

Genotypic Variation for Low Striga Germination Stimulation in Sorghum “*Sorghum bicolor* (L.) Moench” Landraces from Eritrea

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench), the second most important staple crop in Sub-Saharan Africa (SSA) after maize, is well adapted to marginal environments of drought stress and high temperatures. But besides drought stress, the obligate root-parasitic flowering plant *Striga hermonthica* is an equally economically important biotic stress in agro-ecological zones where soils are marginal. Notwithstanding widespread and intense *Striga* infestation, genetic variations in defence mechanisms against the parasite have been reported. Sorghum variants, producing low levels of chemical stimulants such as sorgolactones that deter the advance of *Striga* seed germination and are therefore deemed resistant to the parasite, have been also reported in a few studies. But the existence of sorghum genetic variation for this resistance especially among farmers' landraces is yet to be demonstrated. The objective of this study was therefore to determine the levels of *Striga* germination stimulants in response to each of the 111 collected sorghum landraces and their progenies from Eritrea. The ability of a sorghum genotype to cause germination of a *Striga* seed as a measure of the amount of the germination stimulant produced was used to assess the resistance of these accessions. The data were recorded as *Striga* germination percentage by counting the number of germinated *Striga* seeds. Landraces EG47, EG1261, EG830, EG1076, EG54 and EG746 with 14.68%, 15.32%, 11.85%, 13.05%, 15.74% and 16.5% germination percentages respectively were found to stimulate low levels of *Striga* germination percentage compared to commercial checks, IS9830, SRN39, Framida, with 22.46%, 22.67%, 23.27% germination respectively. While these variants did not show complete resistance against *Striga* seed germination, the low level

production of stimulant indicated their high level of resistance to *Striga*. These results implied that these accessions are likely potential sources of resistance against *Striga* infestation in SSA sorghum breeding programs.

Keywords

Eritrea, Landrace Sorghum, *Striga hermonthica*, *Striga* Germination Stimulants, Seed, Parasitic Plants

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is an important staple crop in Sub-Saharan Africa (SSA) that can meet the increasing demand of food [1]. Although, sorghum consumption is high in most SSA countries, the grain yield at the farm level is low due to the effect of biotic and abiotic stresses [2] [3].

The obligate root-parasitic flowering plant *Striga hermonthica* affects the lives of over 100 million people and infests about 40% of arable land in the savanna region [4]. *Striga* causes 75% of its damage before it emerges above the ground making its control more difficult [5]. Mechanical and chemical control options are less effective because they affect *Striga* after it has already attached and damaged the host [5]. Different control measures such as hand weeding, crop rotation trap crop, catch crops, intercropping, fertilizers and herbicides have also been suggested but with limited success. The many herbicides that have been tried have not been effective, and are costly and in most cases may not be available to resource-poor farmers in SSA.

In Eritrea, *Striga hermonthica* affects the majority of farmers especially in the western part of the country, where continuous mono-cropping is practiced [6]. A report by the African Agricultural Technology Foundation [7] indicated that 30,000 to 90,000 tonnes of grain sorghum is lost annually due to *Striga* in Eritrea. Annual yield losses due to *Striga* in neighbouring countries, for example, Sudan, Ethiopia, Kenya and Uganda are estimated at 1,060,000, 500,000, 50,000 and 40,000 tonnes respectively [7]. To minimize such yield losses, there is a need to devise control measures against the parasite.

In the past, crop improvement efforts have concentrated on host plant resistance as means of breeding against *Striga*. The use of resistant variety is considered to be more efficient and practical option for controlling *Striga* infestation. However, conventional breeding against the parasite has been slow and arduous [8]. A combination of host plant resistance mechanisms with molecular marker assisted selection (MAS) application will most likely yield promising results as shown in previous experiments [6].

Several mechanisms of resistance to *Striga* in sorghum have been reported that probably operate singly or in various combinations [9] [10]. Using *in-vitro* laboratory techniques, four specific mechanisms of resistance to *Striga* which included low production of germination stimulant, low production of the haustoria initiation factor, hypersensi-

tive response, and incompatible response were reported in cultivated sorghums and some wild accessions [5] [11].

Low germination stimulant variants of sorghum produce insufficient amounts of the exudates required for germination of conditioned *Striga* seed. Reduction in amounts of germination stimulants produced by host plants provides the means to reduce numbers of seeds germinating [12]. Low or no stimulant production by cereal roots has been reported to be a mechanism of host plant resistance to *S. hermonthica* infections [13] [14]. Sorghum variants that produce low levels of the germination stimulants have been found to be resistant to *Striga* in field tests [15]. Highly susceptible sorghum variants appeared to be high producers of the germination stimulants [5]. This study tested the germination stimulant production reaction of landraces from Eritrea and that of commercial cultivars and identified genotypes with low levels that may be described as having resistance to *Striga*.

2. Materials and Methods

2.1. Plant Materials

Seeds of *Striga hermonthica* were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) sub-station Kibos. They were collected in 2011 from sorghum growing fields at Kibos (00°04'S, 34°48'E, 1214 m altitude) using standard protocols [16]. At the time of use the *Striga* seeds were 4 years of age. Sorghum landraces were sourced from National Agricultural Research Institute (NARI) of Eritrea which was collected from sorghum growing zones of Gashbarka, Anseba, Southern zone and Northern red sea regions of the country [17]. Elite backcross lines, improved varieties and commercial checks were included in the experiment as indicated in **Table 1**.

2.2. *Striga* Seed Conditioning

Striga hermonthica seeds, to respond for a germination stimulant, have to be conditioned by exposing them to favorable moisture and temperature for two weeks [18]. To condition *Striga* seeds, they were initially surface disinfected for 5 minutes in a mix of 1% sodium hypochlorite containing 0.02% (v/v) Tween 20 [19]. Floating seeds and debris were discarded. The remaining seeds were rinsed using sterile distilled water and

Table 1. Summary of sorghum germplasm used in the study.

Germplasm	Number	Source
Landraces	86	NARI
Improved varieties	5	ICRISAT-Nairobi
Elite crossed lines	17	NARI, ICRISAT
Commercial check	3	ICRISAT-Nairobi
Total	111	

NARI = National agricultural research institute, ICRISAT = International Crops Research Institute for the Semi-Arid Tropics.

later air dried under laminar flow hood. Moistened double layer of 90 mm diameter Whatman no.1 filter papers were placed in a 90 mm sterile petridish. The air dried *Striga* seeds were sprinkled on the glass- fiber discs (Whatman GF/C) so that each disc had 20 - 30 *Striga* seeds and then incubated at 30°C for 14 days [12] [16].

2.3. Experiment Setup

The experiment was conducted in laboratory and screen house at BecA-ILRI Hub, Nairobi, Kenya. Each sorghum accession was planted in a screen house in a 10 cm diameter pot containing sand that was sterilized in a preheated oven at 85°C for 30 minutes. Each pot carried 8 - 10 plants which allowed harvesting at least 1 gram of root. Planting was done at the same date where *Striga* seeds were placed in an incubator for conditioning to synchronize for maximum stimulant production which occurs during the early stage of root development [12]. The seedlings were grown for two weeks. The two weeks old sorghum seedlings were then gently removed from the pot and the roots washed.

For testing germination of *Striga* seeds, the washed roots were cut in to small pieces of about 0.5 cm and 1gram was weighed. Four radial rows of fiber-glass-discs containing conditioned *Striga* seeds were arranged around 1.5 cm diameter aluminum foil ring centered on double layer of Whatman no.1 filter paper moistened with 3 ml of double distilled water in a 90 mm petridish [20]. Then 1 gram of the cut root pieces was placed in the aluminum foil ring and 3 ml of double distilled water added to defuse root exudates across the filter paper as described by [12] [21]. GR24 and double distilled water were used as positive and negative controls, respectively. The Petri dish was then sealed using parafilm then wrapped with aluminum foil and placed at 30°C for 48 hours in an incubator for *Striga* germination [16]. GR24 is a synthetic germination stimulant which is available commercially, is a chemical analog of strigolactones. The stock was prepared as 100mg of GR24 in 10ml of acetone and then diluted with sterile distilled water, a 1 litter stock solution (100 mg·L⁻¹) was made and used at a final concentration of 0.01 mg·L⁻¹.

2.4. Data Recording and Analysis

Following after 48 hours of receiving the *Striga* germination stimuli, *Striga* germination count was done under dissecting microscope by counting the number of *Striga* seeds in each fiber glass discs that had germinated as described by [16]. A seed was considered as germinated if the radicle was seen protruded through the seed coat.

Percentage germination of *Striga hermonthica* seeds were calculated for each treatment. Analysis of variance (ANOVA) was carried out using Genstat®15th Edition (<http://www.vsnl.co.uk>). Treatment means were separated using the least significance difference test at 5% level. Statistical analysis for percent *Striga* germination data was performed after logarithmic transformations using the formula (log (X + 1), where X is the original individual observation) [22]. Correlations between percent *Striga* germination and distance from the source of *Striga* germination stimulant were also performed.

3. Results and Discussion

All sorghum accessions used in this study germinated well in the pots. This enabled the harvesting of at least 1 gram of root from each accession which was required as source of *Striga* germination stimulant in the study. As defined by Ramaiah *et al.* 1990, [23] the term stimulant, refers to that component of the sorghum root exudates that germinate the strain of *Striga hermonthica*. Analysis of variance for *Striga* germination revealed that highly significant differences ($P < 0.001$) were observed among the sorghum accessions tested for their ability to cause *Striga* germination with a range of 11.8 to 40.6% (Table 2). *Striga* seeds germinated at different levels along the radial position in the petri dish in all sorghum variants, indicating the presence of different levels of germination stimulants. This is in agreement with the work of Karaya *et al.* 2012, who studied the variability of *Striga* germination stimulant levels in maize [12].

Accession EG1168 stimulated the highest germination of *Striga* seeds ($40.3\% \pm 4.9$) compared to the rest of accessions. On the contrary accession, EG830 induced the lowest level of *Striga* germination ($11.85\% \pm 2.4$). Such low *Striga* germination percent may indicate a potential for resistance to *Striga*. No *Striga* germination was observed in the negative control (double distilled water) while the positive control GR24 exhibited 43.73%, which was not significantly different from germination observed with sorghum accession EG1168 ($40.6\% \pm 4.9$). However, all the rest of the sorghum accessions induced significantly lower *Striga* germination compared to the GR24. Similar results were reported by [12].

The top 10 genotypes induced less than 18% *Striga* germination (Figure 1), while the commercial checks, IS9830, SRN39 and Framida caused 22.46, 22.67 and 23.27% germination, respectively. No significant differences were observed among these commercial checks. However, *Striga* germination in at least one of the tested landraces, namely accession EG830 had significantly lower ($\text{Prob} \leq 0.05$) germination than that of the commercial varieties. The five sorghum accessions with the lowest *Striga* germination were EG830, EG1076, EG473, EG 1261 and EG546 which caused *Striga* germination percentages of 11.85, 13.05, 14.68, 15.32 and 15.74, respectively. Even though these five accessions did not show total immunity against *Striga* seed germination, as there is no reported complete resistance to *Striga* so far in sorghum [5], the expression of low percentage level of stimulant production was an indication of their high level of resistance to *Striga*. Low *Striga* germination suggests low germination stimulant production. Low level of germination stimulant produced by host plant may 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].

The level of *Striga* germination and the distances from which stimulants were released is shown in Figure 2. Germination percent was high near to the source of stimulant, which suggests that the higher the concentration of the stimulant, the higher the *Striga* germination percent. As the distance from the source of *Striga* stimulant increased, the germination percent was significantly reduced to below 15%. In this study,

Table 2. Levels of *Striga* germination percent exhibited by the sorghum accessions tested.

Rank	Entry	Accession name	Accession source	<i>Striga</i> germination percent (%)
1	83	EG830	GB	11.85
2	94	EG1076	AN	13.05
3	2	EG473	GB	14.68
4	70	EG1261	GB	15.32
5	14	EG546	AN	15.74
6	86	EG898	GB	16.24
7	92	EG746	S	16.5
8	67	EG1256	GB	17.12
9	93	L2P3	NARI-cross	17.66
10	32	EG801	AN	18.22
11	85	EG1258	GB	18.23
12	73	EG2457	NRS	18.37
13	109	IESV 23010	ICRISAT	18.37
14	62	EG1208	NRS	18.39
15	111	L2P5P15	NARI-cross	18.72
16	64	EG1235	GB	19.05
17	91	ICSV111	ICRISAT	19.25
18	69	EG1259	GB	19.3
19	65	EG1237	S	19.87
20	112	L2P5P35	NARI-cross	20.19
21	88	EG806	GB	20.2
22	3	EG480	GB	20.44
23	82	EG881	GB	20.47
24	56	EG896	GB	20.74
25	81	EG1246	S	20.93
26	5	EG497	AN	21.09
27	29	EG789	GB	21.12
28	71	EG2161	NRS	21.23
29	28	EG787	GB	21.37
30	33	EG745	S	21.56
31	61	ICSV 111-2	ICRISAT	21.6
32	57	EG1224	GB	21.81
33	54	Hamelmallo	AN	21.99
34	89	EG896	GB	22.14
35	97	L3P3	NARI-cross	22.41
36	12	IS9830	ICRISAT	22.46
37	1	EG469	GB	22.65
38	101	SRN39	ICRISAT	22.67
39	78	EG786	GB	22.98
40	26	EG779	S	22.99

Continued

41	47	EG873	GB	23.04
42	113	L2P7	ICRISAT	23.17
43	100	Framida	ICRISAT	23.27
44	84	EG1076-2	AN	23.39
45	30	EG791	GB	23.83
46	43	EG2456	NRS	23.87
47	51	EG889	GB	23.9
48	72	EG2453	NRS	23.92
49	76	EG794	GB	24.03
50	53	EG893	GB	24.17
51	38	EG845	GB	24.33
52	40	EG849	GB	24.33
53	87	EG864	GB	24.41
54	55	EG1075	NRS	24.44
55	105	L2P2P8	NARI-cross	24.7
56	36	EG836	AN	24.79
57	44	EG858	S	24.94
58	37	EG843	GB	25.26
59	68	EG1257	GB	25.41
60	95	L1P5	NARI-cross	25.42
61	31	EG797	GB	25.47
62	110	L2P6	NARI-cross	25.64
63	90	Kibra	AN	25.71
64	17	EG717	GB	25.88
65	13	EG554	S	26
66	104	L2P5P25	GB	26.01
67	34	EG813	GB	26.05
68	8	EG540	GB	26.07
69	39	EG846	GB	26.23
70	103	L2P5P20	S	26.26
71	15	EG557	S	26.41
72	20	EG750	S	26.41
73	108	L1P4	NARI-cross	26.41
74	66	EG1239	NRS	26.65
75	41	EG850	GB	26.66
76	98	L2P3	NARI-cross	26.72
77	4	EG494	GB	26.83
78	16	EG584	GB	26.83
79	46	EG870	GB	26.88
80	52	EG890	GB	27.14
81	63	EG1233	GB	27.16
82	19	EG855	GB	27.18

Continued

83	75	EG806	GB	27.24
84	96	Macia × IS2205	ICRISAT	27.51
85	27	EG782	GB	27.56
86	49	EG883	GB	27.91
87	77	EG532	S	28.09
88	35	EG815	GB	28.1
89	106	L2P2P24	NARI-cross	28.1
90	99	L1P2	NARI-cross	28.11
91	11	EG547	GB	28.2
92	80	EG735	GB	28.33
93	79	EG726	S	28.41
94	58	EG1157	NRS	28.64
95	7	EG537	S	28.88
96	48	EG875	GB	28.92
97	107	L3P1P4	NARI-cross	29.1
98	18	N13	ICRISAT	29.51
99	10	EG544	S	29.85
100	102	Hariray × IS2205	ICRISAT	29.95
101	74	EG538	S	31.38
102	21	EG756	AN	31.62
103	9	EG526	AN	31.67
104	6	EG519	GB	31.9
105	50	EG885	GB	31.93
106	45	EG859	S	32
107	22	EG711	NRS	32.22
108	42	EG857	GB	32.67
109	60	EG1172	NRS	32.96
110	23	EG723	AN	33.4
111	59	EG1168	NRS	40.6
	24	GR24 (positive control)		43.73
	25	Water (negative control)		0
		Mean		24.45
		L.S.D		8.838
		CV (%)		26
		SIG		***

*** = highly significant ($P < 0.001$), L.S.D = least significant difference, CV = coefficient of variation, AN = Anseba, GB = Gash Barka, NRS = Northern red sea, S = South.

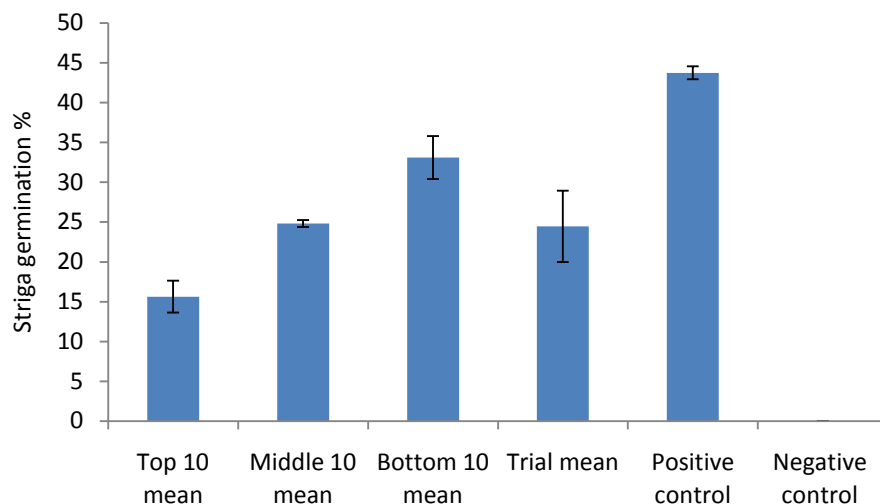


Figure 1. Percent *Striga* seed germination category of sorghum accessions and their control.

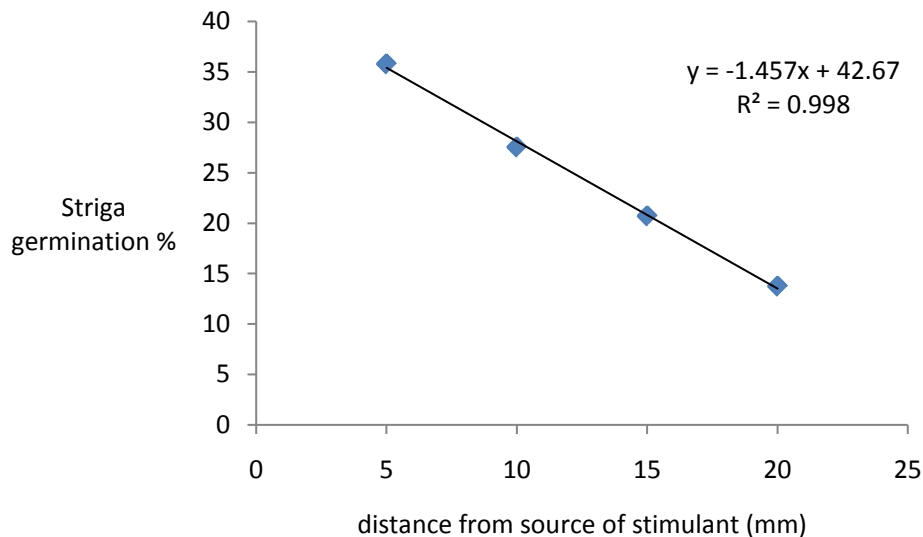


Figure 2. Correlation between *Striga* percent seed germination and the distance (mm) from the source of *Striga* germination stimulant.

the highest germination was recorded on discs which were nearer to the source of stimulant compared to those farther off. Highly significant ($P < 0.001$) and positive correlation coefficients were observed between *Striga* germination and the distances from the source of the stimulant. An indication that the closer the *Striga* seeds to the source of stimulant the higher the amount of seeds stimulated to germinate and vice versa. This result corroborates previous work on variation in *Striga* germination stimulants production in maize [12]. Similarly, reports by [25] indicated that germination stimulant produced by the host plant is mainly exuded in a distance close to radius from the root apex. Support for 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 active to elicit germination was documented [26].

The regression equation $y = -1.4576x + 42.67$ in **Figure 2** implies that for every unit increase of distance from the stimulant, the germination percent of the *Striga* seed is expected to decrease by about 1.4576 percent. The negative slope of the fitted line in **Figure 2** also suggests that decrease in *Striga* germination percent were associated with increased distance from the source of *Striga* germination stimulant. The high coefficient of determination ($R^2 = 0.998$) indicates the variation in germination percentage was almost all explained by the variation in the distance of concentration of *Striga* germination stimulants.

In sorghum, four compounds of root exudates which include sorgoleone, sorgolactone, strigol and a water-soluble compound with a quantitative biosynthetic pathway are reported as germination stimulants [27]. Therefore, it is possible that these stimulants were also produced by the accessions used here.

Low *Striga* germination levels observed in some of the accessions tested in this study may be due to low production of germination stimulant, which is one of the best known mechanisms of resistance in *Striga* [11]. This low germination stimulant production is of special interest in breeding for resistance to *Striga* in sorghum. Low induction of seed germination has been successfully used in sorghum breeding for resistance to *Striga hermonthica* [28]. Ejeta and coworkers selected sorghum lines with reduced induction of germination in their breeding programs [29]. A wide range of sorghum of low stimulant lines has shown resistance in the field which indicates the usefulness of low stimulant form of resistance [23]. Identification of genotypes with low germination stimulant from the current study will play a crucial role in the improvement of sorghum cultivars for *Striga* resistance. Since the identified accessions are landraces which are adapted to the local environmental conditions of the country, they can be included directly in the sorghum breeding program for *Striga* resistance.

4. Conclusion

The accessions with low *Striga* germination stimulant producers identified in this study, namely EG830, EG1076, EG473, EG 1261 and EG546 caused lower germination percent of *Striga* compared with the commercial controls. These accessions may be useful potential sources of resistance to *Striga* as such or in a backcross breeding program. It would be interesting to confirm whether the mechanical type of *Striga* resistance that has been mapped using Quantitative Trait Loci (QTL) and reported elsewhere [10] would be found in genotypes with low stimulants production. In order to consolidate this resistance, these accessions of low stimulant production could be crossed with the already identified backcrosses with intro-gressed *Striga* resistance QTL from a previous study [6]. Such resistance to *Striga* in sorghum, resulting from a combination of two mechanisms, would be more durable and stable across ecological zones than one based on single gene resistance sources.

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