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Research Article
Determination of Resistance to *Striga asiatica* L. Kuntze Using Agar Jel Analysis and Sand Culture in *Sorghum bicolor* L. Moench and *Sorghum arundinaceum* in Zimbabwe

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Abstract

Background and Objective: Resistance through reduced strigolactones is one of the sustainable ways of managing *Striga asiatica*. To verify the existence of reduced strigolactone production in sorghum genotypes, an agar jel assay was carried out on seven sorghum bicolor lines and one *Sorghum arundinaceaum* sourced in Zimbabwe. **Methodology:** In the first experiment, pre-germinated *Striga* seeds were pipetted into a Petri dish with drying agar jel and pre-germinated sorghum seedlings were grown across the Petri dish. The eight sorghum genotypes were also grown in a sand culture and the number of *Striga* that attached were recorded. **Results:** The results indicated that sorghum genotypes varied significantly ($p<0.05$) with respect to maximum germination distance (Mgd) with wild sorghum and SC sila having the largest mgds indicating that they produced the largest quantities of strigolactones. The genotypes Mukadziusaende had the highest tiller numbers while SC sila had the lowest. *Striga* counts were highest on Wild Sorghum, Ruzangwaya and Hlubi. There was a negative correlation between mgd and tiller number showing that the highest strigolactone producers had low tiller numbers. A correlation coefficient of 0.564 between mgd and *Striga* counts showed that as Strigolactones increase *Striga* counts also increase. **Conclusion:** It can therefore be concluded that resistance through reduced strigolactones was found in the sorghum genotype Mukadziusaende. The direct relationship between mgd means that tiller number can be used to select for reduced strigolactone production in the field.

Key words: *Sorghum*, *Striga asiatica*, resistance, strigolactones, Zimbabwe

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The average *Sorghum* productivity in the Sub-saharan Africa subcontinent is low as a result of a myriad of production constraints of which *Striga asiatica* is a major obstacle. *Striga asiatica* is an obligate hemi-parasite that attach to the roots of several crop species leading to severe yield loss[1]. This *Striga* spp. is geographically the most widespread species with large populations having been reported throughout sub-saharan Africa, south east China and the Indian subcontinent while smaller isolated populations have been reported in Arabia, Indonesia, Philippines, north and east Carolina (USA) and Australia[2]. The *S. asiatica* is the predominant species towards the east African coast and southern Africa[3]. According to Bouwmeester *et al.*[4] these parasites especially the *Striga* spp. infests about two thirds of the 70 million ha used for cereal production in Africa. Jamil *et al.*[5] asserts that about 20-80% yield losses or even complete crop failure can occur due to parasitism.

The root parasitic weed has developed the ability to germinate only when they are exposed to germination stimulants released from the host roots, thus synchronising their life cycle to those of their potential hosts only germinating when a suitable host seed is in proximity to the *Striga* seed[6]. Fernadez-Aparacio *et al.*[7] reported that the synchrony is vital for parasitic weed survival because they have an absolute requirement for nutritional support from the host.

According to Bouwmeester *et al.*[4] and Akiyama and Hayashi[8], the first critical step in the life cycle of *Striga*, the germination of its seed, is regulated by strigolactones. The dependence on strigolactones could be exploited for *Striga* management through selecting for low strigolactones producing cultivars. The seeds of these parasitic plants will only germinate after perceiving a germination stimulant of their host[9]. After radicle emergence, the haustoria attaches and penetrates the host roots[10]. Cardoso *et al.*[11] reported that once germination has been triggered, the radicle protrudes from the testa, elongates towards the root and develops haustorium, an organ that can attach to and penetrate roots of the host plant. The parasitic plant grows underground for 4-7 weeks prior to emergence and utilises host water, nutrients and photosynthates[12]. Much of the damage will have occurred by the time the *Striga* emerges above the ground.

Jamil *et al.*[12] found significant variation among NERICA rice cultivars and their parents for strigolactones production and *Striga* germination. Production of low germination stimulants results in low numbers of *Striga asiatica* attachments thereby producing a resistant phenotype. Studies done in *Sorghum* have also shown that genotypes with low production of germination stimulant have demonstrated to be resistant to *Striga* in the field[13-15]. Since the root parasites affect the crop from the time they attach to the root, the development of new control strategies should focus on the initial steps in host parasite interaction[16].

Ejeta *et al.*[17], Wilson *et al.*[18], Gurney *et al.*[19] and Gurney *et al.*[20] demonstrated that the near relatives of cereals could provide new sources of tolerance and or resistance to the parasite infection and may provide the way forward for the control of *Striga* spp. According to Doggett[21,22] and De Wet[23] the cultivated *Sorghums* of today primarily originated in Africa from the wild *Sorghum bicolor* spp. *Arundinaceaum*. Southern Africa has more *sorghum* landraces and whilst the quest to find a land race that produce the least strigolactones is still on, there is need to look at the wild *Sorghum* and the vast number of *Sorghum* landraces that are under cultivation in the African savannah. Given the high genetic diversity of the *Sorghum* spp. including wild *Sorghum*, there is need to quantify strigolactones in most of these cultivated lines as they are grown in *Striga* infested fields. According to Ejeta and Butler[24] different *Sorghum* genotypes differed by as much as a billion fold in the amount of germination stimulants they produce. Jamil *et al.*[5] asserts that strigolactones have a triple role which is underground communication between the plant and AM fungi and parasitic plants and the regulation of tillering. According to Umehara *et al.*[25] and Lopez-Raez *et al.*[26] strigolactones inhibit tillering in plants and therefore the ability to tiller can be used as a selection criteria for reduced strigolactones production. Therefore, the objective of this study was to quantify strigolactones in *Sorghum* lines using agar jel analysis to determine *Sorghum* lines that produce the least strigolactones and therefore confers the highest resistance. The research also aimed at correlating the maximum germination distance (MGD) and tillering and *Striga asiatica* counts.

MATERIALS AND METHODS

Experiment 1: Agar jel assays

Germplasm and chemicals: Seeds of 8 *Sorghum bicolor* varieties were obtained from the gene bank, Harare research Station in Zimbabwe. The seeds of *Sorghum arunaceaum* were obtained from Gwebi agricultural college fields which is 27 km west of the city of Harare.

Experimental design

Surface sterilisation and *Sorghum* seed germination: *Sorghum* seeds were soaked in 1% sodium hypochlorite solution for 60 min and rinsed in double deionised water. The seeds were soaked in an aqueous solution of 10% captan overnight. Seeds were rinsed with deionized water three times and then incubated in moist filter paper at 27°C. At 48 h germinating seeds were placed in agar plates as outlined by Hess *et al.*[14]. The *Sorghum* genotypes whose strigolactones were quantified are shown in Table 1.

Surface sterilisation and conditioning of *Striga* seed: *Striga asiatica* seeds were placed in 30 mL sample bottles and rinsed 3 times by adding 3-5 drops of the detergent tween 20 into 10 mL of distilled water. Sonication was done using an ultra sonic cleaner 3 min during the first rinse[27]. The *Striga* seeds were incubated at 27°C for 3 days prior to transferring them into the fresh sterile flasks containing 15 mL of 0.001% aqueous benomyl solution. The sample bottles were re-incubated at 25°C for 35 days before they were ready for use in the agar jel assay.

Assay set up: Pre-conditioned *Striga* seeds were pipetted into Petri dishes. Water agar was then poured over the seed. The roots of the germinating *Sorghum* seeds were placed in the solidifying agar with the root tip pointing across the plate. The plates were incubated in the dark for 5 days. The maximum germination distances (mgd): distance between the host root and the furthest germinated *Striga* seed were used as indicators of the quantities of strigolactones produced.

Sand culture: About 1 g of *S. asiatica* seeds were weighed for every treatment and mixed thoroughly with 2 kg of washed river sand. The sand was sterilized before the start of the experiment by heating in an oven at 120°C for 48 h to kill any *Striga* weed seeds that could be in the sand. The method was adapted from Jamil *et al.*[5]. Plastic pots of dimensions 18 cm diameter and 20 cm height were used.

Table 1: *Sorghum* genotypes used in strigolactone quantification

Sorghum cultivar	Cultivar name
1	Mukadziusaende
2	Wild *Sorghum*
3	Sc Sila
4	Chiredhi
5	Ruzangwaya
6	Isifumbathe
7	Hlubi
8	Zambia

Experimental design: There were 8 cultivars (Table 1) and the experiment was replicated four times and laid down as a completely randomized design.

Planting and data collection: Five *Sorghum* seeds were planted in pots at a depth of 0.5 cm the seedlings were thinned to one plant per pot at 2 WACE. The *Sorghum* were allowed to grow in pots for 15 weeks and the *Striga* that emerged were counted. The number of tiller in the *Sorghum* plants were also counted. The data was analyzed according to the model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where, Y_{ij} is the measured parameter e.g., tiller number, μ is general mean, T_i effect of the treatment and e_{ij} is the error term. Correlation of the measured parameters were determined using SPSS.

RESULTS

Maximum germination distance (MGD): The *Sorghum* cultivars differed strongly ($p = 0.001$) with regard to maximum germination distance which is indicative of the strigolactones quantity produced (Fig. 1) at 120 h of incubation time at 30°C. The minimum germination distance was 1.225 cm and it was for Mukadziusaende whilst the maximum was 2.775 cm for SC Sila (Fig. 1). The higher the maximum genetic distance the higher the quantity of strigolactones production.

Tillering: *Sorghum* varied strongly ($p<0.01$) in tillering and the average number of tillers varied from 1.5 for SC Sila to 4.5

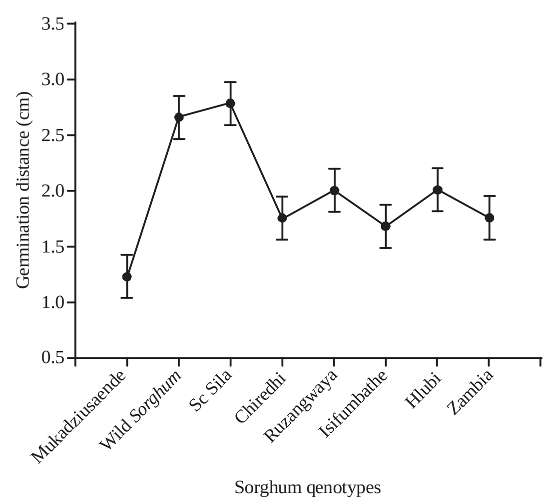

Fig. 1: Maximum germination differences for various *Sorghum* cultivars

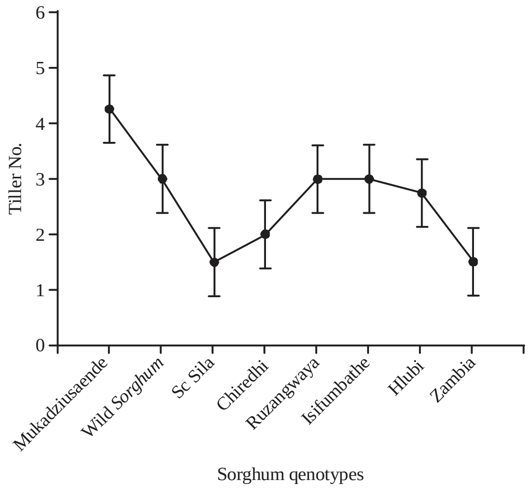

Fig. 2: Effect of *Sorghum* cultivars on tiller numbers

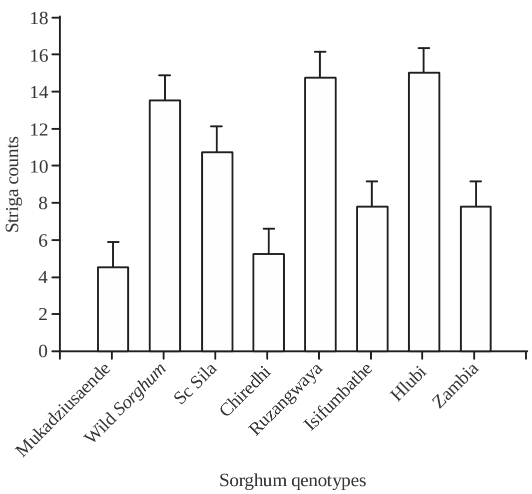

Fig. 3: Effect of *Sorghum* cultivars on *Striga* counts

for Mukadziusaende (Fig. 2). The cultivars that had the highest number of tillers were Mukadziusaende and wild *Sorghum* while the lowest were SC Sila and Zambia (Fig. 2).

***Striga* counts:** The *Sorghum* cultivars varied strongly in eliciting *Striga* germination (counts) ($p<0.01$) the cultivars that had the highest number of *Striga* counts were Ruzangwaya and Hlubi while the lowest were Mukadziusaende and Chiredhi (Fig. 3).

Correlations between maximum germination distance, tillering and *Striga* counts: A correlation coefficient between germination distance and tiller numbers shows a negative relationship with a correlation coefficient of -0.191. When the maximum germination distance increases the tiller number decreases. The correlation coefficient between germination distance and *Striga* counts has a value of 0.564 which shows a relatively strong relationship between the two variables. As mgd increases, *Striga* counts also increase ($p<0.001$).

DISCUSSION

The objective of this study was to determine the *Sorghum* genotype that had produced the lowest strigolactones and the correlate strigolactones production to tillering. The *Sorghum* genotypes with lowest maximum germination distance were Mukadziusaende, Chiredhi and Isifumbathe whilst wild *Sorghum* and SC Sila had the biggest maximum germination distance. All the genotypes were susceptible to *Striga asiatica*. This is based on the stipulation that genotypes with a germination distance of less than 1 cm are resistant while those with more than 1 cm are susceptible to *Striga asiatica*[14]. However, the level of susceptibility differs with the genotypes.

These results are consistent with previous observations in cereals like rice[5]. The results confirm the existence of large genetic variation among the *Sorghum* cultivars. According to Jamil *et al.*[5] up to about 500-fold difference exist in the amounts of strigolactones exuded by rice Germplasm. Ejeta and Butler[24] confirmed the same results in *Sorghum* and reported differences as much as a billion fold in the amounts of stimulants produced in *Sorghum*.

Tillering varied among *Sorghum* genotypes with Mukadziusaende having the highest tiller number whilst the lowest was SC Sila (Fig. 2). In *Sorghum*, tillering has been proposed to be under genetic and environmental control and according to Kim *et al.*[28] tillering has not been comprehensively addressed by the carbohydrate supply and demand framework. In this study the *Sorghum* genotypes were subjected to the same environmental conditions hence the environmental influences were eliminated. Therefore the differences suggested that hybrids may also differ in their propensity to tiller which is independent of the carbon supply demand[28]. The differences could be due to differences in hormonal signaling as the plants were in the same environment. According to Umehara *et al.*[25] the hormone strigolactones reduced tillering in plants so plants that produce less strigolactones have profuse tillering compared to those that produce more. The results of this study support the propensity to tiller hypothesis as the genotypes were grown in the same conditions.

Taken together the results of this study suggested that elevated levels of strigolactones contribute to the inhibition of tiller buds. Therefore tiller growth is promoted when strigolactones are decreasing in the plant. The same results were supported by Umehara *et al.*[25] and Dun *et al.*[29]. The implications of this study are that when high tillering *Sorghum* cultivars are introduced *Striga* can be managed.

CONCLUSION

The genotype Mukadziusaende had the least mgd and more tiller numbers and also was attached to less *Striga asiatica* plants. Tiller numbers can be used to select for reduced strigolactones production in the field since they is an inverse relationship between tillers and strigolactones. Tillering potential of *Sorghum* becomes a marker of the plant's susceptibility to *Striga* infection. The selection of high tillering cultivars could be helpful strategy to reduce *Sorghum* loss to *Striga*. Therefore, farmers in Zimbabwe can utilize tiller number to select for moderately resistant *Sorghum* lines.

SIGNIFICANCE STATEMENT

Sorghum landraces vary with respect to strigolactones production Strigolactones quantities are inversely linked to tillering. The quantity of strigolactones produced is directly related to the number of *Striga* attachments in *Sorghum* Farmers and researchers can use tiller numbers to select for low strigolactones producers.

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