

## EVALUATION OF SORGHUM GENOTYPES FOR *Striga* (*S. hermonthica*) TOLERANCE

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### ABSTRACT

*Striga* infestation and transmission, and the adverse impact of *Striga* on crop yield can essentially be diminished through selection of resistant genotypes. The study was carried out to screen sorghum genotypes for *Striga hermonthica* (*Striga*) tolerance based on their physiological responses to the parasitic effects of this weed. Seventy-five grain sorghum [*Sorghum bicolor* (L.) Moench] genotypes were subjected to three-levels (0 mg, 2.5 mg and 5 mg/pot) of *Striga* at Rattary Arnold Research Institute in Zimbabwe. One way analysis of variance results show that there were significant difference among genotypes on plant height, chlorophyll content, leaf number, field weight, grain weight, panicle height for *Striga*\*genotype interaction for all the 75 sorghum genotypes ( $P<0.001$ ). The correlation matrix show that fresh panicle weight and grain dry weight showed a very high correlation (0.948) at  $P<0.05$ . Panicle height and fresh panicle weight are highly correlated (0.736) at  $P<0.05$ . Similarly, panicle height and grain dry weight were also highly correlated (0.718) at  $P<0.05$ . The heatmap analysis shows that 45%, 31% and 24% of the cultivars exhibited high, medium and low plant heights, respectively. Chlorophyll content showed that 80% and 20% of the cultivars showed medium and low amounts, respectively. All the 75 genotypes recorded low leaf numbers when compared to the control experiment. It was observed that 25%, 63% and 12% for the genotypes produced high, medium and low fresh panicle weight and dry grain weight values, respectively. The panicle size for most (87%) of the genotypes was medium while 13% of the cultivars showed very small sized. Principle component analysis using the scree plot Eigen values shows that the first factor contributes 58% of the cumulative variation. Two principal axes (F1 and F2) were selected, which explained about 74.45% of the total variation. Neighbour-joining hierarchical clustering analysis led to the formation of five groups for *Striga*\*genotype interaction. It can be concluded that *Striga hermonthica* affects negatively crop morpho-physiological aspects such as plant height, chlorophyll content and leaf number as well as yield determining components such as field weight, panicle height which ultimately reduce the yield of sorghum. The existence of high variability in the response to *Striga hermonthica* infestation in the 75 sorghum genotypes gives the possibility to breeding interventions to improve tolerance to this parasitic weed.

**Keywords:** agronomic traits, resistance, infestation, parasitic weed, witch weed.

### INTRODUCTION

The use of *Striga*-resistant/tolerant sorghum cultivars can have little useful effects if soil nutrients are very low. These cultivars ought to be a main element to be included in breeding programs. Future research efforts must be directed in the direction of understanding host resistance mechanisms, improvement of field screening and infestation techniques, and improvement of strong high yielding *Striga* resistant varieties which might be applicable to farmers.

In Ethiopia, it was discovered that some sorghum genotypes support considerably fewer *Striga* plants and give higher grain

yield than others (Bejiga, 2019). On the other hand, some cultivars showed considerably higher yield reductions than others under the same level of infestation. Some are highly susceptible and would not give yield at all. The presence of this wide range of variability in *Striga* resistance and tolerance traits among sorghum genotypes suggests an opportunity to develop high yielding and resistant/tolerant genotypes through hybridization or genetic engineering to improve on the resistance traits (Bejiga, 2019).

Similarly in Nigeria, negative correlations were observed between *Striga* and height of infected Sorghum (-0.371), as well as between number of *Striga* and number of

Sorghum plants (-0.818) (Akomolafe et al., 2018). All these findings point to the need to develop breeding strategies to increase cultivar tolerance to *Striga* attack. *Striga* is additionally notoriously recognized as witchweed causing a yield loss of up to a 100% (Kountche et al., 2019). It has been reported that more than 60% of arable land under development in sub-Saharan Africa (SSA) is pervaded with at least one types of *Striga*, which influences more than three hundred million farmers in more than twenty five nations resulting in a yield loss of more than seven billion dollars (Kountche et al., 2019).

Three types of *Striga* are especially ruinous - *S. hermonthica* (Del.) Benth, also *S. asiatica* (L.) Kuntze, which assault oats, and *S. gesnerioides* (Willd.) Vatke, which is parasitic to cowpea plants. *Striga* infestation and transmission, and the adverse impact of *Striga* on crop yield can essentially be diminished through selection of resistant genotypes. Above ground, germinated *Striga* number is a solid measure for tolerance. *Striga* inflorescence dry weight can be utilised to recognize varieties that decrease *Striga* multiplication. The most extreme relative yield loss is an appropriate choice measure for resilience in susceptible genotypes, while for progressively tolerant genotypes, the relative yield loss per *Striga* contamination appears to be progressively suitable.

For these resistance measures, yield appraisal of close by uninfected controls is fundamental. Chlorophyll fluorescence, all the more exactly photochemical extinguishing and electron transport rate, may empower screening for resistance without this necessity. A two-fold pot system to screen an enormous assortment of sorghum genotypes for low germination stimulant-based protection from *Striga* was developed. In this approach, seven day-old sorghum seedlings were developed in sterile quartz sand in a pot with a punctured base, which was set in another pot without apertures to gather root exudates (Ennami et al., 2020).

An aliquot of the root exudate was applied to precondition *Striga* seeds to evaluate its germination-instigating action. Utilization of

the two-fold pot and other comparative methods brought about the distinguishing proof of a few low-stimulant genotypes. Similarly, pot trials have been used in this research to evaluate tolerance to *Striga* (*Striga aciata*) for 75 sorghum cultivars. *Striga* germination stimulants, strigolactones, in the root exudates of 36 sorghum genotypes were analysed and assessed for *Striga* germination and infection in Sudan. The study shows that the strigolactone profile in the root exudate of sorghum has a large impact on the level of *Striga* infection. High concentrations of 5-deoxystrigol result in high infection, while high concentrations of orobanchol result in low infection (Mohemed et al., 2018).

This knowledge should help to optimize the use of low germination stimulant-based resistance to *Striga* by the selection of sorghum genotypes with strigolactone profiles that favour normal growth and development, but reduce the risk of *Striga* infection or genetic manipulation of related genes responsible for the production 5-deoxystrigol (Mohemed et al., 2018). The aim of this research was to identify high *Striga acaita* stimulated sorghum lines and cultivars to be used to for genetic engineering process using Crispr Cas9 technology to confer resistance to these lines. The specific objectives of this study was to evaluate the effects of *Striga hermonthica* on selected sorghum cultivars based on growth parameters.

## MATERIAL AND METHODS

### *Site description*

The pot trials were carried out at Rattary Arnold research station (RARS) in Harare, Zimbabwe in December 2020-April 2021. The site is located 30 km north east of Harare central business district along Shamva road, with coordinates 17°40'06"531°12'55"E. The station falls under ecological region II b with an altitude of 1354 masl, mean annual temperature range of 16-19°C and mean annual rainfall of 800 mm. The soil type are red clay soils.

***Planting material***

*Striga hermonthica* seeds were collected in 2019 from parasitized sorghum plants in Rushinga, Mt Darwin, Mashonaland West province, Zimbabwe. Sorghum seeds were stored in a black plastic bag in the dark at room temperature until in use. Seventy-five sorghum genotypes were used for this experiment. These included fifty genotypes from Seedco private limited Zimbabwe and 25 grain sorghum genotypes from South Africa Agricultural Research council.

Sorghum seeds were surface sterilized by immersion for 2 min in 70% ethanol followed by 2 min in 5% sodium hypochlorite solution containing 0.02% (v/v) Tween20 and rinsed three times in sterilized distilled water.

***Planting procedure***

The experiment was conducted in pots to investigate the effects of three levels (0 mg, 2.5 mg and 5 mg) of *Striga hermonthica* on different sorghum genotypes growth parameters. Disinfected sorghum seeds (3/pot) were planted in plastic pots (40 cm in diameter) filled with ferruginous soil collected from *S. hermonthica* free area and immediately irrigated. In each pot, 15 g of compound D (7:14:7) fertiliser was applied and thoroughly mixed with the soil in the pot. *S. hermonthica* inoculation was achieved by mixing 2.5 and 5 mg of *S. hermonthica* seeds in the top 6 cm soil in each pot, respectively. The pots were watered every day to prevent moisture deficit. After emergence, the plants were thinned to two plants per pot. Weeds other than *S. hermonthica* were regularly handpicked.

***Experimental design***

Pots were laid out in a split plot design with three *Striga hermonthica* levels (0, 2.5 and 5 mg/pot) as the main factor and 75 cultivars (sub factor) and with two replications.

***Measurements***

Data were collected from each pot from both plants. *Striga hermonthica* un-infested

control was included for comparison purposes. Chlorophyll content was measured starting from week three after emergence to week 10 after emergence using a photometer. The MC-100 chlorometer was used to directly measure and display chlorophyll concentration from intact leaf samples without damaging the plant material. The meter was calibrated to measure chlorophyll concentration with units of  $\mu\text{mol}$  of chlorophyll per  $\text{m}^2$ .

Sorghum height and leaf number were measured at 4, 5, 6, 7, 8, 9 and 10 weeks after sowing (WAS). Sorghum panicle fresh weight, panicle length and grain dry weight were also measured.

***Data analysis***

All data were subjected to descriptive statistics and analysis of variance (ANOVA) using computer program SAS (XLSTAT 2020.5.1.1046). For the statistical analysis, one-way analysis of variance (ANOVA) was used, followed by Least significant differences of means (5% level). Principal component analysis (PCA) of agronomic traits was conducted, Pearson's correlation coefficient for all variables was also determined, heatmap analysis was carried out and Agglomerative hierarchical clustering (AHC) was also conducted using the Ward method.

**RESULTS AND DISCUSSION*****Analysis of Variance***

A one way analysis of variance was carried out to investigate the effects of three levels of *Striga hermonthica* (0 mg, 2.5 mg and 5 mg) on 75 sorghum genotypes. The results show that there were significant difference ( $P < 0.001$ ) for *Striga*\*cultivar interaction for plant height, chlorophyll content, number of leaves, fresh weight, grain dry weight and panicle height for the sorghum genotypes (Table 1).

Table 1. Summary of ANOVA of sorghum genotypes agronomic attributes to *Striga*

Source	DF	Plant height	Chlorophyll	Leaf number	Fresh weight	Grain dry weight	Panicle height
<i>Striga</i>	2	0.008	<0.001	0.065	<0.001	<0.001	0.011
Cultivar	74	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>Striga</i> x Cultivar	148	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Error	219	4.947	1.961	0.5355	2.406	2.531	1.0452

Considering plant height, some sorghum genotypes are inherently short while others are tall. However, in this research, *Striga hermonthica* reduced significantly the plant heights of susceptible sorghum genotypes which later affected the other agronomic attributes of those susceptible ones. Chlorophyll changes were noted even before the *Striga* had started emerging above ground. This indicates that *Striga* damage occurs prior to *Striga* emergence above ground. The number of leaves varied significantly with each response of different lines and cultivars to *Striga hermonthica* infestation. Some of the lines and cultivars developed shorter than normal leaves at the same time showing delayed growth as evidenced by reduced plant height and fewer leaves compared to the control experiment. The fresh weight reduced drastically for those genotypes which are highly susceptible to *Striga*.

Contrastingly, the genotypes which exhibit some level of tolerance did not show very huge decreases in fresh panicle weight at

harvesting. The panicle heights varied in most of the highly susceptible lines and cultivars. They exhibited short, prematurely terminated panicle development and in some cases failed to even produce the panicles. The dry weight of the grains varied significantly amongst the genotypes. The susceptible genotypes produced fewer, smaller and light weight grains while the tolerant ones produce well matured grains. This resulted in the difference in the grain weight among the genotypes.

#### *Pearson's correlation matrix*

The correlation matrix shows that all the variables had positive correlation for all the parameters that were measured. A reduction in the plant height, chlorophyll content and leaf number had a negative impact on the yield components of sorghum genotypes. However, the yield determining components showed that they were highly correlated. Fresh panicle weight and grain dry weight showed a very high correlation (0.948) at  $P < 0.05$  (Table 2).

Table 2. Summary of correlation matrix of sorghum genotypes agronomic attributes to *Striga hermonthica*

Variables	Height	Chlorophyll	Number of leaves	Fresh panicle weight	Grain dry weight	Panicle height
Height	1					
Chlorophyll	0.404	1				
Number of leaves	0.446	0.407	1			
Fresh panicle weight	0.469	0.475	0.353	1		
Grain dry weight	0.429	0.475	0.355	0.948**	1	
Panicle height	0.404	0.403	0.371	0.736**	0.718**	1

Values in bold are different from 0 with a significance level  $\alpha = 0.05$ , \*\* shows very high correlation.

The highly susceptible varieties failed to produce good panicle and in some instances did not even form. A reduction in the panicle size translated to a decrease in the grain weight and vice versa was also true. Panicle height and fresh panicle weight are highly

correlated (0.736) at  $P < 0.05$ . The panicles which were short failed to produce grain and in some instance produced fewer grain which made them to be lighter in weight. The well-developed panicles produced good panicle height and grain filled properly allowing an

increase in the fresh grain weight for the tolerant genotypes. Panicle height and grain dry weight were also highly correlated (0.718) at  $P < 0.05$ .

**Heatmap analysis for agronomic components**

Plant growth characteristics were measured and results are as shown in Figure 1. Results from the heatmap show that for plant height 36% of the plants were severely affected by *Striga* at 0.5 mg application rate, 12% were severely affected at 2.5 mg of *Striga* while it is clear that only 4% of the sorghum genotypes have inherently short plant heights. It was also observed that

85% of the genotypes were severely affected in terms of chlorophyll content when 5 mg of *Striga* were applied, 73% were severely affected with 2.5 mg of *Striga* applied. However, it was noted that 37% of the genotypes have inherently low chlorophyll content as well. The number of leaves was counted and it was observed that 44% of the genotypes showed severe reduction in number of leaves when 5 mg of *Striga* was applied while 36% of the genotypes exhibited a reduction in number of leaves at 2.5 mg of *Striga*. It was observed that 4% of the genotypes have few number of leaves even in the absence of *Striga* damage.

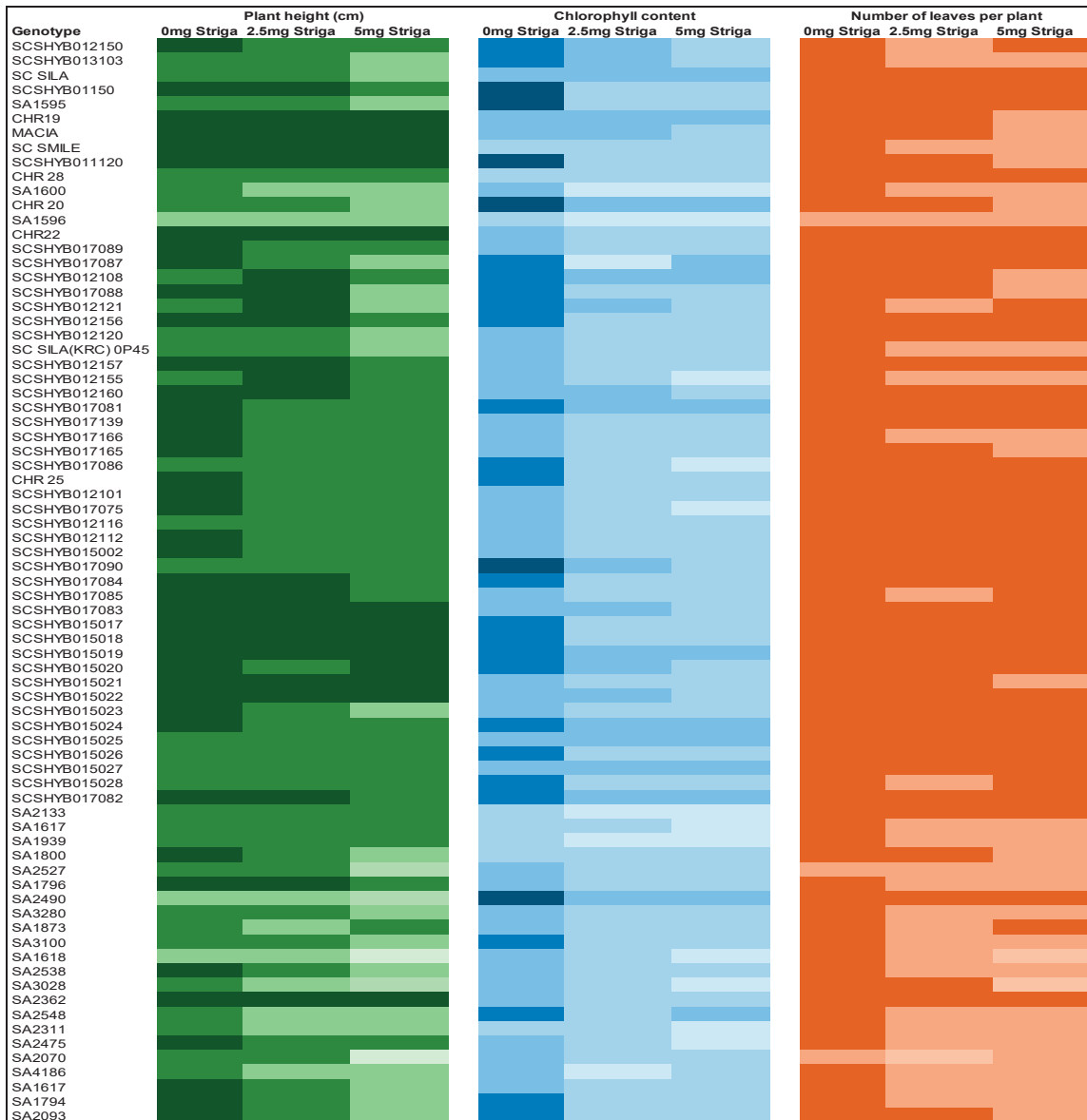


Figure 1. Heatmap analysis for plant height, chlorophyll content and number of leaves per plant for 75 sorghum genotypes exposed to *Striga*



### Principle component analysis

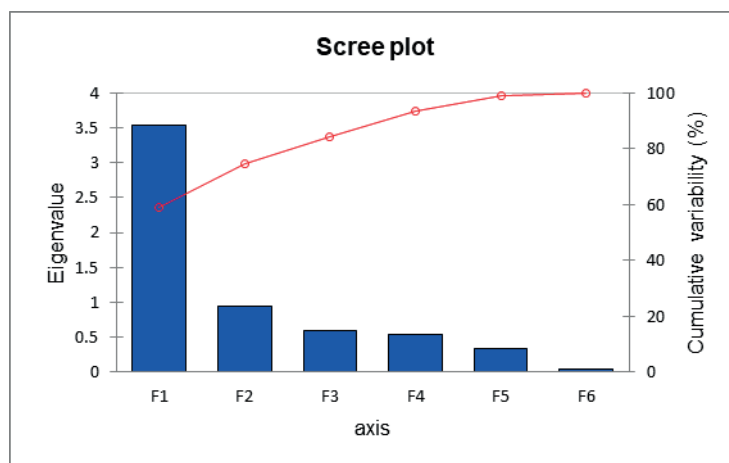


Figure 3. Scree plot for eigenvalues for sorghum physiological and yield components

The first factor contributes 58% of the cumulative variation. This shows how the *Striga hermonthica* infestation contributes highly to the total variability accounted for in this research. F2, F3 and F4 contributed 16%, 10% and 9%, respectively. It can be seen that 94% of the total variation is coming from

these four factors. Cultivar difference also plays a very critical role in explaining the difference in their ability to tolerate *Striga* damage. It is therefore very important to come up with breeding strategies which will leverage on the natural defence mechanisms to reduce the effects of *Striga hermonthica*.

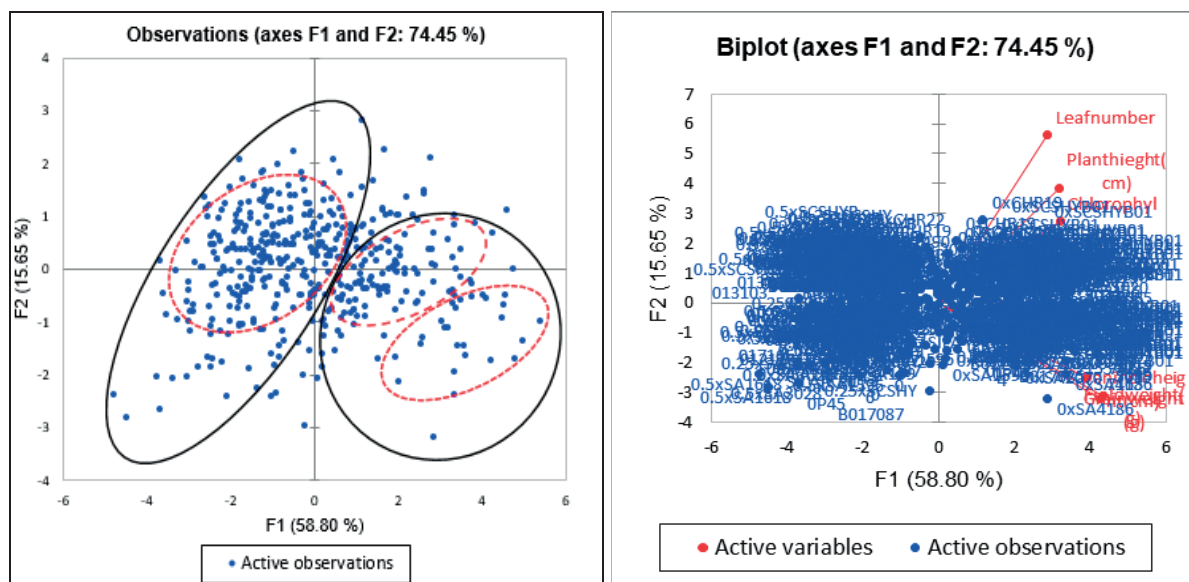


Figure 4. Active observations and box plot for sorghum agronomic attributes of 75 lines and cultivars

Two principal axes (F1 and F2) were selected, which explained about 74.45% of the total variation (measured by the inertia). The contribution of variables to the first principal axis (F1) (also shown on the principal plane spanned by F1 and F2 in Figure 3) is due mostly to the fresh panicle

weight, dry grain weight and panicle height. The second principal axis (F2) mainly contributes plant height, chlorophyll content and leaf number. The findings from the PCA analysis computed with the complete dataset it show that a clear clustering along the first component (describing 58.80% of the total

variance) based on cultivar tolerance to *Striga hermonthica*. Most of cultivars were found on the negative axis and interestingly with a negative loading for fresh panicle weight, dry grain weight and panicle height.

### ***Neighbour-joining hierarchical clustering analysis***

Neighbour-joining hierarchical clustering analysis led to the formation of five groups (Figure 5). Group 1 contained 132 interactions between *Striga* and cultivar (86~0.5 mg *Striga*, 40~0.25 mg *Striga* and 8~0 mg *Striga*). This group includes lines and cultivars which were highly affected by *Striga* infestation. The other 8 interactions with 0 mg shows that there are some lines or cultivars which have inherently low yields even before being attacked by *Striga*. Table 1 supports the results shown in this neighbour joining clustering as shown by the significant difference ( $P < 0.001$ ) on *Striga* for fresh grain weight and dry grain weight. Differences have also be noted for the level of *Striga*

(Figure 5). Group 2 contained 135 interactions between *Striga* and cultivar (40~0.5 mg *Striga*, 53~0.25 mg *Striga* and 42~0 mg *Striga*). This includes lines and cultivars which were moderately affected by *Striga* infestation. Group 3 contained 92 interactions (19~0.5 mg *Striga*, 33~0.25 mg *Striga* and 40~0 mg *Striga*).

This group includes interactions with low susceptibility to *Striga* infestation. Group 4 contained (5~0.5 mg *Striga*, 24~0.25 mg *Striga* and 60~0 mg *Striga*). This group indicates interactions were lines and cultivars are highly tolerant to *Striga* infestation and the control. It is clear that 5 interactions show lines and cultivars that are highly tolerant to *Striga* damage (SCSHYB015022, SCSHYB015017 and SCSHYB015020). The other 24 interactions show that there are lines and cultivars which will show tolerance to *Striga* when the *Striga* levels are low. The other 60 interactions show the control with no *Striga* application.

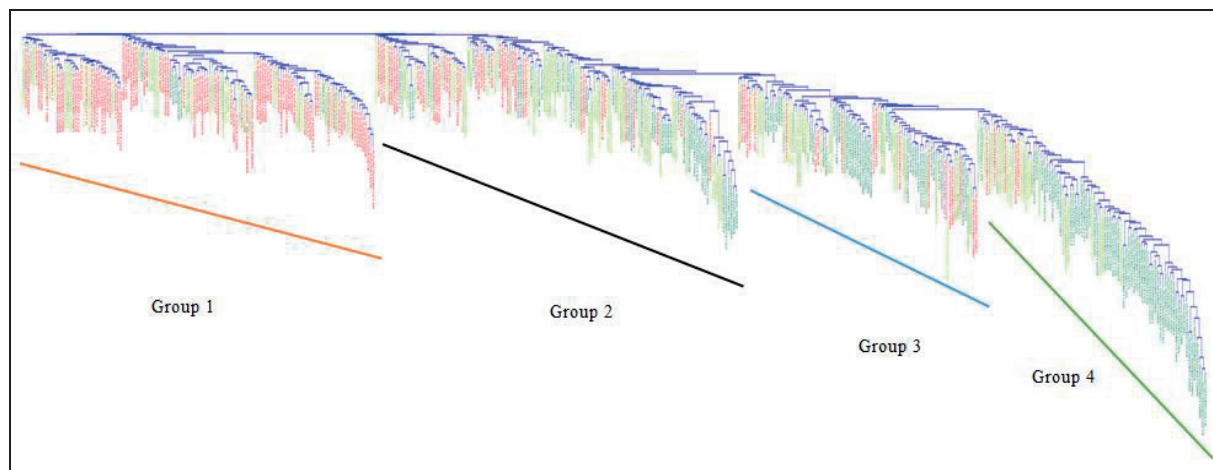


Figure 5. Neighbour join clustering analysis for *Striga*\*cultivar interaction for 75 sorghum genotypes

The genotypes which are found in Group I are the ones which were selected for further studies to perform genetic engineering. Those lines or cultivars which appeared in Group I and also were selected using heatmap analysis for yield components were then selected for further research. The selected varieties for further screening using secondary metabolite analysis are SCSHYB013103, SCSHYB012112, SC Sila, Macia, SA 1617, SA 1618, SA 3028 and SA 2311. These

variety have shown that they are highly susceptible to *Striga* attack from the heatmap analysis and neighbour join clustering.

One way analysis of variance was carried for *Striga*\*genotype interaction of 75 sorghum genotypes. The results show that there were significant difference ( $P < 0.001$ ) for *Striga*\*cultivar interaction for plant height, chlorophyll content, number of leaves, fresh weight, grain dry weight and panicle height for the sorghum genotypes. The differences



in plant height are as a result of varietal differences as well as the contribution made by *Striga hermonthica* infestation. In a similar research which was carried out in Zimbabwe, it was also observed that *Striga hermonthica* influenced sorghum metabolism, regardless of the amount of parasitism to which the host is exposed, causing a reduction of the processes affecting carbon acquisition, and eventually growth. Plant height is regarded as one of the utmost delicate parameters which shows the effects of witch weed on plants (Gwatidzo et al., 2020). It was also demonstrated that some cultivars show some levels of tolerance to *Striga hermonthica* infestation. For example, it was observed that the height and internode lengths of Mahube and ICSV 111 IN varieties were not negatively affected by *Striga hermonthica* (Gwatidzo et al., 2020). This clearly shows that some varieties do not accede to the dwarfing effects of the parasitic weed *Striga hermonthica*.

The reduction in the chlorophyll content especially in the early stages of crop remains key in the ability of *Striga hermonthica* to successfully parasitize its host. It therefore means to minimise damage, there is need to develop cultivars that disrupt the parasite-host interaction in the early stages of crop growth. The existence of this wide range of inconsistency in *Striga hermonthica* resistance and tolerance traits amongst sorghum genotypes proposes a chance to develop high yielding and resistant/tolerant genotypes through hybridization (Seyoum et al., 2019). The number of leaves varied significantly in this research. Contrastingly, in another similar research, it was reported that leaf number was not changed due to *Striga hermonthica* invasion in the development of crop in both susceptible and resistant sorghum varieties. It was observed that there were no substantial reductions in stem height in SRN 39 (Gebremedhin et al., 2000). There were tolerant genotypes which produced normal and good panicles which then later translated into the high fresh weight. The fresh weight of the sorghum panicles differed significantly in this study.

Some of the panicles failed to produce any grains completely, while others had premature abortion of grain and others did produce grain in differing quantities.

The correlation matrix show that fresh panicle weight and grain dry weight showed a very high correlation (0.948) at  $P < 0.05$ . Panicle height and fresh panicle weight are highly correlated (0.736) at  $P < 0.05$ . Similarly, panicle height and grain dry weight were also highly correlated (0.718) at  $P < 0.05$ . These variables are the ones which are very important in the determination of the final yield of different genotypes. In a similar trial in Ethiopia, some sorghum genotypes which supported the germination of considerably fewer *Striga hermonthica* plants and produced greater grain yield than others. It was also reported that various genotypes resulted in slighter yield reductions than others under the same level of infestation (Seyoum et al., 2019). This supports results obtained in this research where some genotypes have shown that they are extremely susceptible and would not give yield at all as evidence by their panicle fresh weight, panicle height and grain dry weights. Heatmap analysis results indicate that *Striga* has the ability to severely affected sorghum plant height, chlorophyll content, and number of leaves per plant. This happens in the early phases of crop growth and in most instances will result in severe crop losses. It can also be reported that yield determining factors such as fresh grain weight, dry grain weight and panicle height are also affected by the parasitic weed *Striga*. It is therefore clear that the parasitic effects of the weed can result in severe loss for most of the genotypes tested.

Principle component analysis using the scree plot Eigen values shows that the first factor contributes 58% of the cumulative variation. Two principal axes (F1 and F2) were selected, which explained about 74.45% of the total variation. It can be reported from results (Figure 3), that the data set has two groups of variables. The first group has leaf number, plant height and chlorophyll content while the other group is comprised of panicle height, field weight and grain weight. The

first group are the morph-physiological features while the second group are the yield determining parameters. In both cases, *Striga hermonthica* significantly affected the cultivars in some diverse ways for those that were susceptible as compared to those that were tolerant. This highlights that *Striga* infection starts even before the parasite itself is still below ground. *Striga hermonthica* germination is not the only import measure of the influence this parasite has on plants. It is very crucial to elucidate activities before *Striga hermonthica* germination as they show to have greater impact to the observed yield components later in the production stage of the crop. Fresh panicle weight and grain weight results show that some of the varieties even failed to produce meaningful grain as a result of the parasitic effects of *Striga hermonthica*. There are promising cultivars which have shown tolerance to *Striga hermonthica* as seen with the grain yield which was slightly affected.

These cultivars also show very few *Striga hermonthica* plants which emerged and the source and mechanism of resistance will be key in improving other varieties. It is highly likely these cultivars have low germination stimulation activity to the *Striga hermonthica* parasite.

Neighbour-joining hierarchical clustering analysis led to the formation of five groups for *Striga*\*genotype interaction. Highly susceptible genotypes were found clustering in the same groups at 0.5 mg of *Striga*. However, it was realised that the behaviour of these genotypes changed when exposed to lower doses of *Striga* (0.25 mg). Grain and stem weight get reduced in susceptible genotypes, while leaf and root biomass are preserved in tolerant ones. Damages in host production result from two processes: export of carbon to the parasite and parasite-induced declines in host photosynthesis. The latter transpires prior to the emergence of *Striga hermonthica* above ground and explains the 80% of the anticipated loss in production over the lifecycle of the association. *S. hermonthica* is reliant on the carbon exported from the host, as the plant has little rates of photosynthesis combined with high rates of

respiration. Host-derived carbon accounts for nearly one-third of the total parasite carbon requirement (Mwangangi et al., 2021).

Producing *Striga hermonthica* resistant genotypes is the utmost promising, practical, and cost effective approach to reduce the effects of *Striga hermonthica*. Furthermore, resistance in cultivars is a key component of integrated control packages. Consequently, impending research efforts should be focused towards understanding host resistance mechanisms, enhancement of field screening and infestation procedures, and development of stable high yielding *Striga hermonthica* resistant varieties that are satisfactory to farmers.

## CONCLUSIONS

It can be concluded that *Striga hermonthica* affects negatively crop morpho-physiological traits such as plant height, chlorophyll content and leaf number as well as yield determining components such as fresh panicle weight, panicle height and dry grain weight which ultimately reduce the yield of sorghum. The existence of high variability in the response to *Striga hermonthica* infestation in the 75 sorghum genotypes gives possibility to breeding interventions to improve tolerance to this parasitic weed. The use of gene editing in manipulating the *Striga hermonthica* germination stimulation activity provides enormous breakthrough for future research in developing resistant cultivars. It is very important to explore natural resistance mechanisms exhibited in the wild type varieties to come up with novel breeding strategies aimed at reducing negative effects of *Striga hermonthica* in sorghum. The sorghum lines which showed all of the three parameters: short panicle height, low fresh panicle weight and low dry grain weight were selected for further screening using secondary metabolites data to confirm their susceptibility to *Striga*. Five of the highly susceptible lines or cultivars were then taken for genetic manipulation using Crispr Cas 9 technology.

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