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In vitro evaluation of marker assisted conversion of adapted sorghum varieties into S*triga hermonthica* resistant versions

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ABSTRACT: *Striga* has long been recognized to infest staple food crops like sorghum in Ethiopia. This study was designed to introgress *Striga*-resistance genes into popular and farmer-preferred varieties through marker-assisted backcrossing and to assess resistance based on Striga germination stimulant activity inagar-gel assay (AGA). The experiment was arranged in completely randomized design with four replications. Genotypes performance, heritability and genetic advance were analyzed and Germination rate was measured. The progeny showed significant genetic variation for maximum germination distance (MGD), germination rate (GR), and germination index (GI). The mean MGD ranged from 0.0 mm to 29.45 mm and GR ranged from 0.0% to 72.38%.Of the 118 backcrossed lines, 22.9% showed less than 10 mm of MGD and GR of <30%, revealing provision of low germination stimulant/*strigolactones* production (lgs). There were significant positive (r = 0.4-0.81) correlations showing the roles of these parameters as selection criteria in breeding for resistance. The existence of higher heritability (h^2_b = 77-83%) and genetic advance (GA = 62-93%) for the germination parameters indicated possibilities for improving resistance against *Striga* through selection. Genotypes that carry different QTLs showed different capacity of producing *Striga* germination stimulants in the AGA. The combined effect of two QTLs (lgs2_SBI-05_60404021 and lgs_3_60629027) at a time showed lower *Striga* germination stimulant activity and better field resistance indicating existence of possible cumulative effects. Thus, the study showed that marker-assisted backcrossing for transfer of lgs QTLs from donor into popular and farmers preferred cultivars has the potential to enhance tolerance/resistance to *Striga* in sorghum.

Key words/Phrases: Agar-gel bioassay, germination index, germination rate, maximum germination distance, *Striga* germination stimulants

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.)Moench] is the fifth most important dry land cereal crop produced worldwide for food, feed, and industrial purposes. Sorghum is among the top 10 crops that feed the world and is a dietary staple food crop of more than 500 million people in more than 30 countries (Kumar, 2016; FAO, 2017; Reddy, 2017; Visarada and Aruna, 2019). One of the centers of diversity for sorghum is Ethiopia, where the crop is grown, among other purposes, for food, local beverages, and feed (Doggett, 1988; Firew Mekbib, 2008). Sorghum takes the third largest share of all cereals grown in Ethiopia after tef [*Eragrostistef* (Zucc.) Trotter] and maize (*Zea mays* L.), be it in area or total annual national production (CSA, 2018). It is one of the principal food crops grown by the majority of the people dwelling in marginal areas where the major production constraints are erratic rainfall, poor soil fertility, and *Striga* infestation Mesfin Abate *et al*., (2014).

Weeds belonging to the genus *Striga* (Family = Orobanchaceae) are economically important obligate, root hemi-parasitic plants that rely on host plants for the acquisition of water, minerals and reduced nitrogen. Besides, to diverting sustenance from their host, they negatively affect host growth and development through toxic effects (Rank *et al*., 2004; Kanampiu *et al*., 2018). *Striga hermonthica* has long been recognized as the most persistent biological constraint to food production, as it infects important staple crops, such as sorghum, maize, finger millet (*Eleusinecoracana*L.), pearl millet (*Pennisetumglaucum* L.) and rice (*Oryzasativa* L.) (Parker, 2009; 2012; Atera *et al*., 2012).During the

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past decade, this parasitic weed has expanded its host-range, becoming increasingly important in other crops, including tef (Fassil Reda *et al*., 2010; AATF, 2011; Welsh and Mohamed, 2011), barley (*Hordeumvulgare* L.) and wheat (*Triticumaestivum* L.) Fassil Reda *et al*., (2010). Generally, the distribution of the parasitic weed is on the increase; it reportedly causes increased damage under low soil fertility and drought conditions (Parker, 2009; AATF, 2011; Welsh and Mohamed, 2011).

It is estimated that more than 50 million ha of the sub-Saharan African arable land is infested with *Striga*, causing enormous yield losses (Gebisa Ejeta, 2007a; Parker, 2012; Westwood *et al*., 2012; Kountche *et al*.,2019) and affecting livelihoods of millions of poor rural families in the semiarid tropical and sub-tropical regions Kountche *et al*., (2016).The global yield losses attributable to *Striga* infestation are immense, ranging from 30% to 90%, with a total crop failure under worst situations, which affects >300 million people (Gebisa Ejeta, 2007a, b; AATF, 2011; Westwood *et al*., 2012; Pennisi, 2015). In Africa, the average annual yield loss has been estimated to exceed 40% (Venne *et al*., 2009; AATF, 2011; Westwood *et al*., 2012; Kountche *et al*., 2019). The yield loss attributable to *Striga* infestation in Ethiopia ranged from 65 to100% (Tesfaye Tesso *et al*., 2007; Mesfin Abate *et al*., 2014). Hence, the control of *Striga* is important in ensuring food security in Africa, in general; and Ethiopia, in particular.

The possible approaches to overcoming the problem of *Striga* include manipulation of the growing environment and genetic manipulation of the crop itself. From the stand points of sustainability and cost effectiveness, the genetic manipulation of the crop to improve resistance to *Striga* is preferred strategy specially for resource-poor farmers. In Ethiopia, the breeding efforts have relied solely on conventional approaches Tesfaye Tesso *et al*., (2007). Nevertheless, it is difficult to conclude that conventional breeding has boosted the resistance to *Striga* as anticipated because of technical difficulties encountered in making major advances, such as long crossing cycles, costs, and influence of genotype by environment interaction.

It is clearly indicated and revealed that when biotechnological tools are properly applied in conjunction with conventional breeding, the long backcrossing cycles to transfer specific genes of interest would be shortened, gene pyramiding would be simpler and the release of varieties and their subsequent use as improved seeds would be enhanced and hastened (Grenier *et al*., 2007; Satish *et al*., 2012; Mohamed *et al*., 2014; Yohannes *et al*., 2015; Ali *et al*., 2016). On the other hand, the application of marker-assisted methods for resistance to *Striga* and tolerance to drought is at rudimentary stages in Ethiopia and thus, leading to an inadequate marker system for the genetic improvement of the crop. In this regard, as an immediate option, it is advisable to validate, refine and adopt molecular markers developed elsewhere for *Striga* resistance and drought tolerance to better serve the needs in Ethiopia. This approach is believed to offer opportunities to Ethiopian breeders there by proffering better solutions for these top priority constraint in sorghum and may even be replicated in other crops like maize.

The Ethiopian local sorghum varieties are highly preferred by the farming communities mostly for their yield, biomass and other morpho-agronomic attributes. Nonetheless, most of these varieties are not desirable, among others, because of susceptibility to *Striga* infection. To this effort, limited works have been made so far to improve the major limitation of the popular and farmers' preferred cultivars. Therefore, it is strategically advisable that breeding efforts should build on the popular improved varieties or landraces to deliver established varieties with protection against *Striga* infestation afforded through a few genes as a stopgap measure to farmers through a marker-assisted backcrossing. Thus, the conversion of the popular varieties or landraces into their *Striga* resistant and drought tolerant versions through incorporation of responsible genes employing marker-assisted backcrossing seems to be the best strategy in terms of time saving, effectiveness and efficiency. This study was, therefore, aimed at introgression of *Striga* resistance genes into the popular sorghum varieties through marker-assisted backcrossing and an *in-vitro* evaluation of pre-attachment resistance mechanisms.

MATERIALS AND METHODS

Plant material

The parental lines used for backcrossing consisted of three donor parents and 12 recurrent parents,

which represented released varieties and known farmers' cultivars (Table 1).

The striga resistant lines used as a gene source were obtained from Purdue sorghum breeding and found resistant and adapted to the lowland sorghum growing environments in Ethiopia. The recurrent parents were derived from the local breeding program that have wider acceptance by the farmers and farmers preferred landraces were used for the backcrossing.

The donor parents possessed *Striga* resistance, while the recurrent parents are high yielding but susceptible to *Striga hermonthica.* Striga hermonthica seeds were collected from Kobo, Humera areas (NABRC), where striga is affecting sorghum production following the standard collection procedure. The *Striga*-resistant line SRN39 with known low germinating stimulant activity was used as a check in the bioassay.

Table 1. **The parental lines used for marker-assisted backcrossing.**

No	Variety	Pedigree	Year of release	Center of release	Parental type
1	Abaere-1	Local	-	-	Recurrent parent
2	America-1	Local	-	-	Recurrent parent
3	Berjokecoll#1	Local	-	-	Recurrent parent
4	Birhan	Key#8566	2002	Srinka ARC†	Donor parent
5	Debir	Debir	2018	Melkassa ARC	Recurrent parent
6	Dekeba	ICSR24004	2012	Melkassa ARC	Recurrent parent
7	Framida	87441	1991	Sudan	Donor parent
8	Gambella1107	Gambella1107	1976	Melkassa ARC	Recurrent parent
9	Gobiye	P-9403	2000	Melkassa ARC	Donor parent
10	Jamiyu	Local	-	-	Recurrent parent
11	Jigurti	Local	-	-	Recurrent parent
12	Teshalle	3443-2-OP	2002	Melkassa/Srinka ARC	Recurrent parent
13	Tseadachimure	Local	-	-	Recurrent parent
14	Wediaker	Local	-	-	Recurrent parent
15	Wetetbegunche	Local	-	-	Recurrent parent

†*ARC= Agricultural Research Center*

Development of backcross lines

The fist backcrossing (BC1F1) was conducted in 2015 at Melkassa based on the genotypes showing homozygosity for the targeted locus. The second backcrossing BC2F1 was conducted in 2016 on the selected BC1F1 crosses that have homozygosity to the target locus and similarity with the recurrent parents. Successive selfing was conducted using the BC2F1 up to BC2F3 from 2016 to 2018 at Melkassa Research station.

Two diagnostic markers mapped on chromosome five (SBI-05) (Satish*et al*., 2012) grouped *Striga* resistance and susceptible sorghum genotypes representing the breeding population were identified. The Ethiopian popular cultivars and farmers preferred cultivars (recurrent parents) were crossed with donor parents to generate 328 F_1 plants using hand pollination method at Melkssa Agricultural Research Center (MARC), Ethiopia. Crossing was done by emasculation of selected plant panicles (recurrent parents) and dusting of pollen from identified plants (donor parents). The resultant 176 F_1 heterozygous plants (out of the 328) with desired quantitative trait loci (QTLs) were backcrossed to the respective recurrent parents to generate 153 BC_1F_1 progeny. BC_1F_1 progeny were screened for the presence of donor parent allele and selected progeny were backcrossed to generate 131 BC_2F_1. The BC_2F_1 conferring the targeted donor parent allele and recovered the genetic background of the recurrent parent through subsequent backcrossing were selected and advanced to 118 BC_2F_3 stage.

In-vitro evaluation procedures

Striga bioassay based on the agar-gel method developed by Hess*et al*., (1992) was conducted at the National Agricultural Biotechnology Research Center (NABRC), Holeta, Ethiopia. A total of 134 lines consisting of the parental lines, 118 BC_2F_3 lines, and SRN39 (resistant check) were evaluated in the agar-gel bioassay (Table 3).

Surface sterilization of Striga seeds

Striga seeds, previously collected by MARC from Humera areas in northern Ethiopia,were sourced from NABRC, Tigray Regional State. *Striga* seeds were surface sterilized following the procedure described by Amusan*et al*.,(2011), and Rich and Daniel Gobena, (2016) with minor modifications, such as replacing working consumables. A cleaned six scoops *Striga* seed was taken to a 50 ml flask recommended for 50 petri dish with a size of 100 mm. The surface sterilization of *Striga* seeds was accomplished by sonicating them in 25 ml 75% (v/v) ethanol and agitated for 2 min by sucking the solution with a sterile glass pipette equipped with an amber bulb. The seeds were allowed to settle in the flask for a while. The mixtures that floated (debris, immature seeds, grasses, etc.) were removed by gently pouring over the waste flask filter funnel. The remaining liquid was sucked with a pipette fitted with amber bulb by squeezing the bulb before putting into the seed slurry and pressing the tip against the inside bottom of the flask. The *Striga* seed were again washed by adding 25 ml of activated MetriCide. The MetriCide (MERICIDE®-Glutaraldehyde 2.5%, a 28-day sterilizing and disinfecting solution, Metrex®Research, USA) was activated by adding the entire contents (35.8 g) of the Activator Plus activator into 946 ml of MetriCide, followed by shaking for a minute. The activation date was labeled on the container and the solution was active for 28 days). The solution was agitated for 2 min by sucking seeds and sterilant in and out of the pipette under the surface of the liquid. The bubbles from the surface of the MetriCide solution were removed with the pipette before emptying the liquid into the waste flask in the same manner as the alcohol was removed, pouring off most, and then sucking the remainder with the pipette. In the same way, floating seeds and debris were removed. The *Striga* seeds were also washed twice by rinsing in sterile double-distilled water (sddH_2O), with each rinse lasting approximately 2 min. After sonication, the remaining sand/debris and water were removed with the pipette.

Preparation of conditioning solutions

First, a Benomyl 10 x stock solution was prepared by dissolving 0.15 g benomylwettable powder [methyle-1-(butylcarbomyl) - 2-benzimidazolecarbamate] in 10 ml activated dimethyl sulfoxide (DMSO: C_2H_6OS, Fisher Chemical, China). Then the solution was heated at 50-70 °C for 5 min to dissolve the fungicide in the solvent and stored at room temperature for subsequent use but not for more than three months. Then, a working solution was prepared by adding 0.5 ml of benomyl solution (from stock solution) in 49.5 ml of sddH_2O.

Conditioning of surface sterilized Striga seeds

Surface-sterilized *Striga* seeds were preconditioned by keeping them in 14.5 ml of sddH_2O and 1.5 ml of 0.015% benomyl in a 50-ml flask. Then flasks were enclosed with aluminum foil and placed in dark in an incubator with a temperature setto 29°C for 5 days. The benomyl solution was changed after one day and then after every two-three days until the seeds were embedded in agar. After the *Striga* seed was soaked in liquid for five days (sddH_2O and benomyl), agar (Bacto™ Agar) was prepared to embed *Striga* seeds. Pyrex bottles (1 L), each containing 700 ml water plus 4.9 Bacto Agar (0.7% w/v), enough for about 20 plates, were prepared and autoclaved for 20 min and allowed to cool in a containment room water bath at 50 ˚C. After changing the benomyl solution, *Striga* seeds was sucked by the pipette from the conditioning flask with aid of an amber bulb and let it settle on the narrow tip for a while. Then, a drop of *Striga* seeds, approximately the size of a sorghum seed, was released from the pipette by touching it at the center of each plate through squeezing the bulb. Thereafter, about 35 ml sterilized agar, cooled to 50°C, was poured over a drop of *Striga* seeds to evenly distribute the seeds in the agar to embed it and allow the conditioning to finish in the agar. The plates with poured *Striga* seeds were allowed to cool before covering and stacking in a Petri-dish bag. Then the seeds were conditioned in this agar in the dark at 29°C for an additional 5-7 days in the incubator.

Striga seed germination test

Plates containing only S*triga* seeds were sprayed on the agar surface with a solution of 10^{-7}M of the synthetic strigolactone, GR24. The GR24 was prepared by adding 0.012 g of GR24 in 10 mm DMSO. From this solution, 250-µ GR24 +100 ml ddH_2O was prepared (10^{-7}M) and sprayed for each

Striga containing plates. Sprayed plates were incubated in the dark at 29°C for three days and plates were observed for germination under a binocular stereomicroscope (10× magnification) fitted with a digital camera. The *Striga* seed was considered to have germinated if it showed a protruded radicle through the seed coat (Prandi *et al*., 2011). The total number of seeds and germinated seeds were counted and germination percentage was determined and expressed in percentage. Mean germination percentage was obtained by calculating the average germination from 16 plates, which ranged from 45-70%, indicating that *Striga* was responsive to the stimulant (GR24) before the actual evaluation of the sorghum genotypes. Then after the germination test on *Striga* was performed in each batch of the bioassay in parallel so as to confirm the sensitivity of *Striga* seeds recommended for genotype evaluation (>35% of germination rate).

Surface sterilization of sorghum seed and pre-germination test

One-hundred thirty-four sorghum entries (118 BC_2F_3 backcrossed lines, 15 parental lines and SRN39) were surface sterilized and pre-germinated according to the method described by Amusan*et al*., (2011) with minor modification, such as replacement of chemical (such as Travo).Twenty cleaned sorghum seeds were counted from each entry and placed in individual glass labeled vials. Once all entries were placed in vials, seeds were soaked by adding 5 ml of freshly prepared 50% bleach and 0.2% Tween 20 solution for 30 min and shaken three times to break surface tension. After the 30 min soak, bleach solution was poured out gently into a waste container, followed by rinsing in sddH_2O for three times. Thereafter, seeds were soaked in a non-systemic fungicide, 5% (w/v) Travo (active ingredient: Azoxystrobin: methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3- methoxyacrylate, 22.9%) and left overnight or at least for more than five hours. The 5% (w/v) Travo solution was prepared by adding 25 g Travo powder to 500 ml sddH_2O and shaking to form a slurry. Then, 5 ml of Travo solution was added to each vial and left overnight. Next day, Travo solution was poured off after shaking the tube to suspend the Travo. About 5 ml of sddH_2O was added to each vial. Each entry was poured into labeled sterile Petri plates containing double filter paper (Whatman #1 90 mm circles). Sorghum containing plates were placed in warm (30°C) dark incubator for 30 hours or until the seed has germinated and the radicle is averagely around 1 cm long (as germination varies among genotypes). After protrusion of both radicle and plumule, only healthy germinated seedlings were gently picked up with forceps and planted on the preconditioned *Striga* seeds.

Agar-gel assay (AGA)

The agar-gel assay method developed by Hess *et al*., (1992) was used to measure *Striga* germination activity based on the capacity of stimulants production of sorghum genotypes. The experiment was conducted in a completely randomized design with four replications. One healthy germinated sorghum seedling from each genotype was gently picked and inserted into the plates containing conditioned *Striga* seeds for 10 days. Seedlings were planted deeply such that the root reaches beneath the agar to where the *Striga* seeds were embedded and that it points toward the center of the plate. The plates were covered and placed into an incubator for incubation set at 29 °C in the dark for 3 days. During each cycle, the same batch(s) of *Striga* blank (no sorghum) and *Striga* batch sprayed with GR24 were included as control.

Data collection

After three days of incubation, the plates were observed under a zoom stereomicroscope at about 10× magnification through the bottom of the plate to determine *Striga* germination stimulant activity. Before going to the sorghum planted plates, the germination rate was calculated from the GR24 treated plates to confirm the germination rate and *Striga* seed is responsive enough at least 30% Rich and Daniel Gobena, (2016) to give a meaningful measure of germination stimulant activity. In this regard, *Striga* germination rate was more than 45%. Three furthest germinated *Striga* seeds from sorghum root were measured at 3 days of incubation to determine maximum germination distance (MGD). MGD is the average of these three measurements on each seedling/plate. Seedlings with an MGD $\geq$ 10 mm have high *Striga* germination stimulant activity, while those with an MGD < 10 mm have low *Striga* germination stimulant production (Hess*et al*., 1992; Haussmann

et al., 2001; Mohamed *et al*., 2010).Germination rate (GR) was obtained from the ratio of germinated *Striga* seeds in the image of selected area of microscopic field (2 cm x 2.5 cm) to the total number of seeds expressed as percentage after three days of incubation. In addition, two images for each plate were taken after GR24 treatment (the day 5 images) was used to determine germination index (GI). First, the same area used to count *Striga* seeds in determination of germination rate "near host root" and secondly a 2 cm × 2.5 cm area was selected "far from host". The germination index was obtained from the ratio of germination rate calculated for the "near-host-root" area to germination rate of the "far-from-host" area.

Statistical analysis

The analysis of variance was performed using R software version 3.6.1 R Core Team(2019).

Estimation of heritability in broad sense

Broad sense heritability (H^2b) was estimated as described by Allard, (1960):

$$H^2b = \left(\frac{\sigma^2 g}{\sigma^2 g + (\frac{\sigma^2 e}{r})} \right) \times 100$$

where, σ^2_g = genotypic variance, $\sigma^2 e$ = environmental variance, and r= number of replications

Estimation of genetic advance

Genetic advance (GA) was calculated with the method suggested (Allard, 1960; Singh and Chaudhury, 1985; Falconer, 1989), assuming the selection intensity of 5%, as:

$GA = K \times \sigma ph \times H^2_b$

Where , GA= expected genetic advance from selection, K= the constant differential (K=2.063 at 5% selection intensity), σph = square root of phenotypic variance and H^2b = broad-sense heritability. Genetic advance as percentage of the mean (GAM) was calculated as described by Johnson *et al*., (1955) as follow: $GAM = \frac{GA}{\bar{x}} \times 100$where $\bar{x}$ = grand mean of a character.

Interrelationships between characters

Correlation coefficients between characters were estimated as described by Miller *et al*., (1958) as: $r = \frac{Covxy}{\sqrt{\sigma x^2 + \sigma y^2}}$

Where, Cov(xy)= co-variance of traits x and y, σx^2= variance of x and σy^2 = variance of y

RESULTS AND DISCUSSION

Performance of the genotypes

Analysis of variance (ANOVA) showed that the introgessed progeny and their parents significantly differed ($p<0.01$) for all the measured variables (Table 2). This unveils the existence of considerable variation in the pre-attachment of *Striga* resistance mechanisms among the developed and parental lines as discussed trait by trait below (Table 3). The variability for the pre-attachment *Striga* related traits were accounted by the variability in response of the test progeny/genotypes as indicated by high coefficient of determination (R^2) ranged from 78.4 to 85.9%.

Table 2.Analysis of variance for measured pre-attachment *Striga* traits

Source of variation	Degree of freedom	Mean square		
		Germination distance	Germination rate	Germination index
Genotypes	133	90.6**	852.7**	13.65**
Residual	402	20.9	190.3	2.25
R^2		85.9	78.4	82.6

*** Significant at p<0.001*

Maximum germination distance

The mean MGD of the converted lines and their parents ranged from the lowest of 0.0 mm to the highest of 29.45 mm in the agar-gel assay (Table 3). Out of the 134 genotypes screened for *Striga hermonthica* resistance, 32 showed low germination stimulant activity (MGD <10 mm), while the remaining 102 showed high germination

stimulant activity (MGD ≥10 mm). Considering the introgressed 118 lines alone, 22.9% of them showed less than 10 mm of MGD values, while the remaining 77.1% showed MGD values of more than 10 mm. Interestingly, the lowest germination stimulant activity (0.0 mm) were recorded from the resistant check, SRN39, followed by the donor parents (Framida, Birhan and Gobiye) which showed MGD values of less than 5 mm. In agreement with this study, a number of previous studies also established the *Striga* germination stimulant activity to be low in Framida (Mohamed *et al*., 2010; Mohemed *et al*., 2018), Gobiye and Birhan (Gebisa Ejeta *et al*., 2007; Grenier *et al*., 2007; Tesfaye Tesso *et al*., 2007; Satish *et al*., 2012), and, SRN39 (Mohamed *et al*., 2010; Satish *et al*., 2012; Daniel Gobena *et al*., 2017; Mohemed *et al*., 2018). It was re-confirmed in this study that the donor parents could further serve as potential sources of genes under the Ethiopian condition for incorporation of resistance genes into the popular sorghum varieties susceptible to *Striga*, but otherwise desirable for other attributes including grain yield and biomass. In fact, the two donor parents (Gobiye and Birhan), not only serve as parents but they have also been in the production system as released varieties in the country (Grenier *et al*., 2007; Tesfaye Tesso *et al*., 2007).

Based on the dividing line of germination stimulant activity of less than 10 mm of MGD, 28 backcrossed lines were found to be resistant to *Striga* (Table 3). The low germination distance of these introgressed progeny revealed their better resistance to the parasitic weed based on the low *Striga* germination stimulants activity. In addition to the low production stimulant activity of these converted progeny and their donor parents, the resistance may also be caused by the altered production of the strigolactoneorobanchol over 5-deoxystrigol, a mutation on sulfotransferase gene (*lgs1*) which reduces the germination of *S. hermonthica*as reported byYoneyama *et al., (*2010) and Daniel Gobena *et al*., (2017). They confirmed that *lgs* sulfotransferase is absent in SRN39 and this is associated with loss of function which results in the production of high orobanchol but low 5-deoxystrigol profile, simulating less *Striga* seed germination. These observations suggest that *S. hermonthica* seeds may distinguish the different variants or composition of strigolactones.

Generally, the practicality of agar-gel assay and the use of MGD in *Striga* resistance breeding is well-established (Gebisa Ejeta *et al*., 1992; 2000a; Haussmann *et al*., 2000a; Omanya *et al*., 2004; Gebisa Ejeta *et al*., 2007; Yoder and Scholes, 2010). Thus, the low *Striga* germination stimulant activity has been an important resistance trait in sorghum improvement programs. To this effect, this useful *in-vitro* assay has resulted in the development and release of several *Striga*-resistant sorghum varieties with low germination stimulant activity (Gebisa Ejeta, 2007b; Ali *et al*., 2016). According to Gebisa Ejeta (2007b) report not all sorghum lines showing field resistance to *Striga* had low *Striga* germination stimulant activity, it can also be due to other means of resistance mechanisms. However, based on previous researches, all low-stimulant sorghums that were field-tested showed *Striga* resistance, indicating the positive correlations between the amount of germination stimulant produced and *Striga* infection levels in the field (Ramaiah, 1987; VasudevaRao, 1987; Hess *et al*., 1992; Haussmann *et al*., 2004; Rich *et al*., 2004; Mohemed *et al*., 2016).

Germination rate of Striga near sorghum root

The GR of *Striga* seeds from the different batch or cycle of bioassay treated with GR 24 (a synthetic analog of strigolactones) ranged from 45% to 70% and with an average of 60%, indicating the responsiveness of the seeds to the germination stimulant (GR24) in each batch of bio-assaying or screening. The results from the screening of sorghum backcrossed lines along with their parents revealed that there were significant variations in *Striga* GR around the sorghum roots in the AGA. The result showed that none of the introgressed progeny were as *Striga* resistance or tolerance as the donor parents, which may be only a result of the transfer of selected low germination stimulant *(lgs)* QTL rather than the full complement of *lgs* QTL in the donor parents. The GR of *Striga* seed in response to the stimulant from the host plant ranged from 0.0% to 72.38%, indicating the clear difference in the production of germination stimulant activity among the backcrossed and parental lines. Of the total (118) converted lines, nearly 22.9% stimulated germination rate of less than 30% and germination distance of less than 10 mm (Table 3). Such low *Striga* germination percent may indicate a potential for resistance to *Striga*. The resistant check, SRN39 (0.0%), followed by the donor parent, Gobiye (1.76%) initiated the lowest

germination stimulant activity. Even though these backcross lines did not show total immunity against *Striga* seed germination, as there is no reported complete resistance to *Striga* so far in sorghum Gebisa Ejeta (2007a), the expression of low percentage level of stimulant production was an indication of their high level of resistance to the parasitic weed suggests to the low germination stimulant production. Consequently, the low level of germination stimulant produced by host plant may result in reduced number of germinated *Striga* seeds. This study is in agreement with previous reports, which recommended selection of sorghum variants with low production of strigolactones as a sound strategy in resistance breeding, based on the relation that sorghum genotypes with low stimulant production have also low germination-inducing activity in their root exudates (Haussmann *et al*., 2001a, Omanya*et al*., 2004; Gebisa Ejeta, 2007a; 2007b; Rich and Gebisa Ejeta, 2008; Satish *et al*., 2012; Daniel Gobena *et al*., 2017; Gwatidzo *et al*., 2020).

On the other hand, 65% of the introgressed lines showed higher *Striga* GR that ranged from 20% to 72.4%, indicating that these backcrossed lines produce higher amount of germination stimulant activity depending on the genetic background (epistasis interaction) or additionally might be related to the incomplete conversion of the generated progeny (87.5%). The recurrent parents showed high GR ranged from 11% to 40% reflecting their high production of *Striga* germination stimulants. Earlier reports showed that not only the amount of exudates they produce but also the type of stimulant (strigolactones) might have resulted in the differences of *Striga* seed GR (Xie *et al*., 2010; Yoneyama *et al*., 2015; Mohemed *et al*., 2016; Tsuchiya *et al*., 2018) among the sorghum genotypes, sorghum produces at least five different strigolactones, that includes: 5-deoxystrigol, sorgolactone, strigol, strigyl acetate, and sorgomol, which varied in *Striga* germination initiation activity (Awad *et al*., 2006; Xie *et al*., 2008; Satish *et al*., 2012 Gwatidzo *et al*., 2020). Other studies, also reported that *Striga* GR might vary with the composition, quantity and nature of the signaling molecules (Yoneyama *et al*., 2010; 2015; Mohemed*et al*., 2018).

It is noteworthy that, genotypes classified as having low *Striga* germination stimulant activity based on the MGD values also showed low GR near their roots in the AGA and *vice-versa*. Previous reports declared that low germination stimulant (*lgs*) gene has been successfully introduced into high yielding and adapted sorghum varieties that have been deployed into several African countries (Gebisa Ejeta *et al*., 1997a; 2007; Grenier *et al*., 2007) including Ethiopia Tesfaye Tesso *et al*., (2007). Although sorghum genotypes that produce little germination stimulants have been shown to be resistant to *Striga* in field tests (Haussmann *et al*., 2000, 2001; Omanya *et al*., 2004; Rodenburg *et al*., 2006) it is, however, important to note that there exist other *Striga* resistant genotypes without low stimulant but yet resistant due to other resistant mechanisms (Gebisa Ejeta *et al*., 2007; Mohamed *et al*., 2010). It was also observed that the germination of preconditioned *Striga* seeds might be influenced by the position, nature or architecture of the root of sorghum and distribution of *Striga* seeds in the agar (Figure 1).

Germination index (GI)

The GI values were determined from the ratio of germination rate in the close (within one cm sorghum near root) to germination rate of the distance (2 cm away from any sorghum roots). The GI values of converted progeny included in this study varied from the lowest of 0.08 to the highest of 16.2. Of the 118 backcrossed lines, only nine (7.6%) of them shows GI values of less than unity (Table 3). In addition, two donor parents (Birhan and Gobiye) also showed GI values less than one. It is logical to assume that inhibitors being exuded from the host root might affect *Striga* seeds close to the host root more than those at a distance, thereby resulting in the germination indices of less than one. Therefore, the low germination events after GR24 spraying could be due to some germination-inhibitory compounds produced by the sorghum genotypes that may interfere with the germination response sequence of conditioned *Striga* seeds. GI values of one indicate that the GRs of the artificially stimulated *Striga seeds* in the proximal position equaled those at the distal positions. Conversely, 92.4% of the introgressed lines and recurrent parents show germination indices of greater than one suggesting more germination events at the proximal than those at the distal positions and hence no-inhibitory induction activity form the host (Table 3).

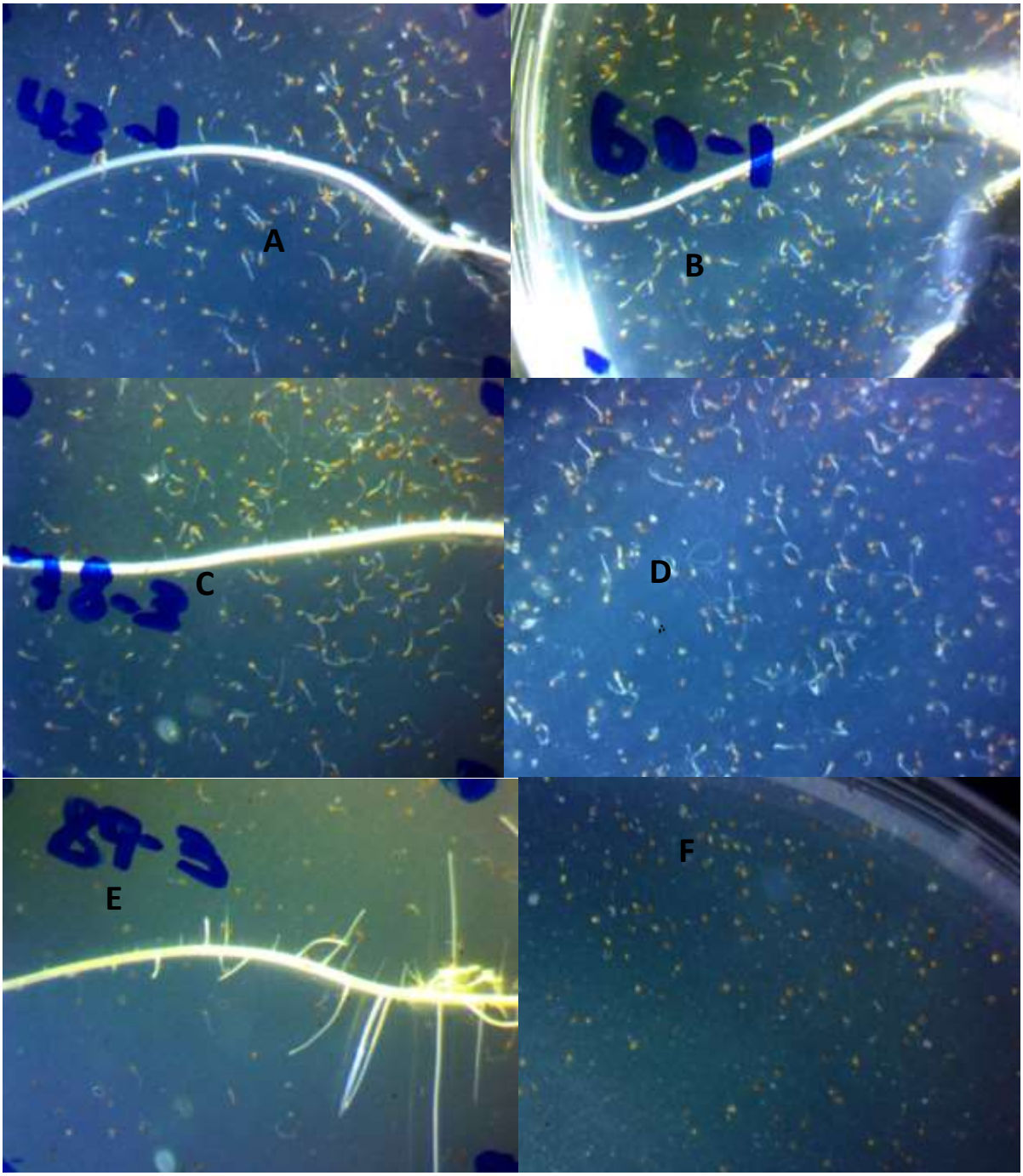

Figure 1. Germination of preconditioned *Striga* seeds embedded in agar surrounding the sorghum root after treatment with GR24. High *Striga* germination stimulant genotypes (A-C); treated with GR24 (D), low *Striga* germination stimulant genotype (E), and pre-conditioned *Striga* seeds with no treatments.

Table 3. The response of sorghum converted progeny and their parents to the capacity of *Striga hermonthica* stimulants activity.

Genotypes	Pedigree/breeder's code	QTL	MGD	GR	GI	Resistance level
Abaere-1	Abaere-1	-	10.67$^{o\text{-}H}$#	11.3$^{x\text{-}H}$	1.08$^{w\text{-}F}$	S††
America-1	America-1	-	8.33$^{v\text{-}I}$	19.64$^{l\text{-}H}$	1.17$^{v\text{-}F}$	R‡‡
BC_2F_3_ETSC_17001	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05† & lgs_3‡	12.56$^{i\text{-}G}$	28.41$^{f\text{-}H}$	1.078$^{w\text{-}F}$	S
BC_2F_3_ETSC_17002	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	13.94$^{g\text{-}E}$	33.68$^{d\text{-}D}$	2.3$^{j\text{-}F}$	S
BC_2F_3_ETSC_17003	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	13.78$^{g\text{-}E}$	28.1$^{f\text{-}H}$	1.78$^{p\text{-}F}$	S
BC_2F_3_ETSC_17004	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	10$^{r\text{-}H}$	20.76$^{j\text{-}H}$	0.88$^{x\text{-}F}$	S
BC_2F_3_ETSC_17005	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	8.67$^{u\text{-}I}$	14.31$^{u\text{-}H}$	1.41$^{t\text{-}F}$	R
BC_2F_3_ETSC_17006	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	14.33$^{f\text{-}E}$	38.61$^{c\text{-}y}$	3.42$^{f\text{-}D}$	S
BC_2F_3_ETSC_17007	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	11.22$^{n\text{-}G}$	9.47$^{z\text{-}H}$	0.72$^{z\text{-}F}$	S
BC_2F_3_ETSC_17008	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	6.89$^{z\text{-}I}$	9.21$^{z\text{-}H}$	2.64$^{h\text{-}F}$	R
BC_2F_3_ETSC_17009	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	6.05$^{D\text{-}I}$	6.72$^{B\text{-}H}$	0.35$^{C\text{-}F}$	R
BC_2F_3_ETSC_17010	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	9.11$^{t\text{-}I}$	16.64$^{r\text{-}H}$	1.03$^{w\text{-}F}$	R
BC_2F_3_ETSC_17011	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	14.39$^{e\text{-}E}$	19.17$^{m\text{-}H}$	1.57$^{s\text{-}F}$	S
BC_2F_3_ETSC_17012	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	13.61$^{g\text{-}E}$	28.44$^{e\text{-}H}$	4.09$^{e\text{-}w}$	S
BC_2F_3_ETSC_17013	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	12.5$^{i\text{-}G}$	9.87$^{y\text{-}H}$	1.04$^{w\text{-}F}$	S
BC_2F_3_ETSC_17014	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	9.33$^{s\text{-}I}$	20.61$^{k\text{-}H}$	2.64$^{h\text{-}F}$	R
BC_2F_3_ETSC_17015	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	20.44$^{a\text{-}n}$	10.35$^{y\text{-}H}$	3.4$^{g\text{-}D}$	S
BC_2F_3_ETSC_17016	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	6.55$^{B\text{-}I}$	14.9$^{t\text{-}H}$	1.2$^{u\text{-}F}$	R
BC_2F_3_ETSC_17017	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	13.33$^{h\text{-}F}$	26.22$^{h\text{-}H}$	4.88$^{e\text{-}p}$	S
BC_2F_3_ETSC_17018	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	12.44$^{i\text{-}G}$	12.64$^{w\text{-}H}$	2.7$^{h\text{-}F}$	S
BC_2F_3_ETSC_17019	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	12.5$^{i\text{-}G}$	34.29$^{d\text{-}C}$	2.74$^{g\text{-}F}$	S
BC_2F_3_ETSC_17020	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	13.45$^{h\text{-}E}$	45.03a-r	1.9$^{n\text{-}F}$	S
BC_2F_3_ETSC_17021	Jamiyu/Birhan///Jamiyu	lgs_3	11.72$^{k\text{-}G}$	33.94$^{d\text{-}C}$	6.78^{cde}	S
BC_2F_3_ETSC_17022	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	12.78$^{i\text{-}G}$	68.78^{ab}	5.4$^{d\text{-}j}$	S
BC_2F_3_ETSC_17023	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	16.44$^{b\text{-}A}$	29.51$^{e\text{-}H}$	2.05$^{m\text{-}F}$	S
BC_2F_3_ETSC_17024	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	7.06$^{y\text{-}I}$	14.04$^{v\text{-}H}$	1.5$^{s\text{-}F}$	R
BC_2F_3_ETSC_17025	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	21.22$^{a\text{-}l}$	30.85$^{e\text{-}G}$	4.64$^{e\text{-}s}$	S
BC_2F_3_ETSC_17026	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	21.61$^{a\text{-}j}$	46.89$^{a\text{-}o}$	5.5$^{d\text{-}i}$	S
BC_2F_3_ETSC_17027	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	14.3$^{f\text{-}E}$	8.98$^{z\text{-}H}$	1.64$^{r\text{-}F}$	S
BC_2F_3_ETSC_17028	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	8.3$^{v\text{-}I}$	9.55$^{y\text{-}H}$	1.98$^{n\text{-}F}$	R
BC_2F_3_ETSC_17029	Teshale/Framida///Teshale	lgs2_SBI-05	18$^{b\text{-}v}$	48.28$^{a\text{-}l}$	2.17$^{l\text{-}F}$	S

Genotypes	Pedigree/breeder's code	QTL	MGD	GR	GI	Resistance level
BC_2F_3_ETSC_17031	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	15.2^{d-D}	35.93^{d-A}	5.2^{d-l}	S
BC_2F_3_ETSC_17032	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	10.06^{r-H}	21.81^{j-H}	4.8^{e-q}	S
BC_2F_3_ETSC_17033	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	7.55^{w-I}	18.44^{o-H}	2.64^{h-F}	R
BC_2F_3_ETSC_17034	Wetetbegunchie/Birhan///Wetetbegunchie	lgs_3	20^{b-q}	60.76^{a-d}	3.6^{f-B}	S
BC_2F_3_ETSC_17035	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	14.3^{f-E}	49.75^{a-j}	5.14^{d-m}	S
BC_2F_3_ETSC_17036	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	18.89^{b-s}	52.2^{a-i}	16.2^{a}	S
BC_2F_3_ETSC_17037	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	9.78^{r-I}	17.81^{p-H}	5.75^{d-h}	R
BC_2F_3_ETSC_17038	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	17.67^{b-v}	40.82^{b-w}	7.94^{cd}	S
BC_2F_3_ETSC_17039	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	8.44^{v-I}	3.64^{E-H}	1.78^{p-F}	R
BC_2F_3_ETSC_17040	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	15.67^{d-D}	6.58^{C-H}	2.58^{i-F}	S
BC_2F_3_ETSC_17041	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	17.44^{b-v}	34.15^{d-C}	6.44^{def}	S
BC_2F_3_ETSC_17042	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	11.3^{m-G}	34.88^{d-C}	9.27^{bc}	S
BC_2F_3_ETSC_17043	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	26^{ab}	53.18^{a-h}	4.79^{e-r}	S
BC_2F_3_ETSC_17044	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	15.78^{c-C}	34.13^{d-C}	3.44^{f-C}	S
BC_2F_3_ETSC_17045	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	24.22^{a-d}	64.65^{abc}	5.83^{defg}	S
BC_2F_3_ETSC_17046	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	17.56^{b-v}	37.77^{c-z}	3^{g-F}	S
BC_2F_3_ETSC_17047	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	14.22^{g-E}	51.98^{a-i}	2.73^{g-F}	S
BC_2F_3_ETSC_17048	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	10.56^{o-H}	23.28^{i-H}	2.2^{l-F}	S
BC_2F_3_ETSC_17049	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	15.55^{d-D}	44.41^{a-s}	3.84^{e-z}	S
BC_2F_3_ETSC_17050	Wetetbegunchie/Gobiye///Wetetbegunchie	lgs2_SBI-05 & lgs_3	17^{b-w}	39.49^{c-x}	3.94^{e-y}	S
BC_2F_3_ETSC_17051	AbaAre-1/Gobiye///AbaAre-1	lgs_3	14^{g-E}	34.72^{d-C}	2.97^{g-F}	S
BC_2F_3_ETSC_17052	AbaAre-1/Gobiye///AbaAre-1	lgs2_SBI-05 & lgs_3	29.45^{a}	42.71^{b-v}	1.94^{n-F}	S
BC_2F_3_ETSC_17053	AbaAre-1/Gobiye///AbaAre-1	lgs_3	19.89^{b-q}	43.33^{b-u}	3.15^{g-F}	S
BC_2F_3_ETSC_17054	AbaAre-1/Gobiye///AbaAre-1	lgs2_SBI-05 & lgs_3	17.5^{b-v}	35.74^{d-B}	4.3^{e-v}	S
BC_2F_3_ETSC_17055	AbaAre-1/Gobiye///AbaAre-1	lgs2_SBI-05 & lgs_3	10.72^{o-H}	18.54^{n-H}	1.08^{w-F}	S
BC_2F_3_ETSC_17056	America-1/Birhan///America-1	lgs2_SBI-05 & lgs_3	11.56^{l-G}	8.65^{z-H}	1.9^{n-F}	S
BC_2F_3_ETSC_17057	America-1/Birhan///America-1	lgs2_SBI-05 & lgs_3	11.44^{m-G}	18.85^{m-H}	5.44^{d-j}	S
BC_2F_3_ETSC_17058	America-1/Birhan///America-1	lgs2_SBI-05 & lgs_3	21.3^{a-k}	34.95^{d-C}	2.48^{i-F}	S
BC_2F_3_ETSC_17059	America-1/Birhan///America-1	lgs2_SBI-05 & lgs_3	9.67^{r-I}	15.44^{s-H}	1.7^{q-F}	R
BC_2F_3_ETSC_17060	America-1/Birhan///America-1	lgs2_SBI-05 & lgs_3	16.89^{b-x}	26.92^{g-H}	5^{e-o}	S
BC_2F_3_ETSC_17061	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	20.22^{b-o}	43.06^{b-v}	4.6^{e-t}	S
BC_2F_3_ETSC_17062	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	24^{a-e}	28.47^{e-H}	1.7^{p-F}	S
BC_2F_3_ETSC_17063	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	8.89^{t-I}	9.5^{z-H}	1.25^{u-F}	R

Genotypes	Pedigree/breeder's code	QTL	MGD	GR	GI	Resistance level
BC_2F_3_ETSC_17064	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	6.78^{A-I}	4.3^{E-H}	0.61^{A-F}	R
BC_2F_3_ETSC_17065	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	6.56^{B-I}	3.27^{FGH}	0.24^{DEF}	R
BC_2F_3_ETSC_17066	America-1/Framida///America-1	lgs2_SBI-05	18.89^{b-s}	22.07^{j-H}	0.89^{x-F}	S
BC_2F_3_ETSC_17067	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	11.44^{m-G}	19.75^{l-H}	2.1^{l-F}	S
BC_2F_3_ETSC_17068	America-1/Framida///America-1	lgs2_SBI-05 & lgs_3	23.22^{a-g}	59.96^{a-d}	1.98^{n-F}	S
BC_2F_3_ETSC_17069	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	19.39^{b-r}	43.72^{b-t}	1.62^{s-F}	S
BC_2F_3_ETSC_17070	Berjokecoll#1/Birhan///Berjokecoll#1	lgs2_SBI-05 & lgs_3	16.16^{c-B}	48.87^{a-k}	2.1^{m-F}	S
BC_2F_3_ETSC_17071	Berjokecoll#1/Framida///Berjokecoll#1	lgs2_SBI-05 & lgs_3	9.94^{r-H}	16.47^{r-H}	1.29^{u-F}	R
BC_2F_3_ETSC_17072	Debir/Birhan///Debir	lgs2_SBI-05 & lgs_3	7.22^{x-I}	6.8^{A-H}	1.63^{r-F}	R
BC_2F_3_ETSC_17073	Debir/Birhan///Debir	lgs2_SBI-05 & lgs_3	20.94^{a-m}	32.25^{d-F}	1.3^{u-F}	S
BC_2F_3_ETSC_17074	Debir/Birhan///Debir	lgs2_SBI-05	15.45^{d-D}	25.43^{h-H}	1.36^{u-F}	S
BC_2F_3_ETSC_17075	Debir/Birhan///Debir	lgs2_SBI-05 & lgs_3	21.89^{a-i}	42.56^{b-v}	2.2^{l-F}	S
BC_2F_3_ETSC_17076	Debir/Birhan///Debir	lgs2_SBI-05 & lgs_3	15.44^{d-D}	30.93^{e-G}	5.03^{e-n}	S
BC_2F_3_ETSC_17077	Debir/Gobiye///Debir	lgs_3	17.2^{b-w}	40.91^{b-w}	2.93^{g-F}	S
BC_2F_3_ETSC_17078	Debir/Gobiye///Debir	lgs_3	18.94^{b-s}	46.11^{a-q}	2.1^{l-F}	S
BC_2F_3_ETSC_17079	Debir/Gobiye///Debir	lgs_3	24.1^{a-d}	72.38^{a}	2.6^{i-F}	S
BC_2F_3_ETSC_17080	Debir/Gobiye///Debir	lgs2_SBI-05 & lgs_3	18.61^{b-t}	57.34^{a-e}	2.8^{g-F}	S
BC_2F_3_ETSC_17081	Debir/Gobiye///Debir	lgs2_SBI-05 & lgs_3	21.55^{a-j}	56.53^{a-f}	1.9^{n-F}	S
BC_2F_3_ETSC_17082	Debir/Gobiye///Debir	lgs2_SBI-05 & lgs_3	15.67^{d-D}	25.78^{h-H}	3.76^{e-A}	S
BC_2F_3_ETSC_17083	Debir/Gobiye///Debir	lgs2_SBI-05 & lgs_3	22.56^{a-h}	53.39^{a-h}	1.6^{s-F}	S
BC_2F_3_ETSC_17084	Dekeba/Framida///Dekeba	lgs2_SBI-05	26^{ab}	64.93^{abc}	1.8^{p-F}	S
BC_2F_3_ETSC_17085	Gambella1107/Birhan///Gambella1107	lgs_3	17.1^{b-w}	47.6^{a-m}	3.1^{g-F}	S
BC_2F_3_ETSC_17086	Gambella1107/Birhan///Gambella1107	lgs_3	25.3^{abc}	55.75^{a-g}	1.79p^{-F}	S
BC_2F_3_ETSC_17087	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	15^{d-D}	24.38^{h-H}	1.82^{p-F}	S
BC_2F_3_ETSC_17088	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	9.78^{r-I}	26.1^{h-H}	2.35^{i-F}	R
BC_2F_3_ETSC_17089	Jamiyu/Birhan///Jamiyu	lgs2_SBI-05 & lgs_3	15.2^{d-D}	42.7^{b-v}	1.6^{r-F}	S
BC_2F_3_ETSC_17090	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	15.4^{d-D}	29.03^{e-H}	2.4^{i-F}	S
BC_2F_3_ETSC_17091	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	21^{a-m}	47.88^{a-m}	1.48^{s-F}	S
BC_2F_3_ETSC_17092	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	14.06^{g-E}	12.48^{w-H}	3.2^{g-F}	S
BC_2F_3_ETSC_17093	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	5.17^{E-I}	4.66^{D-H}	2.5^{i-F}	R
BC_2F_3_ETSC_17094	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	5^{E-I}	8.16^{A-H}	1.8^{p-F}	R
BC_2F_3_ETSC_17095	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	16.55^{b-z}	13.26^{w-H}	1.5^{t-F}	S
BC_2F_3_ETSC_17096	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	14.17^{g-E}	40.49^{b-w}	2.05^{m-F}	S
BC_2F_3_ETSC_17097	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	17.56^{b-v}	47.47^{a-o}	3.1^{g-F}	S
BC_2F_3_ETSC_17098	Jamiyu/Framida///Jamiyu	lgs2_SBI-05 & lgs_3	10^{r-H}	14.52^{u-H}	3.15^{g-F}	S
BC_2F_3_ETSC_17099	Jamiyu/Framida///Jamiyu	lgs2_SBI-05	10.45^{p-H}	8.23^{A-H}	0.5^{B-F}	S

Genotypes	Pedigree/breeder's code	QTL	MGD	GR	GI	Resistance level
		& lgs_3				
BC_2F_3_ETSC_17100	Jigurti/Birhan///Jigurti	lgs_3	$14.3^{f\text{-}E}$	$29.61^{e\text{-}H}$	$1.4^{t\text{-}F}$	S
BC_2F_3_ETSC_17101	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	$20.1^{b\text{-}p}$	$55.22^{a\text{-}g}$	$1.99^{m\text{-}F}$	S
BC_2F_3_ETSC_17102	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	$14.2^{g\text{-}E}$	$28.54^{e\text{-}H}$	11.5^{b}	S
BC_2F_3_ETSC_17103	Jigurti/Birhan///Jigurti	lgs2_SBI-05 & lgs_3	$12^{j\text{-}G}$	$13.28^{w\text{-}H}$	$1.84^{p\text{-}F}$	S
BC_2F_3_ETSC_17104	Jigurti/Birhan///Jigurti	lgs_3	$13.3^{h\text{-}F}$	$32.73^{d\text{-}E}$	$3.28^{g\text{-}E}$	S
BC_2F_3_ETSC_17105	Jigurti/Gobiye///Jigurti	lgs2_SBI-05 & lgs_3	$8.55^{v\text{-}I}$	$9.76^{y\text{-}H}$	$1.36^{u\text{-}F}$	R
BC_2F_3_ETSC_17106	Tseadachimure/Birhan///Tseadachimure	lgs2_SBI-05 & lgs_3	$8.78^{u\text{-}I}$	$20.59^{k\text{-}H}$	$1.2^{u\text{-}F}$	R
BC_2F_3_ETSC_17107	Tseadachimure/Birhan///Tseadachimure	lgs2_SBI-05 & lgs_3	$6.89^{z\text{-}I}$	$12.53^{w\text{-}H}$	$0.77^{y\text{-}F}$	R
BC_2F_3_ETSC_17108	Tseadachimure/Birhan///Tseadachimure	lgs2_SBI-05 & lgs_3	$7.56^{w\text{-}I}$	$8.58^{A\text{-}H}$	$1.1^{w\text{-}F}$	R
BC_2F_3_ETSC_17109	Tseadachimure/Birhan///Tseadachimure	lgs2_SBI-05 & lgs_3	$12.4^{i\text{-}G}$	$28.73^{e\text{-}H}$	$2.7^{h\text{-}F}$	S
BC_2F_3_ETSC_17111	Wediaker/Birhan///Wediaker	lgs2_SBI-05	$10.3^{q\text{-}H}$	$28.21^{f\text{-}H}$	$1.47^{s\text{-}F}$	S
BC_2F_3_ETSC_17112	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$9.78^{r\text{-}I}$	$16.78^{r\text{-}H}$	$1.5^{s\text{-}F}$	R
BC_2F_3_ETSC_17113	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$17.2^{b\text{-}w}$	$25.57^{h\text{-}H}$	$1.8^{p\text{-}F}$	S
BC_2F_3_ETSC_17114	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$18.3^{b\text{-}u}$	$32.5^{d\text{-}E}$	$4.35^{e\text{-}u}$	S
BC_2F_3_ETSC_17115	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$15.78^{c\text{-}C}$	$30.31^{e\text{-}H}$	$4.4^{e\text{-}u}$	S
BC_2F_3_ETSC_17116	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$9.1^{t\text{-}I}$	$10.15^{y\text{-}H}$	$0.98^{w\text{-}F}$	R
BC_2F_3_ETSC_17117	Wetetbegunchie/Birhan///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$6.2^{C\text{-}I}$	$17.69^{q\text{-}H}$	$2.51^{i\text{-}F}$	R
BC_2F_3_ETSC_17119	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$11.78^{k\text{-}G}$	$27.08^{g\text{-}H}$	$3.98^{e\text{-}x}$	S
BC_2F_3_ETSC_17120	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$16.67^{b\text{-}y}$	$46.76^{a\text{-}p}$	$2.27^{k\text{-}F}$	S
BC_2F_3_ETSC_17121	Wetetbegunchie/Framida///Wetetbegunchie	lgs2_SBI-05 & lgs_3	$17.67^{b\text{-}v}$	$40.93^{b\text{-}w}$	$4.35^{e\text{-}u}$	S
Berjokecoll#1	Local	-	$11.67^{k\text{-}G}$	$27.24^{g\text{-}H}$	$1.85^{o\text{-}F}$	S
Birhan	Key#8566	lgs2_SBI-05 & lgs_3	3.2^{GHI}	$9.13^{z\text{-}H}$	0.15^{EF}	R
Debir	Debir	-	$15.^{4d\text{-}D}$	$27.81^{f\text{-}H}$	$1.83^{p\text{-}F}$	S
Dekeba	ICSR24004	-	$21^{a\text{-}m}$	$60.35^{a\text{-}d}$	$2.2^{l\text{-}F}$	S
Framida	87441	lgs2_SBI-05 & lgs_3	$3.72^{F\text{-}I}$	$12.68^{w\text{-}H}$	$1.16^{v\text{-}F}$	R
Gambella1107	Gambella1107	-	$23.89^{a\text{-}f}$	65.6^{abc}	$2.01^{m\text{-}F}$	S
Gobiye	P-9403	lgs2_SBI-05 & lgs_3	1.3^{HI}	2.76^{GH}	0.09^{F}	R
Jamiyu	Local	-	$10.1^{r\text{-}H}$	$17.75^{p\text{-}H}$	$5.38^{d\text{-}k}$	S
Jigurti	Local	-	$19.2^{b\text{-}r}$	$43.27^{b\text{-}u}$	$2.5^{i\text{-}F}$	S
SRN39	-	-	0.0^{I}	0.0^{H}	$1.54^{s\text{-}F}$	R
Teshalle	3443-2-OP	-	$16.1^{c\text{-}B}$	$29.24^{e\text{-}H}$	$0.92^{w\text{-}F}$	S
Tseadachimure	Local	-	$17.89^{b\text{-}v}$	$47.58^{a\text{-}n}$	$2.06^{m\text{-}F}$	S
Wediaker	Local	-	$15^{d\text{-}D}$	$25.91^{h\text{-}H}$	$1.19^{u\text{-}F}$	S
Wetetbegunche	Local	-	$13.67^{g\text{-}E}$	$28.65^{e\text{-}H}$	$2.29^{k\text{-}F}$	S
Mean			14.18	29.46	2.68	
LSD(0.05)§			4.5	13.5	4.45	
CV (%) ¶			14.5	19.3	25.2	

†lgs2_SBI-05 (lgs2_SBI-05_60404021) with allele AT, ‡lgs_3: (lgs_3_60629027) with allele CT, § Least significant difference, ¶ Coefficient of variation, #Genotypes with the same letter are not significantly different at a 0.05, ††Susceptible lines, ‡‡Resistant line.

Comparison of lines with markers and parental lines for Striga stimulant activity

The donor parents consistently showed lowest *Striga* germination stimulant activity followed by 32.4% of backcrossed progeny with double QTLs. Of the 118 developed lines, 86.44%, 9.32%, and 4.24%, were comprised of QTLs (*lgs2_SBI-05_60404021 + lgs_3_60629027), lgs_3_60629027,* and *lgs2_SBI-05_60404021*, respectively (Table 3). About 32.4% of the converted progeny consisted of both markers at a time showed low *Striga* germination stimulants as explained by less than 10 mm MGD and low germination rate indicating the cumulative effects of the two QTLs in reducing the stimulation of *Striga* seeds germination. This clearly showed that these progeny had *lgs* resistance genes from the donor parents. Earlier reports also showed that the identification of different genes controlling low stimulation of *Striga* seed germination and their introgression into a single genotype enhanced not only the degree but also the durability of resistance to *Striga* (Ramaiah *et al*., 1990; Yohannes *et al*., 2015). Conversely, none of the backcrossed progeny with individual QTL showed resistance to the parasitic weed as revealed by the high production of *Striga* germination stimulants, perhaps due to the genetic background (epistasis interaction).Likewise, the seed parents were dominantly high producers of *Striga* stimulant activity.

Heritability and genetic advance from selection

Broad-sense heritability (H^2b) for the traits; MGD, GR, and GI were 77, 79, and 83%, respectively. The magnitude of broad sense heritability was generally high for most of the characters. According to Singh, (2002), heritability values greater than 80% were grouped as very high, values from 60-79% were moderately high, values from 40-59% were medium and values less than 40% were low. In this regard, the estimate of H^2b on GR was categorized as very high, and MGD and GI as moderately high indicating progress from selection can be attained agreeing with previous reports (Haussmann *et al*., 2001; Singh, 2002). It is normally concluded that heritability coupled with genetic advance is more useful and effective for selection of superior individuals than either of the parameters alone Johnson *et al*., (1955). The genetic advance (GA) as percentage of the mean was 61.97%, 93.2% and 71.3% for MGD, GR, and GI respectively. According to Johnson *et al*., (1955), GA is categorized as low (< 10%), moderate (10-20%), and high (> 20%). Based on the findings from this research, it could be concluded that each character had high GA and selection based on these characters could be fairly easy and effective.

Interrelationships among traits

Correlation coefficients (r) showed positively significant association among the three traits studied. MGD maintained significantly strong positive correlations ($p<0.01$) with GR ($r = 0.81$) and GI ($r = 0.4$). This indicated that the use of low levels of MGD and/or GR values could be considered as the best selection criteria for improving *Striga* resistance in sorghum. It is also interesting to note that the phenotype could reflect the genotype for these traits. GR also showed significant and positive ($r = 0.48$) correlation with GI. This might indicate that low germination rate could be associated with the induction of inhibitory compounds from the host roots. Previous report Hess *et al*., (1992) also revealed existence of high positive correlation ($r = 0.93$) between percent of germinated *Striga* and the distance from the host root to the further most germinated *Striga* seeds .

CONCLUSION

This study revealed that, the donor parental lines (Framida, Gobiye and Birhan) were good sources of resistance to *Striga* and can be recommended for future and continuous use in breeding programs. The result also indicated that *Striga* pre-attachment characters in the agar-gel assay could be effective for selection of resistant genotypes, as these traits were found to be indicative for existence of low germination stimulant mode of resistance mechanism. The result also clearly showed the combined expression of the two QTL s (*lgs*2_SBI-05_60404021 and *lgs*_3_60629027) showed better performance in MGD and GR values than those with any of the individual markers thus indicating that it is highly important to integrate different putative QTL probably with different mode of action.

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REFERENCES

1. AATF (African Agricultural Technology Foundation).(2011). Feasibility study on *Striga* control in Sorghum. AATF, Nairobi, ISBN 9966-775-12-9, pp 1-78.
2. Ali, R., Hash, T.C., Damaris, O., Elhussein, A. and Mohamed, H.A. (2016). Introgression of *Striga* resistance into popular Sudanese sorghum varieties using marker assisted selection. *World Journal of Biotechnology*1(01): 48-55.
3. Allard, R.W. (1960). Principles of Plant Breeding. John Willey and sons, Inc. New York
4. Amusan, I., Rich, P., Housley, T. and Gebisa Ejeta (2011). An In Vitro method for identifying post-attachment *Striga* resistance in maize and sorghum. *Agronomy Journal* 103: 1472-1478.
5. Atera, E.A., Itoh, K., Azuma, T. and Ishii, T. (2012). Farmers' perspectives on the biotic constraint of *Striga hermonthica* and its control in western Kenya. *Weed Biology and Management*. 12: 53-62.
6. Awad, A.A., Sato, D., Kusumoto, D., Kamioka, H., Takeuchi, Y. and Yoneyama, K., 2006. Characterization of strigolactones, germination stimulants for the root parasitic plants *Striga* and *Orobanche*, produced by maize, millet and sorghum. *Plant Growth Regulation* **48**(3): 221-227.
7. CSA (Central Statistical Agency) (2018). Area and production of major crops (private peasant holdings, meher season). Statistical bulletin 586, Addis Ababa, Ethiopia. pp 1-10.
8. Daniel Gobena, Mahdere Shimels, , Rich, P.J., Ruyter-Spira, C., Bouwmeester, H.J., Satish, K. Tesfaye Mengiste and Gebisa Ejeta.(2017). Mutation in sorghum low germination stimulant1 alters strigolactones and causes *Striga* resistance. *Proceedings of the National Academy of Sciences* 114 (17): 4471-4476.
9. Doggett, H. (1988). Sorghum, 2nd edn. Longman, Green Co.Ltd., London, pp. 512.
10. Falconer, D.S. (1989). Introduction to Quantitative Genetics. 3rd ed. Longman, London, England
11. FAO, (2017). Food and Agriculture Organization of the United Nations, Rome, Italy.
12. Fasil Reda , Dierick, A. and Verkleij, J.A.C. (2010). Virulence study of *Striga hermonthica* populations from Tigray region (Northern Ethiopia). *World Journal of Agricultural Science* 6(6): 676-682.
13. Firew Mekbib (2008). Genetic erosion of sorghum (*Sorghum bicolor* (L.) Moench) in the centre of diversity, Ethiopia. *Genetic Resources and Crop Evolution*55(3): 351-364.
14. Gebisa Ejeta, Butler, L.G., and Babiker, A.G., 1992. New approaches to the control of *Striga*. *Striga* Research at Purdue University, Agricultural Experiment Station, Purdue University, Research Bulletin, RB-991
15. Gebisa Ejeta (2007a). The *Striga* scourge in Africa: A growing pandemic. In: Integrating New Technologies for *Striga* Control: Towards Ending the Witch-Hunt, Ejeta, G. and Gressel,J. (eds) (World Scientific, Singapore), pp 3–16.
16. Gebisa Ejeta (2007b). *Striga* resistance in sorghum: exploitation of the intricate host-parasite biology. *Crop Science* 47:S216–S227.
17. Gebisa Ejeta, Butler, L.G. Hess, D.E., Obilana,A.T. and Reddy, B.V. (1997). Breeding for *Striga* resistance in sorghum. In: Proc, IntConf on Genetic Improvement of Sorghum and Pearl Millet, September 22–17, 1996, Lubbock, Texas, INTSORMIL/ICRISAT, Publication No. 97–105. Lubbock, Texas. pp. 504–516.
18. Gebisa Ejeta, Rich, J.P. and Mohamed, S. (2007). Dissecting a complex trait to simpler components for effective breeding of sorghum with a high level of *Striga* resistance. In: Gebisa Ejeta and Gressel, J. (eds). Integrating New Technologies for *Striga* Control: Towards Ending the Witch-Hunt. (World Scientific, Singapore). pp 87-97.
19. Grenier, C., Ibrahim, Y., Haussmann, B.I.G., Kiambi, D. and Gebisa Ejeta (2007). Marker-assisted selection for *Striga* resistance in sorghum. In: Gebisa Ejeta and Gressel, J. (eds). Integrating New Technologies for *Striga* Control: Towards Ending the Witch-Hunt. (World Scientific, Singapore). pp, 159-171.
20. Gwatidzo, V.O., Rugare , J.T., Mabasa, S., Mandumbu, R., Chipomho, J., and Chikuta, S., (2020). In Vitro and In Vivo Evaluation of Sorghum (*Sorghum bicolor* L. Moench) Genotypes for Pre- and Post-attachment Resistance against Witch weed (*Striga asiatica* L. Kuntze). *International Journal of Agronomy* 2020: 1-16.
21. Haussmann, B.I.G., Hess, D.E., Omanya, G.O., Folkertsma, R.T., Reddy, B.V.S., Kayentao, M.,

Welz, H. G. and Geiger, H. H. (2004). Genomic regions influencing resistance to the parasitic weed *Striga hermonthica*in two recombinant inbred populations of sorghum. *Theoretical and Applied Genetics* 109(5): 1005-1016.

22. Haussmann, B.I.G., Hess, D.E., Omanya, G.O., Reddy, B.V.S., Welz, H.G. and Geiger,H.H. (2001). Major and minor genes for stimulation of *Striga hermonthica* seed germination in sorghum, and interaction with different *Striga* populations. *Crop Science* 41:1507-1512

23. Haussmann, B.I.G., Hess, D.E., Reddy, B.V.S., Welz, H.G. and Geiger, H.H. (2000). Analysis of resistance to *Striga hermonthica* in diallel crosses of sorghum. *Euphytica* 116:33-40

24. He, C., Holme, J. and Anthony, J. (2014). SNP genotyping: the KASP assay. In: Crop breeding. Humana Press, New York, NY. pp. 75-86.

25. Hess, D.E., Gebisa Ejeta and Butler, L.G. (1992). Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to *Striga*. *Photochemistry* 31: 493-497.

26. Johnson, H.W., Robinson, H.F. and Comstock, R.E.(1955). Estimates of Genetic and Environmental Variability in Soya beans. *Agronomy Journal* 47: 314-318.

27. Kanampiu, F.,Makumbi, D., Mageto, E., Omanya, G., Waruingi, S., Musyoka, P. and Ransom, J. (2018). Assessment of management options on *Striga* infestation and maize grain yield in Kenya. *Weed Science* 66(4): 516-524.

28. Kountche, B.A., Al-Babili, S. and Haussmann, B.I.G. (2016). *Striga*: A Persistent Problem on Millets. In Biotic stress resistance in Millets. Academic Press. pp. 173-203.

29. Kountche, B.A., Jamil, M., Yonli, D., Nikiema, M.P., Blanco-Ania, D., Asami, T., Zwanenburg, B. and Al-Babili, S. (2019). Suicidal germination as a control strategy for *Striga hermonthica* (Benth.) in smallholder farms of sub-Saharan Africa. *Plants, People, Planet*, **1**(2):107-118.

30. Kumar, A.A. (2016). Botany, Taxonomy and Breeding. In: The Sorghum Genome. Springer, Cham. pp 27-45.

31. Mesfin Abate, Firew Mekbib, Temam Hussien,Wondimu Bayu and Fassil Reda (2014). Assessment of genetic diversity in sorghum (*Sorghum bicolor* (L.) Moench) for reactions to *Striga hermonthica* (Del.) Benth. *Australian Journal of Crop Science* 8(8):1248-1256.

32. Miller, P.A., Williams, C., Robinson,H.F. and Comstock, R.E. (1958). Estimates of genotypic and Environmental variances and co-variances in upland cotton and their implications in selection. *Agronomy Journal* 50:126 -131.

33. Mohamed, A., Ali, R., Elhassan, O., Suliman, E., Mugoya, C., Masiga, C.W., Elhusien, A. and Hash,C.T. (2014). First products of DNA marker-assisted selection in sorghum released for cultivation by farmers in sub-Saharan Africa. *Journal of Plant Science and Molecular Breeding*3(3): 1-10.

34. Mohamed, A.H., Housley, T.L. and Gebisa Ejeta (2010). An in vitro technique for studying specific *Striga* resistance mechanisms in sorghum. *African Journal of Agricultural Research* 5: 1868-1875.

35. Mohemed, N., Charnikhova, T., Bakker, E.J., van Ast, A., Babiker, A.G. and Bouwmeester, H.J. (2016). Evaluation of field resistance to *Striga hermonthica* (Del.) Benth. in *Sorghum bicolor* (L.) Moench. The relationship with strigolactones. *Pest Management Science* 72: 2082-2090

36. Mohemed, N., Charnikhova, T., Fradin, E.F., Rienstra, J.,Babiker, A.G. and Bouwmeester, H.J. (2018). Genetic variation in Sorghum bicolor strigolactones and their role in resistance against *Striga hermonthica*. *Journal Experimental Botany*69(9): 2415-2430.

37. Omanya, G.O., Haussmann, B.I.G., Hess, D.E., Reddy, B.V.S., Kayentao, M., Welz, H.G. and Geiger, H.H. (2004). Utility of indirect and direct selection traits for improving *Striga* resistance in two sorghum recombinant inbred populations. *Field Crops Research* 89: 237-252

38. Parker, C. (2009). Observations on the current status of *Orobache* and *Striga* problems worldwide. *Pest Management Science* 65: 453-459.

39. Parker, C. (2012). Parasitic weeds: a world challenge. *Weed Science* 60: 269-276.

40. Pennisi, E. (2015). How crop-killing witchweed senses its victims. *Science* 350: 146-147.

41. Prandi, C., Occhiato, E.G., Tabasso, S., Bonfante, P., Novero, M., Scarpi, D., Bova, M.E. and Miletto, I. (2011). New potent fluorescent analogues of strigolactones: synthesis and biological activity in parasitic weed germination and fungal branching. *European Journal of Organic Chemistry*(20-21): 3781-3793.

42. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

43. Ramaiah, K.V.(1987). Breeding cereal grains for resistance to witchweed. In: Musselman, L.J. (ed.), Parasitic Weeds in Agriculture, Vol I, *Striga*. CRC Press, Boca Raton, FL, pp. 227-242.

44. Ramaiah, K.V., Chidley, V.L. and House, L.R. (1990). Inheritance of *Striga* seed-germination stimulant in sorghum. *Euphytica* 45: 33-38.

45. Rank, C., Rasmussen, L.S., Jensen, S.R., Pierce, S., Press, M.C. and Scholes, J.D. (2004). Cytotoxic constituents of Alectra and *Striga* species. *Weed Research* 44 (4): 265-270.

46. Reddy, P.S. (2017). Sorghum, *Sorghum bicolor* (L.) Moench. In: Patil, J.V. (Eds), Millets and Sorghum: Biology and Genetic Improvement. pp.1-32.

47. Rich, P.J. and Daniel Gobena (2016). Laboratory Methods for *Striga* resistance Screening in Sorghum. pp38.

48. Rich, P.J. and Gabisa Ejeta (2008). Towards effective resistance to *Striga* in African maize. *Plant Signaling and Behavior*3(9): 618-621.

49. Rich, P.J., Grenier, C. and Gabisa Ejeta (2004). *Striga* resistance in the wild relatives of sorghum. *Crop Science* 44: 2221-2229.

50. Rodenburg, J., Bastiaans, L., Kropff, M.J. and Van Ast, A.. (2006). Effects of host plant genotype and seed bank density on *Striga* reproduction. *Weed Research* 46: 251-263.

51. Satish, K., Gutema, Z., Grenier, C., Rich, P.J. and Gabisa Ejeta (2012). Tagging and validation of microsatellite markers linked to the low germination stimulant gene (*lgs*) for *Striga* resistance in sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical Applied Genetics* 124: 989-1003.

52. Singh, B.D. (2002). Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi-Ludhiana.

53. Singh, R.K. and Chaudhary, B.D. (1985). Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi-Ludhiana.

54. Tesfaye Tesso, Zenbaba, Gutema,Aberra Deressa, and Gebisa Ejeta (2007). An integrated *Striga* management option offers effective control of *Striga* in Ethiopia. In: Gebisa Ejeta and J. Gressel. (Eds.), Integrating New Technologies for *Striga* Control: Towards Ending the Witch-Hunt. World Scientific Publishing Co. Pvt. Ltd., Singapore, pp. 199-212.

55. Tsuchiya, Y., Yoshimura, M. and Hagihara, S. (2018). The dynamics of Strigolactone perception in *Strigahermonthica*: a working hypothesis. *Journal of experimental Botany* 69(9): 2281-2290.

56. VasudevaRao, M.J. and Musselman, L.J. (1987). Host specificity in *Striga* spp. and physiological strains. In: Musselman, L.J. (Ed.), Parasitic Weeds in Agriculture, Vol. I, *Striga*. CRC Press, Boca Raton, FL, pp. 13_25.

57. Venne, J., Beed, F. and Watson, A. (2009). Integrating *Fusariumoxysporum* f. sp. *strigae* into cereal cropping systems in Africa. *Pest Management Science* 65: 572-580.

58. Visarada, K.B.R.S. and Aruna, C. (2019). Sorghum: A Bundle of Opportunities in the 21st Century: In: Aruna C., K.B.R.S. Visarada, B.VenkateshBhat, and V.ATonapi. (eds). Breeding Sorghum for diverse end uses. pp 1-10.

59. Welsh, A.B. and Mohamed, K.L. (2011). Genetic diversity of *Striga hermonthica*populations in Ethiopia: evaluating the role of geography and host specificity in shaping population structure. *International Journal of Plant Science* 172: 773-782.

60. Westwood, J.H., Depamphilis, C.W., Das, M., Fernández-Aparicio, M., Honaas, L.A., Timko, M.P., Wafula,E.K., Wickett, N.J. and Yoder, J.I. (2012). The parasitic plant genome project: new tools for understanding the biology of *Orobanche* and *Striga*. *Weed Science* 60(2): 295-306.

61. Williams, C.N. (1958).The parasitism of witchweed. a review. *West African Journal of Biological Chemistry*2: 4-7.

62. Xie, X., Yoneyama, K., Kusumoto, D., Yamada, Y., Yokota, T., Takeuchi, Y. and Yoneyama, K., 2008. Isolation and identification of alectrol as (+)-orobanchyl acetate, a germination stimulant for root parasitic plants. *Phytochemistry* **69**(2): 427-431

63. Xie, X., Yoneyama, K. and Yoneyama, K. (2010). The strigolactone story. *AnnualReviewof Phytopathology*pp 48 (1): 93-117.

64. Yohannes, T., Tesfamichael, A., Kiambi, D., Folkertsma, R., Hash, C.T., Ngugi, K., Mutitu, E.,Abraha, N., Weldetsion, M., Mugoya, C. and Masiga, C.W. (2015). Marker-assisted introgression improves *Striga* resistance in an Eritrean Farmer-Preferred Sorghum Variety. *Field Crops Research* 173: 22-29.

65. Yoneyama, K., Arakawa, R., Ishimoto,K., Kim, H.I., Kisugi, T., Xie, X., Nomura T.,Kanampiu, F., Yokota, T., Ezawa, T. and Yoneyama, K. (2015). Difference in *Striga*-susceptibility is reflected in strigolactone secretion profile, but not in compatibility and host preference in Arbuscular mycorrhizal symbiosis in two maize cultivars. *New Phytologist* 206(3): 983-989.

66. Yoder, J.I., and Scholes, J.D., 2010. Host plant resistance to parasitic weeds; recent progress and bottlenecks. *Current opinion in plant biology* **13**(4): 478-484.

67. Yoneyama, K., Awad, A.A., Xie, X., Yoneyama K., Takeuchi, Y. (2010). Strigolactones as germination stimulants for root parasitic plants. *Plant Cell Physiology* 51(7):1095-1103.