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Bio-Assay Screening of Sorghum [*Sorghum bicolor* (L.) Moench] Inbred Lines for Resistance to *Striga* [*Striga hermonthica* (Del.)] in Ethiopia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was carried out to validate the reaction of sorghum inbred lines for low germination stimuli using bio-assay in the laboratory. Twenty-two (22) sorghum genotypes with two resistant checks (Gobiye and SRN-39) and one susceptible check (Teshale) were conducted in a completely randomized design (CRD) with three replications at National Agricultural Biotechnology Research Center of Holetta. During the laboratory study, four parameters were measured among which germination rate of Striga around the host root and maximum germination distance from the host root was used as the index of resistance. Maximum germination distance (MGD), germination percentage near the host roots, germination index, and haustorial initiation percentage were recorded and significant differences were observed among the genotypes tested. Germination rate of Striga around the host root and maximum germination distance from the host root was also used as the index of resistance. Sorghum genotypes 2006 MW 6044, ETSC 300080, ETSC 300081, 05 MW 6019, ETSC 300086, 2006 MW 6123, ETSC 300003, ETSC 300082, and 05 MW 6028 induced

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less Striga seed germination and lower maximum germination distance, which was not significantly different from the resistant checks Gobiye and SRN-39. Out of the 22 genotypes, eight had a maximum germination distance of less than the threshold value of MGD (< 10 mm) that made the genotypes of low germination stimulant of Striga. These genotypes also showed the lowest Striga germination percentage near the host root which indicated the strong and positive correlation of MGD and GP %. Therefore, these sorghum genotypes were low in pre-attachment to Striga and are said to be resistant and/or tolerant to Striga infestation and thus important in increasing production and productivity of sorghum in Striga infested areas.

Keywords: Extended agar gel assay; genotypes, germination stimulant; parasitic weed; resistant; screening techniques.

1. INTRODUCTION

"Sorghum is a cereal of a remarkable genetic variability; with more than 30,000 selections present in the world genetic collections" [1]. "The morphological characteristics change with genotype and growing conditions. Most of the tropical sorghums are short day plants and their response to day length is an important adaptation. However, the selection of early-maturing varieties and hybridization helped its spread in the USA" [2]. "Globally, sorghum is the fifth most important staple food crop after wheat, rice, maize and barley and the third most important cereal crop grown in the Ethiopia" [3]. "In the sub-Saharan Africa, sorghum is the second most important food crop after maize, and is predominantly grown by small-scale and subsistence farmers. More importantly, sorghum is the major food and cash crop for the most food insecure farmers in the semi-arid areas which experience low and unreliable rainfall patterns, and which are not suitable for most other crops, including maize" [4]. It is also used for animal feed and nowadays, sorghum has emerged as a 'smart' crop for production of ethanol (bio-fuel), especially in the two main sorghum producing countries, Brazil and India.

"In Ethiopia, sorghum is the most important cereal crop, particularly in lowland areas where rainfall is unreliable and crop failures due to recurrent drought are common. It plays a significant role for millions of food-insecure people living in such environments. Ethiopia is the third largest sorghum producer in Africa next to Nigeria and Sudan" [3]. "The crop is one of the major food cereals like tef, wheat, maize and barley" [5]. "It ranks second after maize in total production, third after wheat and maize in productivity per hectare, and after tef and maize in area cultivated" [5]. "It is cultivated in almost all regions, covering a total land area of 1.8 million ha and grown mainly in dry-lands and semi-arid areas of Ethiopia where drought and poor harvests are common, and it is considered as the principal crop providing means of survival. It is a multipurpose crop because the grain is used to prepare food and beverages, and the juicy sweet stem of sorghum is often chewed by humans particularly in rural Ethiopia. The stem is also used for various purposes such as animal feed, construction of houses and as fuel wood for cooking" [6,7].

"However, various biotic and abiotic factors contributed to the low productivity of sorghum. Among the biotic factors, weed, mainly *Striga* is the most important cause of yield loss in most regions of Ethiopia. Drought and *Striga* weed are the most important constraints in the Northern and North Eastern parts of Ethiopia" [8]. "The problems of various invasive and devastating weeds including striga are the major constraints especially in Africa and Ethiopia in particular" [9]. "Striga spp. are obligate hemi-parasitic plants that attack to the roots of their host to obtain water, nutrient and carbohydrates" [10]. "*Striga* weeds, particularly *Striga hermonthica* (Del.) Benth inflicted significant yield losses in cereal crops including sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), maize (*Zea mays*), and rice (*Oryza sativa*)" [11]. "The weed is endemic to sub-Saharan Africa and infests about 26 to 50 million ha, causing annual crop losses ranging from 30 to 90% and sometimes leading to complete yield loss" [12]. "In Ethiopia, losses of 65-100% are common in heavily infested fields" [13]. "Its effect depends on the crop genotype, degree of infestation, rainfall pattern, and fertility of the soil" [14].

"Effective, affordable, and sustainable control options are needed to enhance small-scale sorghum productivity in areas where the parasite occurs. Several control options have been recommended to reduce *Striga* damage, including the use of crop rotation, intercropping with legumes, late planting, use of trap crops,

application of organic and inorganic fertilizers, herbicides, and biological control" [15]. "Furthermore, these control options when applied individually are not effective and are sometimes affected by environmental conditions. Therefore, several options need to be integrated to achieve sustained and successful *Striga* control. The use of resistant cultivars is a most robust and effective approach to control parasitic weeds. Host-plant resistance in adapted, productive cultivars is a central component of integrated *Striga* management. The resistant cultivars offer an economically feasible and culturally sustainable technology for smallholder farmers" [16]. "This has been demonstrated in multi-location field tests conducted in Ethiopia and Tanzania" [17,18]. Previous research has shown that "there is significant genetic variation for *Striga* resistance in sorghum" [19]. "However, advances in breeding have been limited due to inadequate information on the genetics of *Striga* resistance and difficulty of evaluating resistance in the field" [19].

"Precise and reliable screening techniques are indispensable in order to select *Striga* resistant lines through breeding" [20]. "The genetics of *Striga* resistance and methodologies for breeding *Striga*- resistant sorghum with special emphasis on direct field evaluation have been reviewed" [21].

"The presence of individual mechanisms conferring resistance to *Striga* may be examined in the laboratory, whereas complex resistance must be assessed under field conditions and screening in pots may include advantages of both, providing some control over environmental conditions, but with the disadvantage of a largely artificial root environment. Field screening for striga resistance is hampered by the following: heterogeneity of natural field infestations, large environmental effects on striga emergence, and complex interactions between host, parasite and environment affecting the parasite's establishment and reproductive success" [22]. The agar-gel assay developed by [23] provides "a simple means for screening host genotypes for low production of striga seed germination stimulant".

In Ethiopia, so far about four varieties have been released for production in *Striga* infested areas of the country. However, with the diversity of sorghum growing environments and the expanding effect of *Striga* on sorghum production, developing varieties that have higher yield potential and *Striga* resistance is one of the priorities. The major problem associated with the use of resistant cultivars is the lack of universal resistance and that resistance is said to decrease with time. Thus, there is always a need for continuous screening of germplasm to be able to ascertain varieties to use in resistance breeding and in production. Thus, the activity was done with the objective to assess the reaction of sorghum inbred lines for low germination stimuli using assay-based screening method.

2. MATERIALS AND METHODS

2.1 Experimental Materials and Description of the study area

The experiment was conducted at the bioassay laboratory during 2017 at National Agricultural Biotechnology Research Center (NABRC) of Ethiopian Institute of Agricultural Research (EIAR) at Holeta, which is located at 20 km away to the west from Addis Ababa, at 9^{o} 4' N latitude, 38^{o} 30' E longitudes, and 2400 m.a.s.l. *Striga* [*Striga hermonthica (*Del.)] seed was used for the study. A total of twenty two (22) sorghum genotypes developed from crosses of improved sorghum genotypes with known sources of *Striga* resistant genes and three (3) standard checks were evaluated in a Completely Randomized Design (CRD) with three replications per treatment. The genotypes were advanced from the pedigree breeding program of the National Sorghum Improvement Program at Melkassa Agricultural Research Center and selfed up to F6 stage, which were screened for resistance to *Striga* in *Striga* infested areas of Ethiopia. The standard checks used were 'Gobiye' and 'SRN-39' as resistant check and 'Teshale' as susceptible check.

2.2 Surface Sterilization of Sorghum Seeds

Sorghum seeds were soaked in 5 ml sodium hypochlorite (NaOCl) solution (50% commercial bleach) for 30 - 60 min and rinsed three times with double distilled water (ddH2O). They were then soaked in the solution of 5% Daconil solution (50% wet table powder) in 5ml of this Daconil slurry to each vial and the seeds were soaked in this solution overnight to imbibe. After at least 5 hours, the Daconil slurry was poured off from the vials and 5 ml of sterile distilled water was added. The seeds and water were poured off into labeled sterile Petri dishes, each containing 2 Whatman # 1 filter paper (90 mm)

circles and incubated in the dark at 30°C for 48 hours. After 48 hours, germinated sorghum seeds were placed in agar plates as outlined by [23].

2.3 Surface Sterilization and Conditioning of *Striga* seeds

Striga seed surface sterilization and conditioning were performed using the procedure described by [24] with some modification [25]. Briefly, seeds were added to 50 ml flask containing 25 ml of 75% ethanol and were agitated with sterile glass pipette equipped with an amber bulb for 2 min. After 2 min agitation in ethanol, 25 ml of activated Metricide was added into the flask containing *Striga* seeds and agitated again for 2 min and poured off the solution after the seeds were settled and the remaining solutions removed with the pipette. Then after, sterile ddH2O was added and the seeds were rinsed 2 times, each time agitating with a pipette for about a minute, and then the liquid was removed. The seeds were then rinsed in 25 ml ddH2O and 25µL of the 1000x Benomyl stock. The flasks were then placed in a 29°C incubator for five days to begin conditioning prior to transferring them into agar plates. The Benomyl solution was changed after one day and then 2- 3 days after until the seeds were embedded in agar and returned to the incubator at 29^{o}C.

2.4 Extended Agar Gel Assay (EAGA)

The EAGA is used to establish genotypic differences in inducing *Striga* germination, haustorial initiation, as well as to detect differences in pre-attachment. The extended agar gel assay (EAGA) is a modification of the agar gel assay developed by [23] and later also modified by [24], and a quick tool to screen genotypes for their ability to stimulate *Striga* seed germination. In the EAGA, large Petri dishes with a thick agar layer were used to support growth of sorghum seedlings for a longer period. Water agar (0.7%) solution was autoclaved for 15-20 min, and then cooled to 50°C for at least one hour. Under the hood, the pre-conditioned *Striga* seeds from the flasks were pipetted to the center of the Petri dishes with the aid of an amber bulb and 50°C warm agar was poured into the Petri-dish containing pre-conditioned *Striga* seeds, which produced an even distribution of seeds. Single sorghum seeds were placed along the edges of each dish so that the radicles just penetrated the gel. The dishes were then covered and placed in a dark incubator at 29°C. The agar Petri dishes were checked three days after inoculation of sorghum seedlings with *Striga* embedded agar and *Striga* seed germination was clearly observed through the bottom of the Petri dish using a dissecting microscope. Each dish was observed under the dissecting microscope for seed germination, parasitic attachment and host root development. The plates containing sorghum were selected and prepared for data collection in which the selection was made based on health of the host plant and proper root penetration to the agar.

The data on germinated *Striga* were taken along the sorghum root beginning 2cm from the kernel and scoring *Striga* germination and haustorial initiation were made under a zoom stereomicroscope (SZ series) at 10× magnification from standard area of 2×2.5cm near and far from root. Maximum germination distance (MGD), that is, the distance between host root and most distant germinated *S. hermonthica* seeds, and germination percentage were used as the best indices of resistance. The furthest germinated *Striga* seed from the host root was marked and the maximum distance between the host root and the most distant germinated *Striga* seed was recorded. The total distance for each of the three seedlings in three Petri dishes was recorded and near host germination rate (2×2.5cm) was recorded on day three and was used for statistical analysis to measure *Striga* germination stimulant activity. After the maximum germination distance and near host germination rate was recorded, the Petri dishes were then treated with GR24 to promote *Striga* seed germination at 29°C. The Petri dishes were observed under a dissecting microscope 48 hour after treatment (5 days after incubation). Germination percentage of *Striga* seeds in the agar were measured in areas of the agar in close proximity to the host root and far away from the host root, which indicates the germination index. A germination index was assigned representing the ratio of proximal and distal *Striga* germination rates. The germination indices of three seedlings in three Petri dishes were recorded and used for statistical analysis. After the germination index was estimated, the Petri dishes were observed under a dissecting microscope for haustorial initiation indicated by the appearance of hair like projections (tubercles). The total number of haustorial formed within an area of 2×2.5cm along the root was recorded as a percentage of total germinated *Striga* with haustoria. The percentages of haustorial formed for each of the three seedlings in three Petri dishes were recorded and used for statistical analysis.

2.5 Data Collection and Analysis

Maximum germination distance (MGD): the maximum distance between host root and the further germinating *Striga* seed (mm) measured three days after incubation.

Germination percentage (%): Number of germinated *Striga* observed three days after inoculation of sorghum seedlings with conditioned *Striga* in an area of 2×2.5cm near host root and the average of three replications was used for analysis. $GP(\%) = \frac{GS}{TNS} \times 100$ where GP is germination percentage, TNS is total number of *Striga* seeds in the area and GS is germinated *Striga* seedling in the area.

Haustoria initiation Percentage (%): The total number of haustoria formed within 2×2.5 cm area along the root was recorded as percentage of total germinated *Striga*; a mean of three replications was used for analysis.

Germination index: The germination indices were taken from an area of 2×2.5cm near and far from host root representing the ratio of proximal and distal *Striga* germination rates. Data were means of three replications.

$$\text{Germination Index} = \frac{GR.nearhost}{GR.far\ from\ host}$$

where;

GR is germination rate.

The collected data were analyzed using Statistical Analysis Systems (SAS, 2003) version 9.1 for both the field and bioassay lab experiment.

Treatment means were separated using the least significance difference (LSD) test at 5% level.

3. RESULTS AND DISCUSSION

The analysis of variance (ANOVA) for the characters studied in laboratory is presented (Table 1). All the characters namely maximum germination distance, germination percentage, haustorial initiation percentage and germination index showed very highly significant (P≤ 0.001) difference among the tested genotypes. The observed significant differences indicate the presence of variability for each of the characters among the tested genotypes.

The first critical step in *Striga* life cycle is the germination of *Striga* seed, after a short conditioning period under sufficient warm and wet conditions triggered by stimulant molecules, which are released by the roots of the host plants and regulated by the Strigo-lactones [26,27]. Many secondary metabolites have been identified as germination stimulants. However, most of them correspond to Strigo-lactones [28]. Germination of *Striga* seed from extended Bagar gel assays (EAGA) has been presented below (Fig. 1).

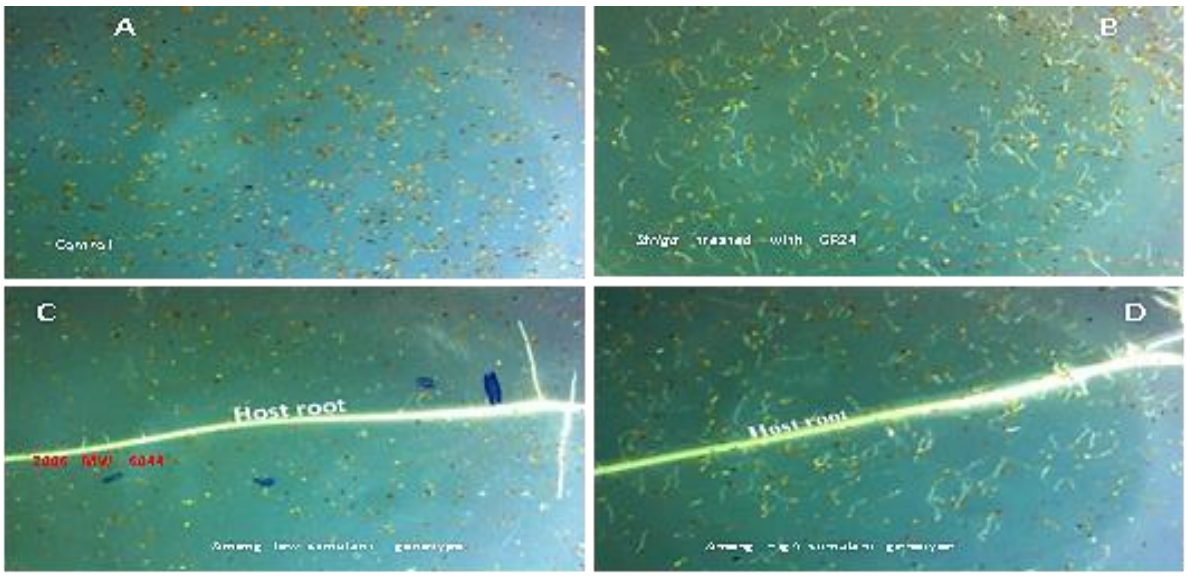

Fig. 1. Germination status of preconditioned Striga embedded in agar around the host root and after treatment with GR24. Pre- conditioned Striga with no GR24 (A); Treated with GR24 (B); low germination stimulant genotype (C) and High germination stimulant genotype (D)

Table 1. Mean squares of different source of variation and corresponding CV (%) for the four traits evaluated in laboratory bioassay during 2017

Traits	Replication (2)	Treatment (24)	Error (48)	CV (%)
MGD (mm)	1.67	110.891**	5.8	23
GP (%)	27.8	428.43**	47.72	46.1
HIP (%)	8.038	256.806**	9.136	26.5
GI	0.04	0.06667**	0.02	11.5

*'**' highly significant difference (P≤ 0.001); MGD= Maximum germination distance (mm); GP= Germination Percentage (%); HIP= Haustorial initiation percentage (%); GI= Germination index; Numbers in parenthesis indicate the degree of freedoms.*

3.1 Germination Percentage (GP%)

The number of *Striga* seed germination ranged between 0.0 to 36.08% (Table 2). Analysis of variance (ANOVA) for *Striga* germination revealed very highly significant (P ≤ 0.001) differences among the sorghum genotypes tested for their ability to cause *Striga* germination. Genotypes have showed significant variation in inducing *Striga* seed germination through the releasing of germination signal. The sorghum genotype 2006 MW 6044 stimulated significantly less *Striga* seed germination (1.95%) next to the two resistant checks SRN-39 and Gobiye. Similarly, genotypes ETSC 300080, ETSC 300081, 05 MW 6019, ETSC 300086, 2006 MW 6123, ETSC 300003, ETSC 300082 and 05 MW 6028 induced less *Striga* seed germination which was not significantly (P > 0.05) different from the resistant checks Gobiye and SRN-39. These genotypes said to be low germination stimulants as low *Striga* seed germination levels were observed in these genotypes but any of the sorghum genotypes did not exhibit zero germination of *Striga* seed plant. This is in line with the report of [29], as there is no complete resistance to *Striga* so far in sorghum, the expression of low percentage level of stimulant production was an indication of their high level of resistance to *Striga*. This finding is also in agreement with the work of [30], who studied the variability of *Striga* seed germination stimulant levels in maize.

The low level of seed germination stimulant production is of special interest in sorghum breeding for resistance to *Striga*. Low *Striga* germination suggests low germination stimulant production. Low level of seed germination stimulant produced by host plants, result in reduced number of germinated *Striga* seeds. However, low germination could also be due to some germination-inhibitory compounds produced by the sorghum accessions that may interfere with the germination response sequence of conditioned *Striga* seeds as reported by [24]. On the contrary, genotype 2006 MW 6067 stimulated the highest (36.08%) germination of *Striga* seeds followed by genotypes 05 MW 6066 (35.16%), 2006 MW 6112 (34.1%), 148 X Framida (31.31%) which were recorded greater than that of susceptible check Teshale (30.13%). Similarly, genotypes ETSC 300087 and 06 MW 6015 were recorded high germination percentage value which was not significantly different from the highest germination stimulant genotype (Table 2).

3.2 Maximum Germination Distance (MGD)

The germination distance is a measure of the amount of *Striga* seed germination stimulant produced by the sorghum genotype. Analysis of variance (ANOVA) indicated that very highly significance (P ≤ 0.001) differences in maximum germination distance (MGD)was observed among the tested genotypes (Table 2). The maximum germination distance ranged from 0 to 21.72 mm was recorded. Eight (8) of the tested genotypes had MGD less than the thresh-hold value (MGD<10) (Table 3). The least MGD was recorded on genotype2006 MW 6044 (2.33 mm), and genotypes ETSC 300081, ETSC300080, 05 MW 6073, ETSC 300086, 05 MW 6019, ETSC 300003and ETSC 300087 had also the lowest germination distances which were not significantly different (P > 0.05) from resistant checks Gobiye and SRN-39. *Striga* seed germination was not observed on the two resistant check varieties (Gubiye and SRN-39) and hence, confirmed their consistent resistance to *Striga*. The result generally confirmed that the aforementioned genotypes contained *Striga* resistant genes. The correlation between *Striga* germination count and maximum germination distance of the *Striga* seeds germinated from their host roots reflected the similarity between the two traits to select *Striga* resistant genotypes (Fig. 2).

Table 2. Mean comparison of the sorghum genotypes tested at bioassay lab during 2017

		Traits
Genotype	**MGD (mm)**	**GP (%)**
1. 2006 MW 6044	2.3i-k	1.95^{ef}
2. ETSC 300003	8.1e-g	7.68^{b-f}
3. 05MW6019	7.2f-h	3.35^{d-f}
4. 05 MW 6073	4.4g-j	14.85^{bc}
5. 2006 MW 6185	13cd	16.11^{b}
6. ETSC 300081	2.78^{i-k}	2.78^{d-f}
7. ETSC 300086	5g-i	3.68^{c-f}
8. ETSC 300080	4.1h-j	2.74^{d-f}
9. 2006 MW 6145	16.34^{bc}	13.93^{b-d}
10. ETSC 300087	9.56^{d-f}	28.28^{a}
11. 2006 MW 6112	18.89^{ab}	34.1^{a}
12. 148 x Framida	21.72^{a}	31.31^{a}
13. 05 MW 6066	18.89^{a}	35.16^{a}
14. 06 MW 6015	16bc	27.65^{a}
15. ETSC 300085	10.25^{d-f}	15.48^{b}
16. 2006 MW 6067	17.56^{b}	36.08^{a}
17. 05 MW 6005	11.67^{de}	16.06^{b}
18. ETSC 300083	10.37^{d-f}	12.77^{b-e}
19. ETSC 300082	10.67^{d-f}	8.49^{b-f}
20. 05 MW 6028	11.78^{de}	8.69^{b-f}
21. 2006 MW 6123	11.78^{de}	5.93^{b-f}
22. SPV-245 X 1(146 X 354)-27 xFramida-7-1	11.67^{de}	16.09^{b}
23. SRN-39 (Resistant)	0.0^{k}	0.0^{f}
24. Teshale (Susceptible)	16.89^{bc}	30.13^{a}
25. Gobiye (Resistant)	0.95^{jk}	1.08^{f}
Mean	10.47±1.965	14.97± 5.640
LSD (5%)	3.952	11.34
CV %	23.0	46.1

Means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance; LSD= Least significant difference; CV= Coefficient of Variation; MGD= Maximum Germination Distance (mm); GP=Germination Percentage (%)

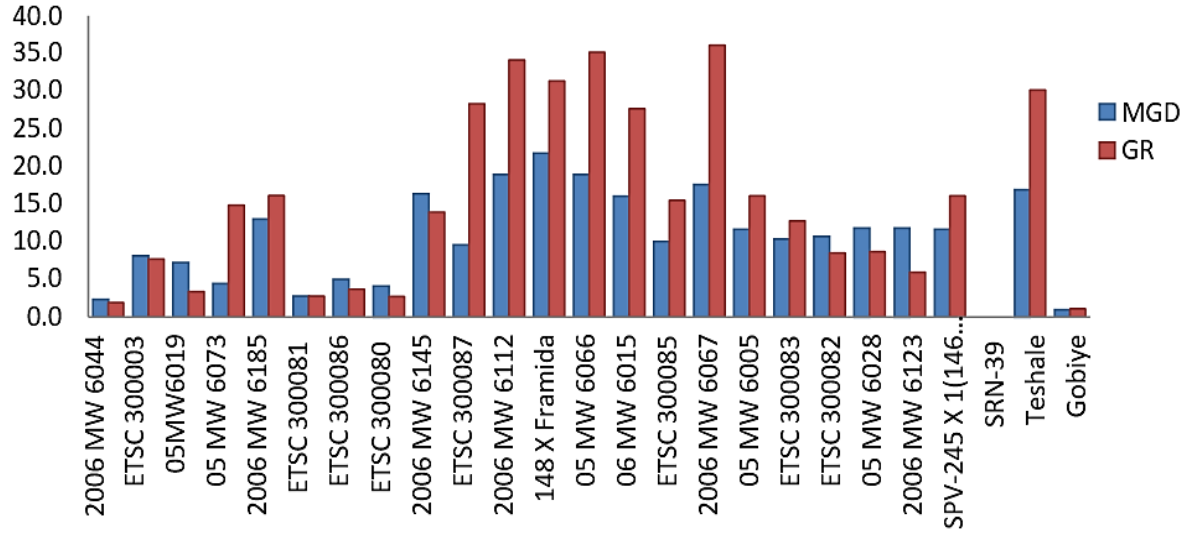

Fig. 2. The *Striga* germination rate and maximum germination distance relations for all sorghum genotypes tested in 2017

On the other hand, genotype 148 X Framida induced the highest (21.7 mm) maximum germination distance of away from its root (Table 3). Similarly, genotypes 05 MW 6066 and 2006 MW 6112 recorded large MDG value of 18.89 mm which was not significantly different from the highest MDG value. However, the value was greater than the susceptible check and hence these genotypes were indicated as susceptible as they stimulate high germination of *Striga* seeds. Generally, sorghum genotypes 148 X Framida, 2006 MW 6112, 05 MW 6066, 2006 MW 6067, 2006 MW 6145, 06 MW 6015, 2006 MW 6185, 05 MW 6028, 2006 MW 6123, 05 MW 6005, SPV-245 X 1(146 X 354)-27 x Framida-7-1, ETSC 300082, ETSC 300083 and ETSC 300085 had MGD > 10 mm and were high *Striga* germination stimulant genotypes, whereas genotypes 2006 MW 6044, ETSC 300081, ETSC 300080, 05 MW 6073, ETSC 300086, ETSC 300086, ETSC 300003 and ETSC 300087 had MGD < 10 mm and are accepted as low *Striga* germination stimulant activity as it has been suggested by [23].

Seed germination percent was high near the source of stimulant, which suggests that the higher the concentration of the stimulant, the higher the *Striga* seed germination percent. Interestingly, the higher concentration of *Striga hermonthica* recorded in sorghum genotypes also made the *Striga* to germinate far away from the host roots.

3.3 Haustorial Initiation Percentage (HIP%)

A germinating *Striga* seed develops a radicle that does not differentiate further until a second host-derived signal is received. Once the *Striga* seed germinated, it must produce the special organ called the haustorium (Fig. 3) to attach to its host. It must be understood that haustorial initiation factors are different from compounds that stimulate *Striga* seed germination. The analysis for haustorial initiation revealed the presence of significant variability among the tested sorghum genotypes. The mean haustorial initiating percentage ranged between 1.89 to 44.36% (Table 3). Among the tested sorghum genotypes, the lowest haustorial initiation percentage of 2.2% was obtained on the genotype05 MW 6019, which was significantly different from the *Striga* resistant check (SRN-39). Similarly, the genotype 05 MW 6028 had the lowest haustorial initiation percentage followed by the improved check variety (Gobiye). This result indicated the possibility of identifying genotypes that supported the lowest *Striga* plant population and increased grain yield in sorghum. On the contrary, the highest (44.36%) haustorial initiation percentage was recorded on the genotype 2006 MW 6185.

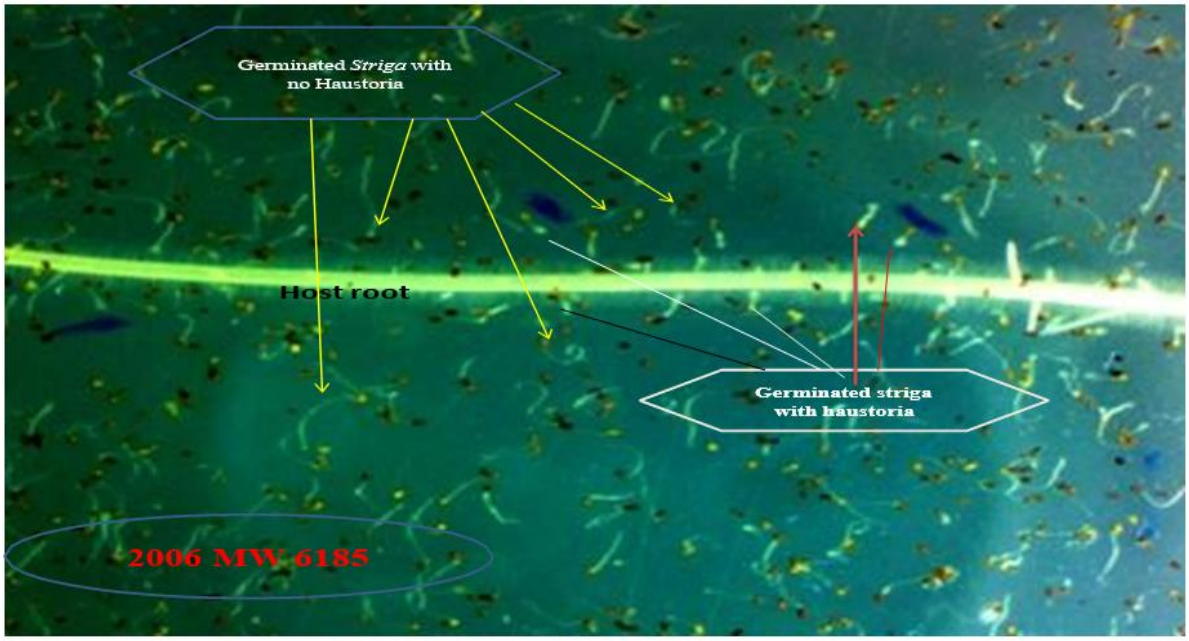

Fig. 3. Germinated *Striga* with haustoria after GR24 treatment

Table 3. Mean *Striga* haustorial initiation percentage and germination index of sorghum genotypes tested using the extended agar gel assay

		Traits	
S.No_	**Genotype**	**HIP (%)**	**GI**
1	2006 MW 6044	8.25^{g-k}	1.01^{f-h}
2	ETSC 300003	26.67^{b}	1.20^{c-f}
3	05MW6019	2.2^{m}	1.02^{f-h}
4	05 MW 6073	13.95^{de}	1.11^{d-h}
5	2006 MW 6185	44.36^{a}	1.56^{a}
6	ETSC 300081	7.28^{h-l}	0.967^{gh}
7	ETSC 300086	6.4h-m	1.07^{d-h}
8	ETSC 300080	16.95^{cd}	1.03^{e-h}
9	2006 MW 6145	4.2j-m	1.12^{d-h}
10	ETSC 300087	16.72^{cd}	1.01^{f-h}
11	2006 MW 6112	13.01^{d-g}	1.25^{b-d}
12	148 X Framida	10.23^{e-h}	1.45^{ab}
13	05 MW 6066	3.91^{j-m}	1.05^{d-h}
14	06 MW 6015	14.25^{c-e}	1.11^{d-h}
15	ETSC 300085	9.3e-i	1.087^{d-h}
16	2006 MW 6067	12.86^{d-g}	1.14d^{e-h}
17	05 MW 6005	3.75^{k-m}	1.03^{e-h}
18	ETSC 300083	13.62^{d-f}	1.24^{b-e}
19	ETSC 300082	18.98^{c}	1.15^{c-g}
20	05 MW 6028	3.07^{lm}	1.16^{c-g}
21	2006 MW 6123	8.76^{f-j}	0.93^{h}
22	SPV-245 X 1(146 X 354)-27 XFramida-7-1	5.25^{i-m}	1.05^{d-h}
23	SRN-39 (Resistant)	1.89^{m}	1.10^{d-h}
24	Teshale (Susceptible)	15.54^{cd}	1.36^{a-c}
25	Gobiye (Resistant)	3.43^{k-m}	1.02^{f-h}
	Mean	**11.39±2.47**	**1.129±0.11**
	LSD (5%)	**4.962**	**0.2138**
	CV (%)	**26.5**	**11.5**

Means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance; LSD= Least significant difference; CV= Coefficient of variation; HIP (%) = Haustorial initiation percentage and GI = Germination index

In comparison to the improved check variety (Gobiye), fourteen (14) of the tested genotypes had the highest percentage of haustorial initiation. Most of the sorghum genotypes that induced low germination rate and low maximum germination distance (Table 3) induced more haustorial initiation and vice versa (Table 3). This current observation agrees with the findings of [31] who stated that, germination signal and the haustorial initiation signal are independent in that neither has any activity of the other type and they are completely independently inherited. This is one of the reasons why low *Striga* seed germination stimulant production was chosen as tangible indicators of the host resistant to the *Striga*. Even though, the genetics of the haustorial initiation factor has not been reported, crop cultivars which produce *Striga* germination stimulants abundantly but that fail to produce the haustorial initiation signal would be uniquely useful. Apart from being resistant to *Striga*, such cultivars should also deplete the *Striga* seed population in the soil by promoting suicidal germination [32].

However, most sorghum host plants probably produce 2, 4-DMBQ since syringic acid is a ubiquitous metabolite of lignin biosynthesis and also because peroxide reaction is involved in most pathogenic processes [21]. This explains why sorghum genotypes differ relatively little in their capacity to produce the haustorial factor, compared to their wide differences in capacity to produce the germination stimulant [33].

3.4 Germination Index (GI)

Analysis of variance (ANOVA) showed very highly significant ($P \leq 0.001$) differences were

recorded on GI among the sorghum genotypes tested (Table 3). The result indicated that, out of twenty-five (25) sorghum genotypes assayed twenty-three (23) recorded a germination index greater than one, suggesting more germination events proximal to the root (Table 3). This showed that these sorghum genotypes had no inhibition influence on the germination of the *Striga* near the host root after the GR24 treatment. However, sorghum genotypes 2006 MW 6123 (0.93) and ETSC 300081 (0.97) recorded the lowest germination index which was less than one. This indicated *Striga* seed germinated near the roots of genotype 2006 MW 6123 and ETSC 300081 showed lower germination rates than the *Striga* of seeds germinated further out from the roots after GR24 treatment which suggested some inhibition of germination proximal to the root.

3.5 Correlation Analysis

Highly significant (P ≤ 0.01) correlations were observed between germination percentage and maximum germination distance (MGD), germination index and MGD, germination percentage and haustorial initiation percentage, using Pearsons' correlation coefficients, which is very important for initiation of any breeding program as it provides a chance for selection of desirable genotypes. However, significant correlation did not observe between haustorial initiation percentage and *Striga* seed germination percentage, maximum germination distance and haustorial initiation percentage (Table 4).

Highly significant (P ≤ 0.01) and positive correlation were observed between *Striga* germination rate and the maximum *Striga* germination distances from the source of the stimulant (Table 4). Sorghum genotypes that accounted for many germinations counts also stimulated *Striga* germination farthest away from the host root, while genotypes that allowed less germination rate exhibited a closer distance to the host root. A positive correlation (r = 0.726) was observed between the total number of *Striga* germination count and the distance from the root to the furthermost maximum germination distance (Table 4). These results are in agreement with the findings of [23] who found a similar agar gel assay with high positive correlation between percent germination of *Striga hermonthica* seeds induced by *Sorghum bicolor* and the distance of germination from the *S. bicolor* root. The positive correlation between maximum germination distance and *Striga* germination rates indicates better *Striga* resistance because of lower Strigo-lactones production which suggests low germination stimulant activity of the genotypes.

Generally, in this study the highest germination percent was recorded in the genotypes which had high maximum germination distance. An indication that the closer the *Striga* seeds to the source of stimulant the higher the amount of seeds elicited to germinate and vice versa. This spatial relationship between host roots and *Striga* seed germination as a function of the distance from the host root to where germination stimulant is still active in large enough concentrations to elicit germination was also reported by [34]. However, correlation observed between haustorial initiation percentage and maximum germination distance, germination percentage and haustorial initiation percentage was not significant.

An indication that the *Striga* germination stimulant signal and haustorial initiation factor were different. Compounds that stimulate *Striga* germination and the distance of *Striga* germination from the source of stimulant is one and the same which had no significant influence on haustorial initiation percentage on the genotypes tested. This is also in line with the work of [31] who stated as germination signal and haustorial initiation compound are independent and they are completely inherited.

Table 4. Correlation coefficient among the four parameters studied in bioassay laboratory

	MGD	GP	HIP	GI
MGD	1			
GP	0.726**	1		
HIP	0.141	0.174	1	
GI	0.378**	0.354**	0.528**	1

***. Correlation is highly significant at the 0.01 level; MGD= Maximum germination distance (mm); GP= Germination percentage (%); HIP= Haustorial initiation percentage (%); GI= Germination index*

4. SUMMARY AND CONCLUSSION

Sorghum (*Sorghum bicolor* L. Moench) is the major staple crop cultivated widely in the drought prone lowland areas of Ethiopia where *Striga* is prevalent. *Striga*, particularly *Striga hermonthica,* is the major parasitic weed affecting sorghum production in Ethiopia. It is difficult to manage this parasitic weed effectively because most of its damage to the host plant occurs underground before the parasitic plant emerges. The use of improved *Striga* resistant sorghum varieties had been suggested as the most feasible and useful controlling options for the small-scale sorghum growing farmers.

In bioassay lab, four parameters for *Striga* resistance included MGD, *Striga* germination rate near the host root, haustorium initiation percentage, and germination index. Maximum germination distance and germination rates of *Striga* near the host root were the tangible indicators of pre-attachment *Striga* resistant hosts. The present study showed a wide variation in pre-attachment *Striga* resistance in the sorghum inbred lines tested. Accordingly, the low germination stimulant sorghum inbred lines were identified. The results indicated significant differences for MGD, germination rates, germination index and haustorium initiation percentage. Sorghum inbred line 2006 MW 6044; ETSC 300080, ETSC 300081, 05 MW 6019, ETSC 300086, 2006 MW 6123, ETSC 300003, ETSC 300082 and 05 MW 6028

induced less *Striga* seed germination similar to the two resistant checks. These genotypes had low germination stimulants as low *Striga* germination levels observed in these genotypes and had potential for resistance to *Striga*. Maximum germination distances ranged from 0 to 21.72 mm were recorded and out of the twenty two (22) inbred lines eight (8) recorded a maximum germination distance of less than 10 mm (MGD<10). The least maximum germination distance (MGD) was recorded on inbred line 2006 MW 6044 (2.33 mm) which had also showed lowest germination rate near the host root. Inbred lines ETSC 300081, ETSC 300080, 05 MW 6073, ETSC 300086, 05 MW 6019, ETSC 300003 and ETSC 300087 also induced low germination distances and were not significantly different from the resistant checks.

The positive correlation (r = 0.726) between maximum germination distance and *Striga* germination rate indicates low germination stimulant activity of the inbred lines and their *Striga* resistance, which may be associated with low strigolactones production. Most of the inbred lines had germination index greater than one, indicating no inhibitory effect on *Striga* germination. Among the twenty-two inbred lines tested. ETSC 300003, 2006 MW 6123, ETSC 300081, 05 MW 6019, ETSC 300080, ETSC 300082 and 05 MW 6028 showed low germination stimulant in bioassay laboratory. Thus, these lines are the most promising sources of resistance to *Striga hermonthica*. The resistant varieties (Gobiye and SRN-39) and susceptible check Teshale were truly confirmed their resistant and susceptible respectively at bioassay laboratory.

Generally, it is recommended that the continuation of the experiments using the promising lines confirm their resistance. Future studies should focus on understanding host resistance mechanisms and improving field screening in numerous hotspot areas. As the response mechanisms of *Striga* resistance in sorghum genotypes are distinct, post-attachment and marker assisted selection should be expected to observe the real resistant genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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