicolor* subspecies *drummondii*, *S. hewisonni* and *S. b. verticilliflorum* [7]. In the incompatible response (IR) mechanism parasite development beyond attachment is discouraged. In host genotypes whose *Striga* resistance is based on IR, *Striga* seedlings that succeed in penetrating host tissue may not develop beyond emergence of the first leaves [8]. Some *Striga* will be observed to develop normally at first but later show signs of stunted growth [3]. The reaction is similar to that observed when *Striga* unsuccessfully infests non-host plants. This study investigated the existence of post- attachment stage resistance (host root penetration) on selected field resistant sorghum lines.

## 2. MATERIALS AND METHODS

Sorghum seeds used in this study comprised four lines (SRS1208/2, SRS3308/5, SRS2408 and SRS2208). These four were picked from nine sorghum lines that were developed and selected for field resistance to *S. hermonthica* in Uganda [9]. They were developed by crossing four *Striga* resistant sorghum lines (Brhan, N13, SRN39 and Framida) with four locally adapted and high yielding sorghum lines (Sekedo, Hakika, Dobbs and Karimtama) according to the North Carolina II mating design [10]. The parent lines were crossed as follows: Brhan x Dobbs (SRS3108), Brhan x Karimtama (SRS4609), N13 x Sekedo (SRS609), N13 x Dobbs (SRS1708), N13 x Karimtama (SR3108), SRN39 x Sekedo (SRS3408), SRN39 x Hakika (SRS2408), Framida x Hakika (SRS1208) and Brhan x Hakika (SRS2208). The seeds were obtained from the National Semi-Arid Resources Research Institute, Serere, Uganda.

The highly resistant parental line Brhan and the commercially available cultivar CSH-1 previously shown to be susceptible to infection by *Striga* species [11,12,13], were used as controls. The CSH-1 was obtained from the animal and plant sciences laboratory of University of Sheffield, UK courtesy of Prof. Julie Scholes. The *Striga* seeds used in this study consisted of four *S. hermonthica* ecotypes, three *Striga* ecotypes were harvested in sorghum and maize infested fields in Kenya, courtesy of Dr. Steven Runo.

### 2.1 Growth and Infection of Sorghum Plants

Sorghum seeds were germinated between two sheets of moistened glass fibre filter paper (GF/A Whatman, BDH, Poole, UK) supported by a block of moistened horticultural Rockwool (Aquaculture, Sheffield, UK). These were placed on potting trays and kept in the dark in a temperature controlled chamber, at 30ºC for two days to stimulate even germination. The seedlings were then transferred to the controlled environment growth room for another four days. The growth rooms operated with a 12 hour photo period and a photon flux density of 800 µmol $m^{-2}$ $s^{-1}$. Day: night temperatures were maintained at 27:20°C and day: night humidity was maintained at 50:70%. After 7 days, a single sorghum seedling was transferred to a root observation chamber (rhizotron). A rhizotron consisted of a 22 cm x 22 cm x 2 $cm^3$ petri dish filled with vermiculite into which a mesh was placed (100 µm polyester, Plastic Group, Birkenhead, UK). The roots of the sorghum grew down the mesh and openings at the top and bottom allowed for shoot growth and root growth respectively while a block of rock wool at the base aided in drainage.

Diagrams of a rhizotron experimental set-up are given in Figs. 1 and 2 respectively. The rhizotrons were wrapped in aluminium foil to prevent light from reaching the roots. The rhizotrons were drip fed with 40% (v/v) Long Ashton solution containing 1 mol $m^{-3}$ ammonium nitrate [14] at four hour intervals during each photoperiod to give a total volume of 200 ml $d^{-1}$. Five replicate plants for each sorghum line were established for each treatment in four independent experiments. Rhizotrons were placed in a completely randomized design.

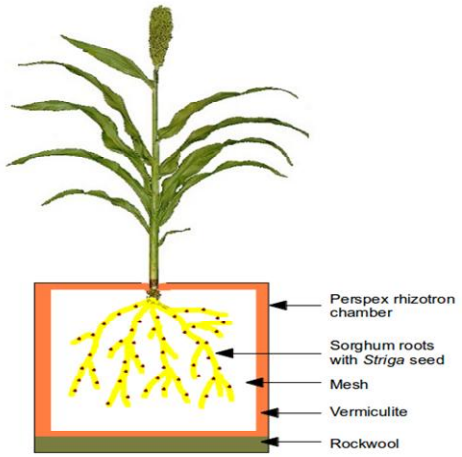


**Fig. 1. Rhizotron setup used in the post-attachment sorghum resistance assays. Once the chamber lid was affixed, the rhizotron was wrapped in aluminium foil to keep the root system in the dark [15]**

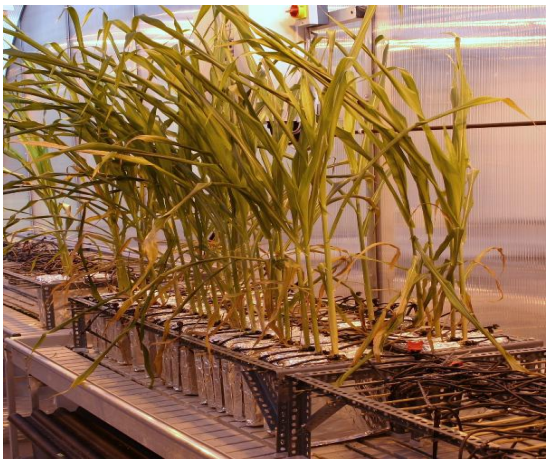

**Fig. 2. A typical rhizotron set- up in a growth room**

## 2.2 Post Attachment Resistance Assays

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

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

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

## 3. RESULTS AND DISCUSSION

Transverse sections through the sorghum roots at the site of haustorial attachment on the 3$^{rd}$ day showed that the parasite had penetrated through the cortex in all the studied sorghum cultivars (Fig. 3). However, at 10 DAI the phenotype of resistance varied between the SRS lines and Brhan. The SRS lines exhibited a compatible interaction whereby the parasite had penetrated the endodermis and begun to form connections with the host xylem (Fig. 3). Eventually, the parasite haustorium formed few xylem-xylem connections with the host. Haustorium growing on the SRS lines showed poor tissue differentiation and the resulting parasites grew slowly and small except in the interaction between *S. hermonthica* Mbita ecotype and SRS 3308/5 (Fig. 3). In the compatible interaction (3308/5 ), *S. hermonthica* parasites penetrated through the cortex and endodermis and by day ten the haustorium was well developed showing three defined regions, a densely stained hyaline body (Hb), the vascular core (Vc) consisting of the xylem vessels and the endophyte that penetrated the host root cortex and endodermis (En) (Fig. 3). In the incompatible interaction (Brhan) the parasite penetrated the host cortex but was unable to traverse the endodermis and form a connection with the xylem vessels of the host (Fig. 3). The parasite grew around the host endodermis and the haustorium failed to differentiate, the parasite vessel cells were not observed in the haustorium and hyaline body was reduced in size at 10 DAI (Fig. 3).

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

The difference in growth rate and size of the parasite may be due to differences in the ability of the host to supply nutrients to the parasites [16], but it may be more likely to reflect genotypic differences in the susceptibility/resistance of the different cultivars.

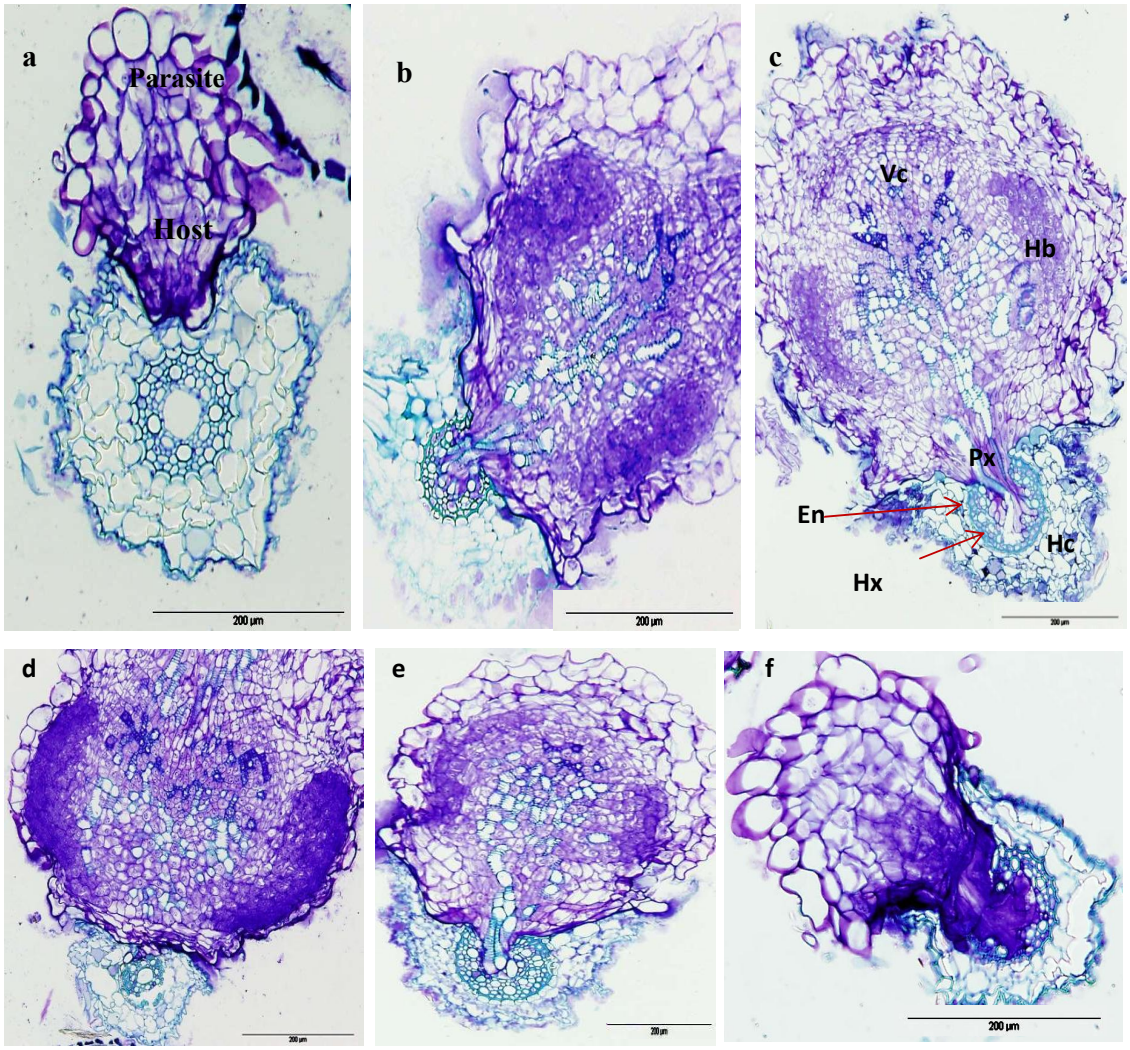


**Fig. 3. Light micrographs of transverse sections of *Striga hermonthica* haustoria penetrating sorghum roots. Technovit embedded tissues were cross sectioned at 3DAI (a) and 10 DAI (b, c, d, e and f). Structures (b, c, e, and f) represent compatible interactions with SRS 1208/2, 3308/5 SRS CSH-1 and SRS 2208, respectively while (d) represent incompatible interaction (Brhan). En, endodermis; Hb, hyaline body; Px, parasite xylem; Hx, host xylem Vc, vascular core. Bar, 200 µm**

**Table 1. Biomass, number and mean lengths of *S. hermonthica* (four ecotypes) attached to the roots of all four SRS Sorghum cultivars and the two controls, Brhan (resistant) and CSH-1 (susceptible) at 21 DAI**

| *Striga* eco type sorghum line | SH Mbita | | | SH Kibos | | | SH Alupe | | | SH Tanga | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Striga* biomass (mg) | Number of attachments | Length of *S. hermonthica* (mm) | *Striga* biomass (mg) | Number of attachments | Length of *S. hermonthica* (mm) | *Striga* biomass (mg) | Number of attachments | Length of *S. hermonthica* (mm) | *Striga* biomass (mg) | Number of attachments | Length of *S. hermonthica* (mm) |
| Brhan | 5.24$^{a}$ | 34.8$^{a}$ | 4.03$^{a}$ | 7.3$^{a}$ | 43.4$^{a}$ | 4.4$^{a}$ | 2.5$^{a}$ | 14.8$^{a}$ | 3.6$^{a}$ | 1.1$^{a}$ | 5.6$^{a}$ | 2.5$^{a}$ |
| 1208/2 | 3.9$^{a}$ | 27.2$^{a}$ | 2.13$^{b}$ | 11.0$^{b}$ | 53.4$^{a}$ | 4.8$^{a}$ | 2.1$^{b}$ | 20.6$^{a}$ | 2.9$^{a}$ | 1.0$^{a}$ | 5.8$^{a}$ | 2.3$^{a}$ |
| 2408 | 16.2$^{b}$ | 62.3$^{a}$ | 5.18$^{c}$ | 12.1$^{b}$ | 58.8$^{a}$ | 6.9$^{b}$ | 2.8$^{a}$ | 29.5$^{a}$ | 2.9$^{a}$ | 3.2$^{a}$ | 13.6$^{b}$ | 6.2$^{a}$ |
| 2208 | 8.9$^{c}$ | 57.8$^{a}$ | 3.7$^{a}$ | 11.7$^{b}$ | 53.8$^{a}$ | 4.6$^{a}$ | 11.5$^{c}$ | 46.5$^{a}$ | 4.5$^{a}$ | 1.5$^{a}$ | 10.0$^{b}$ | 2.5$^{a}$ |
| 3308/5 | 8.8$^{c}$ | 42.2$^{a}$ | 3.9$^{a}$ | 15.6$^{c}$ | 60.4$^{a}$ | 3.9$^{a}$ | 14.2$^{d}$ | 41.0$^{a}$ | 4.0$^{a}$ | 8.4$^{b}$ | 36.0$^{b}$ | 3.8$^{a}$ |
| CSH-1 | 29.5$^{d}$ | 80.3$^{a}$ | 7.8$^{d}$ | 21.1$^{d}$ | 85.2$^{a}$ | 5.9$^{c}$ | 18.8$^{e}$ | 41.2$^{a}$ | 8.3$^{b}$ | 12.3$^{b}$ | 43.4$^{c}$ | 6.0$^{a}$ |
| MEAN | 10.7 | 47.8 | 4.2 | 13.2 | 61.7 | 5.0 | 8.78 | 29.1 | 4.3 | 4.64 | 18.96 | 4.08 |
| P value | .000 | .193 | .000 | .000 | .173 | .001 | .000 | .074 | .000 | .00 | .005 | .246 |

*Means within a column not followed by the same upper case letter show significant difference (Tukey's HSD) at 5% probability level*

### 3.1 Multiple Resistance

SRS 1208/2 had the lowest biomass of attached *Striga* at 21 DAI in three ecotypes of *Striga* i.e Mbita (3.9 mg), Alupe (2.1mg) and Tanga (1.0 mg). Brhan however had lower biomass of attached *Striga* from Kibos. There was significant difference (P= .000) in the biomass of attached *Striga* in the roots of all sorghum lines. There was no significant difference (P≥ .05) in the number of attached *Striga* seedlings in the roots of all the sorghum lines. SRS1208/2 had the lowest average length of attached *Striga* (2.83 mm). There was a significant difference (P< .05) in the average lengths of attached *Striga* from Kenya (Table 1).

The three different post-attachment parameters (biomass, length and number) used in this study may reflect different underlying aspects of the host-parasite interaction. Rhizotron studies of *Striga*-host interactions have used a variety of scoring methods that have either categorized attachments into different parasite developmental stages, and have interpreted these directly and/or analysed derivatives of these [16,17,18], or have used parasite length and/or dry weight [16] and [19]. Different cellular and molecular interactions may lie behind different phenotypic measures of a parasite's success on its host. For example, in rhizotrons, the number of attachments on a host may be high, but individual parasites may not develop to any great size [19]. Furthermore these studies found that using *Striga* total dry weight as the response metric in a quantitative genetic study of rice resistance led to clearer identification of QTL than when using the number of *Striga* attachments.

## 4. CONCLUSION AND RECOMMENDATION

SRS 1208/2 had very high post- attachment resistance to the *S. hermonthica* ecotypes used in this study, SRS 2408 and SRS 2208 exhibited intermediate resistance while SRS 3308/5 had low resistance. The difference in biomass, number and length of attached *Striga* seedlings upon infection with the different *Striga* ecotypes clearly indicate genetic variability for *Striga* resistance in the selected lines. Similarly, the results reported here indicate that parasite length and total dry weight provide a greater separation in parasite resistance between the different hosts than the number of attached parasites, although all three responses differentiated hosts to some extent; this suggests the choice of a response metric is important when reaching conclusions about host-parasite interactions. Among the four sorghum lines studied, cultivar SRS 1208/2 was the most promising source of resistance to obligate root parasite *S. hermonthica* and can be recommended for future use in sorghum breeding programs in East Africa. Another phenotype of resistance observed was in the interaction between the SRS lines and SH-Tanga, the parasites that had elicited a resistant response were turning brown, dying and the host root showed intense necrosis at the point of attachment.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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