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Introgression of *Striga* resistance gene into Sudanese Sorghum Variety Arfagadamek-8 (AG8)

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Abstract: *Different control strategies for the parasite were developed but they were either expensive or complex for farmers to afford or adopted. Resistance crop cultivars are found to be the most economical and effective control option. Sorghum wild relatives are recognized as the main genetic resource for novel genes to tackle untractable problems. The objective of this study was to improve sorghum production aided by introgression of Striga resistance genes from wild relatives into farmer preferred varieties. Nine sorghum wild relatives' accessions were screened for Striga resistance in infested plot at Gezira Research Station Farm of the Agricultural Research Corporation (ARC), during season 2013/14. Wild sorghum accessions were crossed with a farmer preferred variety AG8, and BC3F3, (113), progenies were generated. BC3F3 derived progenies were screened for Striga resistance in field infested plot at Gezira Research Station Farm of the ARC during season 2016/17 and 2017/18. Wild accessions and BC3F3 derived progenies were also screened for their Striga resistance in the laboratory, using the Extended Agar Gel Assay (EAGA). No Striga emerged plants were mostly observed in wild sorghum accessions, WSA-1 and WSA-4 at 75 and 120 days, while all wild accessions with expectation of WSB-1 and WSB-2 showed a few Striga emerged plants compared to the checks. Results indicated that BC3F3 progenies grain yield were negative and significantly correlated with Striga count at 60, 75 and 90 days after sowing. Laboratory studies revealed that sorghum wild relatives WSA-1 and WSA-2 have the lowest Striga germination index (0.94 and 0.71), respectively. The wild accessions WSA-1, WSB-2, WSB-1 and WSA-2 have the lowest Striga germination distance (0.77, 0.89 and 0.98 cm) respectively. The laboratory studies showed that BC3F3 progenies 29, 35 and 47 have low Striga germination, less than 3%, progenies 58, 71 and 115 less than 10% and zero germination distance for progenies 88 and 96.*

Keywords: *Striga*, wild sorghum, AG8

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L). Moench) is the fifth most important cereal crop worldwide (FAOSTAT, 2011). It is a diploid grass (2n=20) belong to the family Poaceae [1]. The total cultivated area mounted to 42 million hectares with a total production of 62 million tons annually [2]. In semi-arid areas, sorghum is used as food, feed and recently as biofuel [3]. It is also used in the manufacturing of different products such as wax and adhesives [4]. In Africa, sorghum production is still low due to constraints associated with, the low use of improved varieties, farmers often use low yielding land races, the use of nutrient depleted soils, low and erratic rainfall and pests and diseases complex.

In Sudan, a number of improved sorghum varieties were released by the ARC for both irrigated and rain-fed. In the irrigated sectors, the major constrains of sorghum production are the minimum adoption of husbandry and protection technologies for both biotic and abiotic stresses.

Striga is a parasitic weed belongs to the family Orobanchaceae (formerly: Scrophulariaceae), is one of the major biotic constraints to cereal production in sub-Saharan Africa [5]. It parasitizes cereal crops such as rice (*Oryza glaberrima* Steudel and *O. sativa* L.), pearl millet (*Pennisetum glaucum* L. R. Br., maize (*Zea mays* L.) and sorghum. Several species from over fifty *Striga* species, affect cereal and legume crops production in sub-Saharan Africa and Asia [6].

Different control measures were suggested, however, the use of a single option on its own has proven to be both sufficiently ineffective and endurable as well as not economically and practically applicable for low-input farming systems [7]. Integration of multiple control options is the best approach for *Striga* management [8]. The integrated *Striga* management approach is cheap, simple and could be adopted for low-input, small scale farmers in Africa [7]. Resistant crop cultivars were recognized as the most cost-effective control option and low input systems compatible [9].

The host plant resistance mechanisms are based on the interactions between the parasite and the host plant [10; 11; 12]. The resistance in the host may limit the number of emerged *Striga* plants [13], or may reduce the impact of *Striga* on the host plants [14]. Tolerance is the ability of the host to support equally severe levels of infestations without associated yield losses [1]. Host plant resistance expression could either before the parasitic attachment (e.g., low germination stimulants and low haustorial initiation factor) or after attachment (e.g., hypersensitive response) [15].

Sorghum wild relatives are recognized as abroad genetic base reservoirs and potential sources for important genes for pest resistance and other adaptation traits [16; 17]. Reference, [17], reported that resistance mechanisms against the parasitic weed *Striga* have been found in wild sorghum. Reference [10], reported that wild sorghum genotype P47121 (*S. bicolor* ssp. *drummondii*) expressed resistance through reduction of haustoria formation. Sorghum wild relatives are resources for natural resistance or tolerance genes that could be used in breeding programs [18]. These genes could be used for different resistance mechanisms for more stable, polygenic

durable resistance and to transfer resistance or tolerance genes into well-adapted genetic backgrounds [19]. The present study was undertaken to improve production and productivity of sorghum aided by introgression of novel genes for *Striga* resistance from wild relatives of sorghum.

2. MATERIALS AND METHODS

2.1 THE PLANT MATERIALS

Gene introgression was based on a series of backcrosses, performed to add *Striga* resistance from a donor parent to adapted Sudanese sorghum cultivar Arfagadamek-8 (AG8) that selected as the recurrent parent for backcrossing. Arfagadamek-8 is a cultivar that has valuable agronomic characteristics, but lacks resistance to *Striga* parasitism. The procedure was carried out by using the cultivar AG8 as the parent while pollen was obtained from the resistant wild sorghum accessions. AG8 was crossed with nine wild sorghum (WS) accessions in seasons 2013-2016. The accessions were designated from WS-1 to WS-9. The generated F_1 plants were backcrossed to the recurrent parent, AG8, to obtain 117 BC_1F_1, 117 BC_2F_1 and 117 BC_3F_1 derived progenies, then for two successive generations to generate 113 BC_3F_3 progenies. Wild accessions were morphologically classified [20] (Table 1).

A total of 113 lines and their checks (Wad Ahmed, Tabat and AG8) were evaluated for their resistance in *S. hermonthica* infested plot at Gezira Research Station farm at the ARC for two consecutive seasons 2016/17 and 2017/18. The standard cultural practices adopted for sorghum were followed. The plot size was arranged in two rows five meters long with inter and intra row spacing of 30 cm 80 cm, respectively. The five seeds were sown in a hill and then thinned to three plants per hill. Urea at 40 kg urea/fed was applied. For artificial infestation, *S. hermonthica* seeds were mixed with soil at 1mg/kg and the mixture planted at 5g/hole. The crop was kept weed-free and irrigated every two weeks or whenever necessary. *S. hermonthica* parameters were total number of emerged *Striga* plants every two weeks start at 45 days after sowing till harvest. Genotypes' grain yield (kg ha^{-1}) was obtained at harvest.

Table 1. Classification of wild sorghum accessions

Wild sorghum accessions	Scientific name
WSB-1	*Sorghum bicolor* (L.) Moench
WSB-2	
WSB-3	
WSA-1	*Sorghum arundinaceum* (Desv.) Stapf.
WSA-2	
WSA-3	
WSA-4	
WSA-5	
WSD-1	*Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet & Harlan.

2.2 LABORATORY SCREENING

The extended agar gel assay (EAGA) was undertaken at the Weed Research Laboratory, ARC to screen for *Striga* resistance among sets of BC_3F_3 lines and the parental genotypes using the EAGA. Mechanisms of resistance intervening before and after parasite attachment notably the low germination stimulant production, low haustorium initiation factor, hypersensitive response and an incompatible reaction. Three seedlings were assayed for each line and the parental genotypes. The germination stimulant production scored as the vertical distance between the host root and the furthest germinated *Striga* in the plate.

2.3 SURFACE STERILIZATION OF SORGHUM SEEDS

Sorghum seeds were soaked in 10 ml 1% sodium hypochloride (NaOCl) solution for 2-3 min and rinsed three times with distilled water. The seeds were transferred to petri-dishes that contained moist filter paper and incubated in the dark at 28°C for 24h. Healthy germinated seeds were selected for the extended agar gel assay [21].

2.4 Surface sterilization of *Striga* seeds and conditioning

Striga seed surface sterilization and conditioning was performed using the procedure described by reference [22]. Seeds were added to 10 mL in a 50-mL flask containing 3 - 5 drops of Tween 20, followed by removal of as much sand and debris as possible with a pipette, and sonicating the seeds for 2 min with occasional swirling. After sonication, the remaining sand/debris and water were removed with a pipette. The seeds were rinsed-3 times with H_2O depending on their cleanliness. Seeds were allowed to settle before pipetting to reduce loss. Seeds were sonicated and swirled 3 - 4 times a minute in a flask containing Metricide diluted 10 times. The seeds were then rinsed 3 times in 10 mL ddH_2O. 4mL of ddH_2O and 1.5 mL of a 0.015% Benomyl [methyl-1-(butylcarbomyl)-2-benzimidazolecarbamate] solution were then added to each flask followed by 10 mL of sterile water. The flasks were then placed in a 28°C incubator to begin conditioning. Every 3- 4 days, under a laminar air hood, seeds were pipetted into fresh sterile flasks containing 15.5 mL of the Benomyl solution and returned to the incubator.

2.5 Extended Agar Gel Assay (EAGA)

The assay is a modification of the agar gel assay described by reference [23]. In the EAGA, large petri dishes with a thick agar layer were used to support growth of sorghum seedlings for a longer period of time. Around 1500 *Striga* seeds (4 drops

of settled seeds) conditioned for 8 - 22 days were pipetted using a sterile 150mm petri dish. A 0.7% agar solution was autoclaved for 15 min, and then cooled to 50°C for at least one hour. The 50°C agar was poured into the petri dish containing conditioned *Striga* seeds, which produced an even distribution of seeds. Three pre-germinated sorghum seeds were placed at even intervals along the edges of each dish so that the radicles just penetrated the gel. The dishes were then covered and placed in an incubator at 28°C.

Three days following inoculation, each dish was observed for germination, attachment and host root development.

Data were collected under the microscope where the entire length of the host seedling root is scanned under a magnification of at least ×25. The dishes were observed at 2, 5, and 7 days after pouring plates for *Striga* attachment, and number of attachment sites was recorded. Sites of attached *Striga* were circled on the petri dish for future observation of necrosis and parasitic discouragement. A *Striga* seedling is counted as having a haustorium only if hairs like projections (tubercles) are present on the radicle. The three most distant *Striga* (with haustoria) from the seedling root were identified and the shortest distance to the primary host was measured to the nearest 0.5 mm.

3. Results and discussion

Breeding for *Striga* resistance has always been a hope for *Striga* management. However, breeding for *Striga* resistance has been very slow due to the limited sources of the resistance in the cultivated sorghum. Also, there is no complete resistance in the cultivated sorghum which makes the reported resistance always inoculum dependent and highly affected by the environment. To enhance *Striga* breeding efforts a large collection of sorghum wild relatives was evaluated under *Striga* pressure.

The result showed that, all wild sorghum accessions exhibited no emerged *Striga* plants at 45, 60 and 75 days after sowing compared to the checks (Fig. 1).

Wild sorghum accessions WSA-1and WSA-4 also observed no emerged *Striga* plants at 120 days (Fig.1). Wild sorghum accessions WSB-1 and WSB-2 showed only emerged *Striga* plants similar to the Wad Ahmed and AG-8 at the 120 days (Fig. 1). Results indicated that the wild sorghum accessions posses' resistance/ tolerance to *S. hermonthica* parasitism even when high number of emerged *Striga* plants was observed.

BC_3F_3 grain yield was correlated with the *Striga* count. In season 2016/17, the grain yield was negatively significantly correlated with *Striga* count at 45 DAS (-0.359 ***), *Striga* count at 60 DAS (-0.417***), *Striga* count at 75 DAS (-0.346***) and *Striga* count at 90 DAS (-0.381***) (Table 2). In season 2017/18, the grain yield negatively and significantly correlated with *Striga* count at 60 DAS (-0.220*), *Striga* count at 75 DAS (-0.264**) and *Striga* count at 90 DAS (-0.365***) (Table 3).

Reference [19] reported that for resistant genotypes a reduced relative yield loss per aboveground *Striga* plant indicates tolerance, whereas for less resistant genotypes the relative yield loss as such provides the best indication. Reference [24] stated that observed reduction and delay in *Striga* emergence may be attributed to reduced germination, reduced haustorium initiation and attachment. Reference [25] pointed out that the genetic differences between sorghum cultivars affect time of parasite attachment.

3.1 Striga seed germination using EAGA

Stimulation of *Striga* seeds germination through the root exudates of wild sorghum accessions was carried out using EAGA. Wild sorghum accessions, WSA-5, WSA-1 and WSA-2 showed *Striga* seed germination index of 2.64, 0.94 and 0.71, respectively (Table 4). The other wild sorghum accessions displayed *Striga* germinate index ranged from 1.00 for WSA-4 to 1.74 for WSD-1 (Table 4). The germination distance means the vertical distance of furthest *Striga* seed germinated away from the host root. The highest germination distance was observed in wild sorghum accession, WSA-3 (1.24) while the lowest germination distance 0.77 observed in wild sorghum accessions, WSA-1 and WSB-2 (Table 4). Wild sorghum accessions WSB-1, WSB-2 and WSB- 6 accessions observed variations in germination distance (Table 4). It worth noting wild sorghum accessions WSA-4 and WSD-1 have same germination distance (Table 4). In conclusion with other reports, wild sorghum as an important gene reservoir for *Striga* resistance, such as low germination stimulant production, germination inhibition and low haustorium initiation factor (Rich *et al.*, 2004). It is also reported *Striga* seed germination in sorghum is linked to the production of sorgoleone [26] and sorgolactones [27].

According to reference [23], a genotype with mean germination distances less than 10mm is considered as a low germination stimulator, and if it more than 10 mm considered as a high germination stimulator

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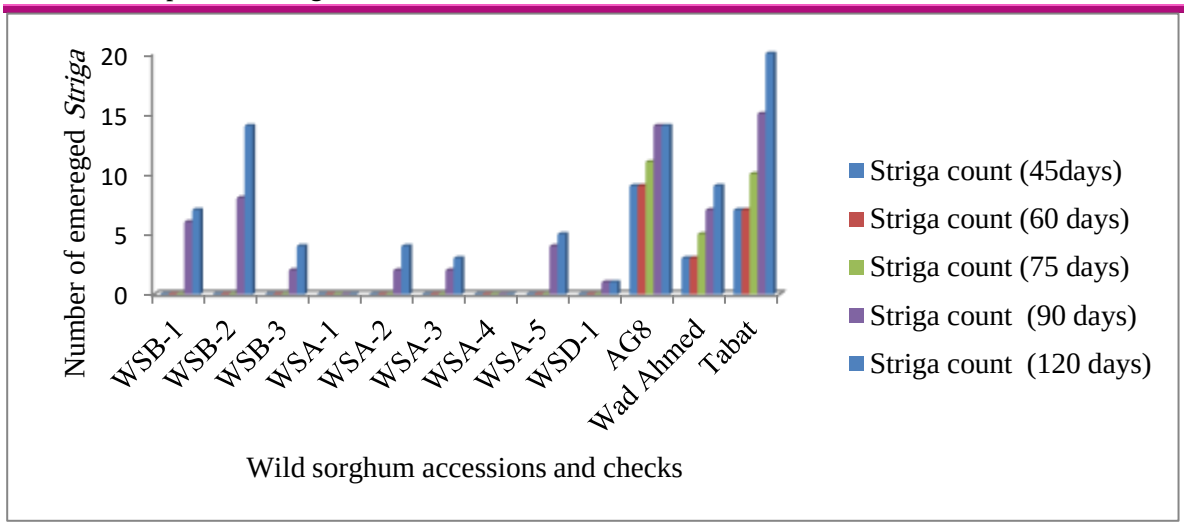

Fig. 1. Number of emerged *S. hermonthica* by wild sorghum accessions in *S. hermonthica* infested plot in comparison to the checks, Wad Ahmed, Tabat and AG8

Table 2. Simple linear correlation coefficients among *Striga* traits on BC_3F_3 sorghum progenies grain yield at ARC, Wad Medani, season 2016/17.

	1st Count	2nd Count	3rd Count	4th Count	Grain yield
1st Count	1				
2nd Count	0.815***	1			
3rd Count	0.782***	0.864***	1		
4th Count	0.709***	0.735***	0.808***	1	
Grain yield	-0.359***	-0.417***	-0.346***	-0.381***	1

*** Significant at P= 0.001 level.

Table 3. Simple linear correlation coefficients among *Striga* traits on BC_3F_3 sorghum progenies grain yield at ARC, Wad Medani, season 2017/18.

	1st Count	2nd Count	3rd Count	4th Count	Grain yield
1st Count	1				
2nd Count	0.602***	1			
3rd Count	0.488***	0.914***	1		
4th Count	0.315***	0.768***	0.840***	1	
Grain yield	0.165*	-0.220*	-0.264**	-0.365***	1

*, *** significant at P=0.05, 0.001 level.

Table 4. *Striga* seed germination and germination distance of wild sorghum accessions using EAGA

Wild sorghum accessions	*Striga* seed germination	Germination distance (cm)
WSB-1	1.14	0.89
WSA-1	0.94	0.77
WSB-2	1.14	0.77
WSA-2	0.71	0.98
WSA-3	1.66	1.24
WSB-3	1.31	1.10

WSA-4	1.00	1.11
WSA-5	2.64	1.12
WSD-1	1.74	1.11
AG-8	2.75	1.3
Tabat	2.9	1.4
CV (%)	45.4	32.5
SE±	0.61*	0.34

Wild sorghum accessions WSB-1, WSB-2, WSA-1 and WSA-2 exposed less mean germination distance (<10 mm) should be classified as low germination stimulator while wild sorghum accessions WSA-3, WSB-3, WSA-4, WSA-5 and WSD-1 had high mean germination distance (>10 mm) classified as high germination stimulator. Reference [21] also reported that *S. drummondii* (P 78) as a low germination stimulator and *S. arundunicum* (P47121) as a high germination stimulor using the EAGA assay. He also confirmed that *S. drummondii* (P78) also had low haustorium initiation factor. Reference [17] found wild sorghum, *S. drummondii* (PQ-434) presented low germination stimulant and low haustorium initiation factor. Reference [28] indicated wild sorghum accessions *S. aethiopicum* and *S. arundunicum* had high levels of post-attachment resistance and low resistance exhibited by *S. drummondii* accessions to three common *Striga* ecotypes in Kenya. He also mentioned that one accession of *S. aethiopicum* and the two accessions *S. arundunicum* have the lowest number of *Striga* attachments and biomass compared to resistant sorghum variety N13. Several studies indicated that *S. drummondii* had least resistance level compared to other wild sorghum accessions [11].

In this study, no *Striga* penetrations into the host endodermis for wild sorghum accessions were observed. The penetration failure is suggested either an active host defense responses or mechanical barriers as suggested by earlier studies. This result is agreement with reference [28] who indicated that mechanical barriers inhibited haustorium penetration into *S. arundunicum* accessions endodermis and released secondary metabolites due to biochemical interaction between the host and the parasite that inhibited haustorium penetration into the host's endodermis of *S. aethiopicum*. He also stated that *S. arundunicum* and *S. aethiopicum* are highly resistance to *Striga* compared to N13.

3.2 BC_3F_3 sorghum progenies

Striga resistant wild sorghum accessions and their BC_3F_3 derived progenies generated from their crosses with a susceptible parent AG8, were evaluated for *Striga* seed germination (Fig. 2-7). Seventeen BC_3F_3 progenies, which resulted from cross between wild sorghum accession (WSB-1) and sorghum variety, AG8. Results indicated that BC_3F_3 progenies13, 14 and 15 gave low *Striga* seed germination percentage (15-23%) while BC_3F_3 progenies 1, 2, 5, 6, 9, 10 and 17 showed germination percentage over 70% (Fig. 2).

BC_3F_3 eighteen progenies were generated from a cross with wild sorghum accession WSA-1and sorghum variety AG8. Results specified BC_3F_3 progenies 18, 22, 26, 32 and 33 have high *Striga* seed germination percentage (Fig. 3). *Striga* seed germination for BC_3F_3 progenies 19, 21, 23, 27 and 28 was less than 15% and BC_3F_3 progenies 29 and 35 have germination percentage of 3% (Fig. 3).

Crossing of wild sorghum accession WSB-2 with sorghum variety AG8 resulted in sixteen BC_3F_3 progenies. Progenies 37, 41, 44 and 45 gave high means of *Striga* seed germination percentage 75-100% (Fig.4). Progeny 47 showed the least germination percentage (3%) compared to WSB-2 (Fig. 4).

For the thirty- two progenies resulted from a cross of wild sorghum accession WSA-2 and AG-8, progenies 56, 60 and 64 resulted in *Striga* seed germination percentage less than 25% (Fig. 5). Progenies 58 and 71 gave *Striga* seed germination percentage less than 10% (Fig. 5).

Twelve progenies were resulted from a cross between WSA-3 and AG-8. Progenies 88 and 96 observed no *Striga* seeds germination (Fig. 6).

Cross of WSA-4 with AG-8 generated nineteen BC_3F_3 progenies. Progenies 109 and 113 had low *Striga* seeds germination percentage (10-20%) and progeny 115 had zero *Striga* seeds germination (Fig. 7).

These finding agreed with reference [28] who revealed that *S. arundunicum* is the most resistance wild sorghum.

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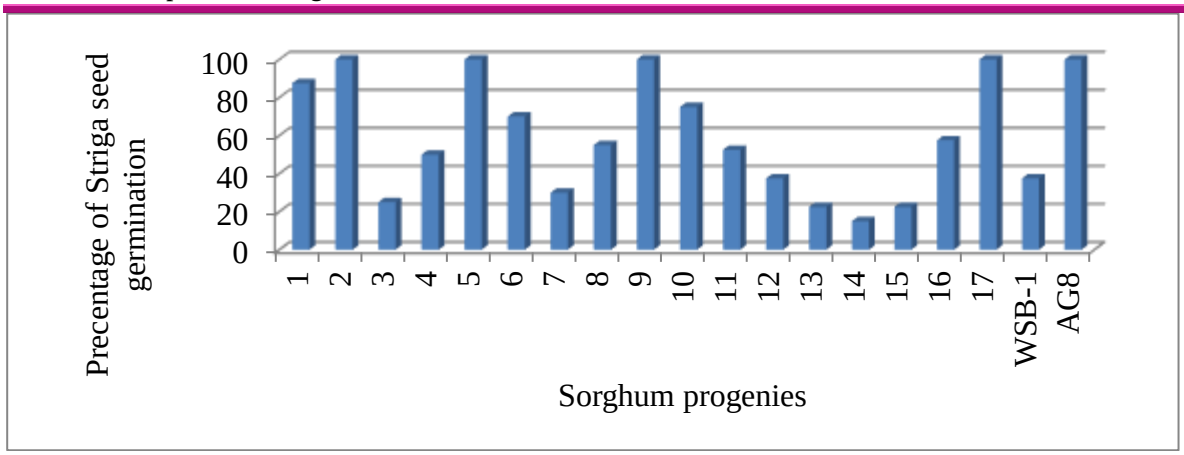

Fig. 2. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSB-1 using EAGA method

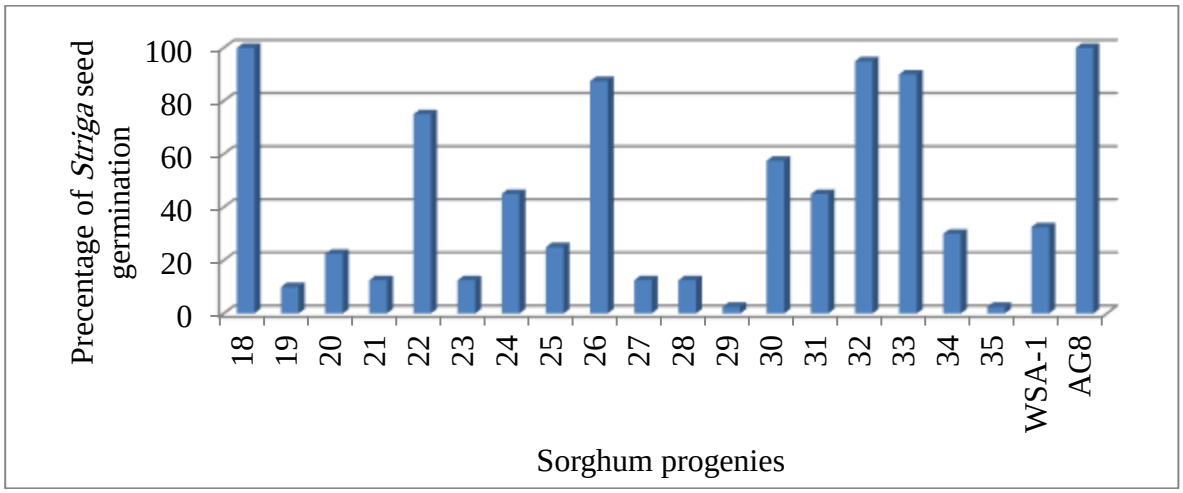

Fig. 3. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSA-1 using EAGA method

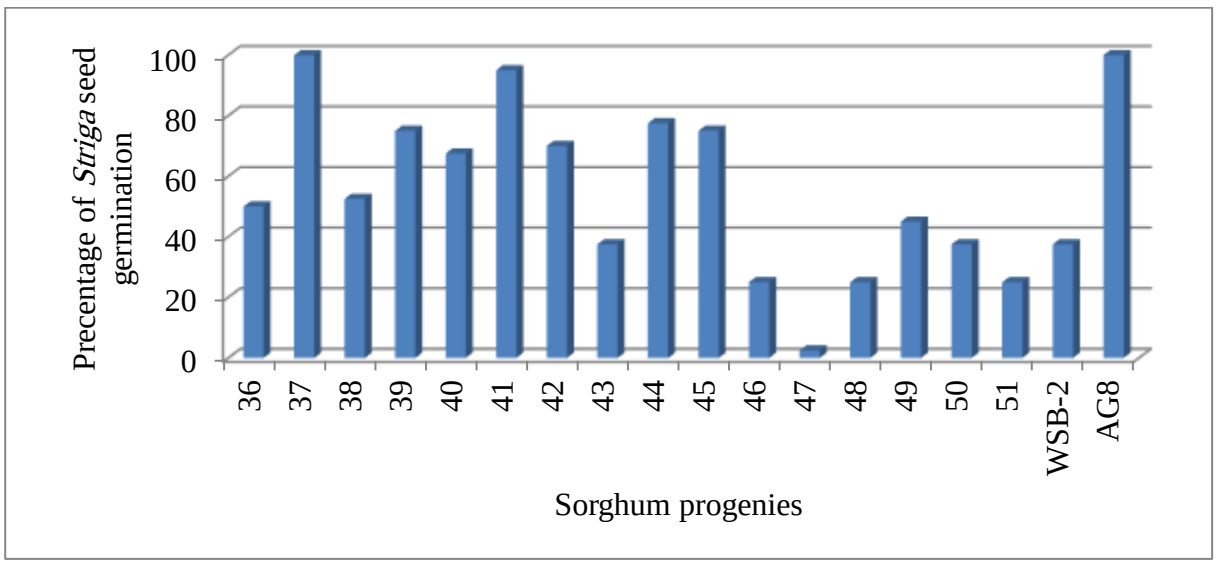

Fig. 4. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSB-2 using EAGA method

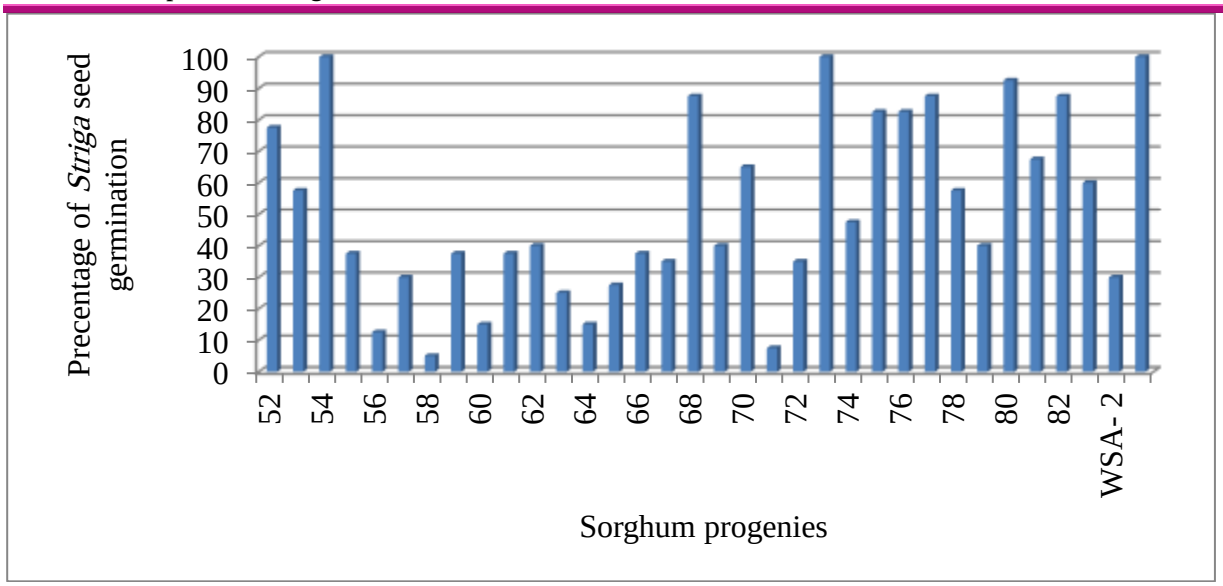

Fig. 5. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSA-2 using EAGA method

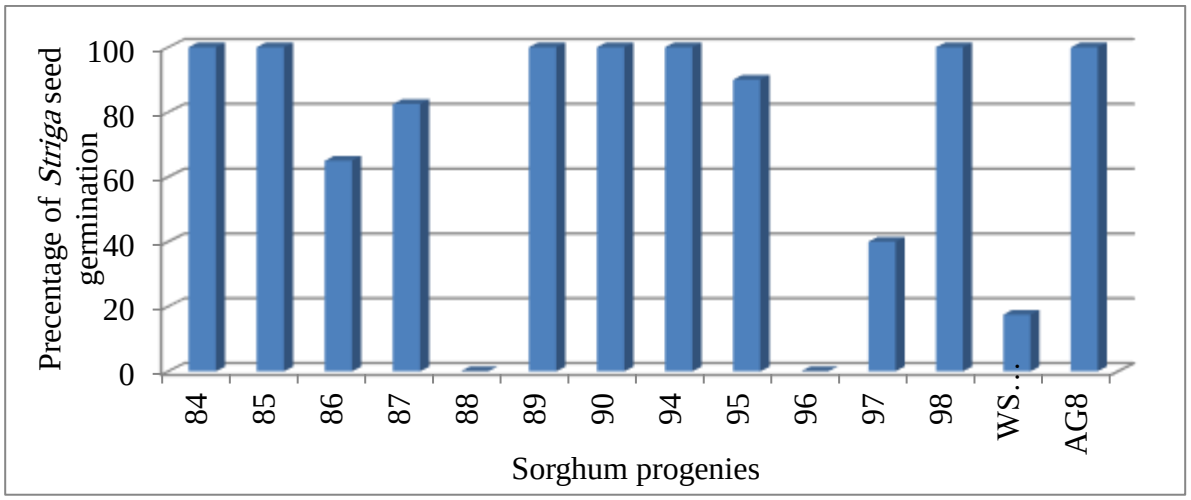

Fig. 6. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSA-3 using EAGA method

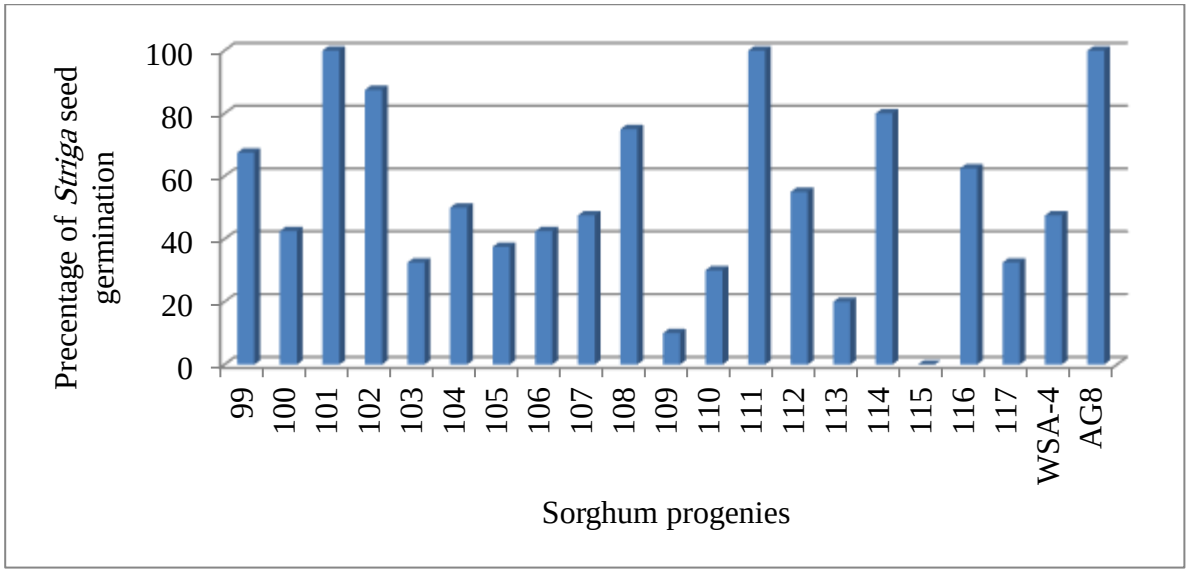

Fig. 7. Percentage of *Striga* seed germination by BC_3F_3 sorghum progenies, AG8 and WSA-4 using EAGA method

Reference [29] used this technique to differentiated maize genotypes in production of germination stimulant and haustoria initiation for *S. asiatica*. He also found no relation between germination distance and haustoria initiation. Reference [30] stated that resistant phenotypes associated with low stimulant production are controlled by single nuclear recessive gene (LGS) with highly additive gene action. Appearances of necrosis at resistant accessions result in parasite death [28]. Similar resistance response was reported in some sorghum cultivars after infection with *S. asiatica* [10].

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