

# Harnessing the strigolactone biosynthesis mutant *lgs1* to combat food insecurity in Africa

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## Abstract

Sorghum is a food staple for millions of people in sub-Saharan Africa, but parasitic weeds of the *Striga* genus greatly diminish its production. An efficient and cost-effective way of managing *Striga* in smallholder farms in Africa is to deploy resistant varieties. Here, we leverage genomics and the vast genetic diversity of sorghum – evolutionarily adapted to cope with *Striga* parasitism in Africa – to identify new *Striga*-resistant sorghum genotypes. We exploit a *Striga* resistance mechanism that hinges on essential communication molecules – strigolactones exuded by hosts to trigger parasite seed germination. We used the Sorghum Association Panel (SAP) to search for sorghum genotypes with a mutation on the *LOW GERMINATION STIMULANT 1* (*LGS1*) locus that makes them ineffective in inducing *Striga* germination. Our analysis led us to identify new *lgs1* sorghum genotypes which we named SAP *lgs1*. SAP *lgs1* had the SL exudation profile of known *lgs1* sorghum whose hallmark is the production of the low inducer of germination, orobanchol. Laboratory and field resistance screens showed that the SAP *lgs1* genotypes also exhibited remarkable resistance against *Striga*. Our findings have far-reaching implications for improving food security in Africa by potentially reducing crop losses due to *Striga* parasitism.

## Introduction

*Sorghum bicolor* (L.) Moench is a well-adapted C4 grass that is a staple food to millions in sub-Saharan Africa (SSA). Despite its great economic value, sorghum cultivation in this region is threatened by *Striga*, a genus of parasitic plants that feeds from its hosts by obstructing nutrients and water through a specialized organ called haustorium (Hearne 2009). *Striga* also inflicts phytotoxicity on its hosts (Fujioka, Samejima, Mizutani, Okamoto & Sugimoto 2019a), severely impairing the growth and yield of infested crops (Frost, Gurney, Press & Scholes 1997). Infestation in SSA is estimated at 100 million ha (Spallek, Mutuku & Shirasu 2013) with a corresponding annual loss of USD 7 billion (Ejeta, 2007), which increases exponentially every year (Rodenburg, Demont, Zwart & Bastiaans 2016). *Striga*'s success is attributed to its well-adapted parasitic lifestyle that includes seed fecundity, longevity, ease of dispersal, and capacity to manipulate host immunity (Runo & Kuria 2018).

For smallholder farmers in SSA, the most efficient, and sustainable *Striga* control method is an integrated approach that incorporates natural host resistance as a major component (Mwangangi *et al.* 2021). Such

resistance can be achieved by interfering with critical communication exchange between the host and the parasite (Jamil *et al.*., 2021).

At the fore of this host to *Striga* dialogue is the parasite’s perception of host-derived chemo attractants commonly known as strigolactones (SLs), Reviewed in (Al-Babili & Bouwmeester 2014). SLs are structurally diverse and differ in their efficiency and specificity in inducing seed germination in root parasitic plants (Guercio, Palayam & Shabek 2023). In sorghum, most genotypes emit 5-deoxystrigol as the dominant SL while a few emit orobanchol (Gobena *et al.* 2017; Mohamed *et al.* 2018). Orobanchol is a poor inducer of *Striga* germination, so accordingly, sorghum genotypes that emit primarily orobanchol are termed *Striga* resistant. Advances in molecular genetics have now shown that sorghum genotypes that emit orobanchol have a mutation in a single recessive gene identified as the *LOW GERMINATION STIMULANT 1 (LGS1)* locus located at the tip of chromosome 5 (Satish, Gutema, Grenier, Rich & Ejeta 2012; Gobena *et al.* 2017). The causative gene encodes a sulfotransferase and is missing in producers of 5-deoxystrigol (Gobena *et al.* 2017). Further evidence has shown that loss-of-function alleles have been subject to selection in sorghum landraces from SSA, where *Striga* and sorghum co-evolved, conferring *Striga* resistance to some African sorghum accessions (Bellis *et al.* 2020). This finding was upheld by the widespread discovery of new *lgs1* African sorghum genotypes harboring *Striga* resistance (Mallu *et al.* 2021).

Encouraged by these results and motivated by the potential of increasing sorghum yields in SSA through the cultivation of *Striga* resistant sorghum, we sought to mine for new alleles of *Striga* resistant sorghum. For this study, we selected a large collection of sorghum accessions, the Sorghum Accession Panel (SAP), maintained by the United States Department of Agriculture Agricultural Research Service (USDA-ARS) (<https://www.ars-grin.gov/>). High quality genotypic data is available for the SAP including genotyping by sequencing, single nucleotide polymorphism (SNP) markers (Morris *et al.* 2013) and whole genome sequencing (WGS) (Boatwright *et al.* 2022) making the SAP suitable for subsequent molecular breeding studies. The SAP provides a further advantage because it comprises converted tropical lines (Klein *et al.* 2008) of diverse genotypes and geographic origin as well as breeding lines that are photoperiod-insensitive, elite inbred, improved cultivars, and landraces selected to capture maximum genetic and phenotypic diversity (Casa *et al.* 2008). We describe how the SAP, and genomics resources helped us to identify new *Striga* resistant, early maturing sorghum. Incorporating this material into breeding programs can potentially alleviate losses caused by *Striga* parasitism and positively impact food security in smallholder farms of Africa.

## Materials and Methods

### Plant materials

Seeds of *Sorghum bicolor* were obtained from the USDA-ARS, Plant Genetic Resources Conservation Unit, and Griffin, Georgia, USA (<https://www.ars-grin.gov/>). SRN39 (PI656027) was used as the resistant control while the Chinese landrace, Shanqui Red (PI 656025) as the susceptible control. Seeds of *Striga hermonthica* were collected from sorghum growing fields in Western Kenya, Alupe (0.45°, 34.13°) during the long rain-growing season in 2017. Seeds were stored and handled under an approved quarantined laboratory facility at Kenyatta University (Nairobi, Kenya).

### DNA isolation and polymerase chain reaction

Sorghum leaf tissues were harvested from 14 days old seedlings for DNA isolation. Total genomic DNA was extracted from three individual plants for each of the 373 genotypes (separate extractions) using a modified CTAB method according to (Mace *et al.* . (2003). DNA was amplified in a multiplex-Polymerase Chain reaction (PCR) targeting Sulfotransferase and Ubiquitin genes. The Sulfotransferase primer pair (PD*Striga* 15b: forward 5’-CAAACCCATCGGACATCTTC-3’ ;PD*Strigalgs* 5b: reverse 5’ -CAGCATGTCCTCGTACCTGA-3’ ) as well as the Ubiquitin primers (forward 5’ -ATGTCCTGCTGCGAAGCTAT-3’ ;reverse 5’ -GCTAGAGCACCCGAGGAGTA-3’ ), have been previously described by Gobena *et al.* . (2017) and Mallu *et al.* . (2021), respectively. PCR reactions were performed in 20 µl volumes using MyTaq DNA polymerase kit (Bioline, Meridian Biosciences). The cycling conditions were 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 62.5°C for 30 seconds, an extension of 72°C

for 1 minute and a final extension of 72°C for 7 minutes using a Bio-Rad thermal cycler. The PCR amplicons were resolved in a 2% (w/v) agarose gel stained with Safe View<sup>TM</sup> Fire Red (Biotium) and visualized under UV light.

### Genetic description of the sorghum accession panel

To visualize the global origin of various sorghum genotypes and *Striga* range, we utilized the Geographical Positioning System (GPS) coordinates available at <https://www.genesys-pgr.org/> and <https://www.pnas.org/content/117/8/4243/tab-figure-data> (Dataset\_S03) and Dataset\_S1 to construct distribution maps using the MapTool (Lemmond 1994) version 2 package in R. We then obtained Single Nucleotide Polymorphism (SNP) data (Morris *et al.* 2013) from the repository of Colorado State University available at <https://www.morislabs.org/data>. These polymorphisms were used to determine the genetic structure of the SAP using phylogenetic relationships, Bayesian-based clustering, and discriminant analysis of principle components (DAPC). To infer phylogenetic relationships of sorghum accessions, we first converted the SNP Hapmap file to a Variant Calling File (VCF) using Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) version 5.0 (Bradbury *et al.* 2007). The resulting file was subsequently used to construct a neighbor-joining (NJ) tree using the Analyses of Phylogenetics and Evolution (APE) package in R (Paradis, Claude & Strimmer 2004). For Bayesian clustering, we used ADMIXTURE in plink (Alexander, Novembre & Lange 2009) by first converting the SNP data in Hapmap format into plink format TASSEL. The resulting file was used to determine clusters of the population through analysis of K values from 1 to 10 with a burn-in period of 50,000 iterations and 500,000 Markov Chain Monte Carlo (MCMC) iterations by assuming the admixture model. The most likely number of clusters were determined from the K value with the least cross validation error. Admixture plots were then visualized using the ggplot2 function in R (Villanueva & Chen 2019). Calculation of principal components was done using Adegenet R package (Jombart 2008) and factoextra in R.

### Striga seed germination bioassays

To evaluate the resistance of sorghum accessions, we screened 11 *lgs1* sorghum genotypes identified from genotypic screening of SAP accessions for *Striga* germination induction. Shanqui Red, PI533839, PI656054 were used as LGS1 controls while SRN 39 was used as the *lgs1* check.

Two *Striga* germination assays were used: (i) germination frequency using extracted sorghum root exudate and (ii) agar gel assays (Hess, Ejeta & Butler 1992). Root exudate germination assays were performed according to Jamil *et al.* (2022). *Striga* seeds were surface sterilized in 10% commercial bleach for 5 minutes followed by rinsing thoroughly with sterile milliQ water and air drying in a laminar flow for one hour. *Striga* seeds were then uniformly distributed (approximately 50-100 seeds each) on 9mm-fibre filter paper discs (Sartorius, Goettingen Germany) and put in 90mm-diameter petri-plates lined with moistened filter paper (Whatman, Maidstone, UK). Twelve (12) discs containing *Striga* seeds were transferred to each plate, sealed with parafilm, and wrapped in aluminum foil. For conditioning, *Striga* seeds were incubated at 30°C for 10 days. Afterwards, conditioned *Striga* seeds were treated with root exudates collected from each sorghum (55 µL per disc). Six discs were used per treatment. The petri plates were sealed, wrapped in aluminum foil, and incubated for 24 h at 30°C to induce germination. Germinated and non-germinated seeds were analyzed using the SeedQuant software (Braguay *et al.* 2021) by observing under a binocular microscope. The germination frequency in percentage (GF %) was calculated for each replicate using the formula:

$$GF = \left( \frac{N_{gs}}{N_{ts}} \right) \times 100$$
 Where  $N_{gs}$  is the number of germinated seeds per disc and  $N_{ts}$  is the total number of seeds per disc.

Agar gel assays were performed according to Hess *et al.* (1992). Sorghum seeds were soaked in 70 % ethanol for 5 minutes and then in 1 % NaOCl solution for 20 mins. Seeds were rinsed thoroughly in sterile double distilled water and transferred in petri dishes lined with moist Whatman GFA filter paper (Meadow, UK). The plates were then wrapped in aluminum foil and incubated at 28 °C for 48 hrs. Conditioned *S. hermonthica* seeds (300 µl) were pipetted into petri dishes then water agar (0.8 %) was dispensed over the

seeds to achieve even distribution. To the solidifying media, a pre-germinated sorghum seedling was placed at the edge of each plate with the tip of the root pointing across the plate. The gel-embedded host-parasite culture was incubated at 28 °C in the dark for 72 hours and scored for induction of *Striga* seed germination. Images were taken using a Leica MZ10F microscope and analyzed for germination using imageJ Version 1.5i (<https://imagej.nih.gov/ij/>). The maximum germination distance (MGD), which is the distance between the host root and the furthest germinated seeds, was used as a measure of resistance.

### LC-MS/MS analysis of root exudates

Analysis of root exudates was conducted on 13 genotypes (9 SAP *lgs1* natural mutants, 2 *LGS1* lines), alongside resistant and susceptible checks (SRN39 and Shanqui Red). Seeds of sorghum were surface sterilized in 50 % sodium hypochlorite and germinated *in vitro* on petri plates lined with moistened filter papers. One-week-old sorghum seedlings were subjected to a hydroponic system containing normal Hoaglands nutrient solution (with phosphate). After one week, the sorghum seedlings were fed with nutrient solution devoid of phosphate (-Pi) for 7 days. On the day of root exudates collection, seedlings were first fed with fresh -Pi solution for 6 h, and then root exudates were collected for LC-MS/MS analysis and *Striga* bioassays.

SL analysis of the root exudates was done according to (Wang *et al.* 2021). Briefly, root exudates spiked with 2 ng of *rac* -GR24 and passed through C<sub>18</sub>-Fast Reversed-SPE column (500 mg/3 mL), and the SLs eluted in 5 mL of acetone. SLs were extracted in ethyl acetate, vacuum dried and the final extract was dissolved in acetonitrile:water (25:75). SLs were quantified by LC-MS/MS using UHPLC-Triple-Stage-Quadrupole Mass Spectrometer. Strigolactones were identified by comparing the MS/MS spectrum and retention time with standards including 5-deoxystrigol, orobanchol and GR24. The characteristic Multiple Reaction Monitoring (MRM) transitions (precursor ion - product ion) were 331.15-216.0, 331.15-234.1, 331.15-97.02 for 5-deoxystrigol; 347.14-329.14, 347.14-233.12, 347.14- 205.12, 347.14-97.02 for orobanchol; 299.09-158.06, 299.09-157.06, 299.09-97.02 for GR24.

LCMS quantification of abscisic acid (ABA) followed the procedure by Wang *et al.* (2021). ABA was analyzed using LC-MS/MS using UHPLC-Triple-Stage Quadrupole Mass Spectrometer. The characteristic Multiple Reaction Monitoring (MRM) transitions (precursor ion - product ion) was 263.2 - 153.1, 263.3 - 204.1, 263.3 - 219.1 for ABA

### Evaluation of *Striga* resistance in pot experiments

The resistance response of 13 SAP *lgs1* sorghum lines alongside controls was further studied in pots under greenhouse conditions as described by Jamil *et al.* (2022b). A mixture of soil (Stender), sand and rock at a 3:1:1 ratio was prepared. About 1 L of uninfected soil was added to the bottom of each 3 L plastic pot. Then, about 120 mg (~70000) *Striga* seeds were mixed thoroughly with 6 L soil mixture which was added equally in six pots (1 L per pot) containing uninfected soil. The pots were irrigated and *Striga* seeds allowed to condition for 10 days. Following conditioning, one-week-old sorghum seedlings (one per pot) were planted. The number of emerged *Striga* seedlings and plant height were scored in each pot 10 weeks after sorghum planting.

### Evaluation of *Striga* emergence under natural field infestation

Sixteen (16) sorghum genotypes consisting of 12 SAP *lgs1* mutants, 2 *LGS1* lines, and a resistant and susceptible check (SRN39 and Shanqui Red) were evaluated in a *Striga* infested field in Alupe, Kenya (0.45deg, 34.7deg). The sorghum entries were planted in four-row plots measuring 4 x 3.2 m. Each plot was surrounded by 1 m path. Spacing was set at 80 cm between rows and 20 cm between plants and the 16 entries were laid in lattice design replicated three times. To ensure uniform distribution of *Striga* seeds in the sick plots, each planting hill was augmented with approximately 3000 *Striga* seeds. 2 g of diammonium phosphate (DAP) fertilizer were applied per hill. Twenty-one days after sowing plants were weeded using a hoe and subsequently thinned leaving 8 plants per row. To avoid disturbing the emerging *Striga* plants, second and third weeding was done by hand pulling. The same experimental set up was used on non-infested (*Striga*free) plots.



Sorghum agronomic traits and *Striga* data parameters were measured. The number of emerging *Striga* plants was counted at 42, 56, 70, 84 and 98 days after sowing in each plot. Data on sorghum included days to 50 % flowering, plant height, weight of 100 grains (HGW). Sorghum panicles were sun dried, threshed and kernel yield was determined.

## Data analysis

Data were analyzed using agricolae and tidyverse R packages for Analysis of Variance and plotting as described in (Wickham 2016). The resulting means were separated using Tukey's honest significant difference (HSD) test at a 5% level of significance, and box plots were generated using ggplot2 (Wickham et al., 2019). Area Under *Striga* Progressive Curve (AUSNPC) was determined based on methods described in (Simko & Piepho 2012) and customized R scripts.

## Results

### Genetic structure of the sorghum accession panel

*Striga* resistance is more prevalent in sorghum landraces from SSA because of co-evolution of the host and the pathogen (Bellis *et al.* 2020). Analysis of the geo-referenced origin of the SAP showed a notable overlap with *Striga* distribution range in SSA (Figure 1a and b). Further, the SAP was genetically diverse with representation from all the major races and subraces of sorghum. The most common race was caudatum and its subraces having 122 accessions with collections mostly from Africa. Durra was also common with 79 collections from eastern Africa and India. The least common race was bicolor with 10 accessions collected from eastern Africa (Figure S1a). To understand the genetic structure of the SAP, we carried out population structure analysis. We used both phylogenetic relationships and hierarchical Bayesian clustering (HBC) to provide information on sorghum race structure. In general, most races formed clusters based on their races rather than geographical status as revealed by phylogenetic analysis (Figure 1c). HBC further resolved the genetic structure of the SAP. At the most likely K value (K=7) HBC grouped the panel into 5 distinct clusters of durra, bicolor, guinea, caudatum and kafir caudatum. The rest of the population was an admixture suggestive of multiple race integrations in the development of the panel (Figure S1b). Based on genetic diversity, and high representation from SSA, we determined that the SAP collection was a good resource for *Striga* resistance allele mining.

### Identification and characterization of new low germination stimulant 1 (*lgs1*) sorghum

We sought to determine if there were *Striga* resistant genotypes in the SAP. The best characterized genetic cause of *Striga* resistance is the *LOW GERMINATION LOCI 1 (LGS1)* that leads to the low germination stimulant phenotype. The causative gene (Sobic.0011G112600) encodes a sulfotransferase whose loss of function causes a shift from high to low potent SLs (Gobena *et al.* 2017). Sorghum genotypes with a deletion in the LGS1 region are poor inducers of germination and are *Striga* resistant.

To determine if any of the SAP genotypes had a deletion on the *LGS1* region, we performed a polymerase chain reaction (PCR) to amplify the sulfotransferase gene. A positive PCR is indicative of an intact gene and a band absence represents a gene deletion. To ensure that the lack of a band is not due to PCR failure, our PCR was designed to simultaneously amplify the sulfotransferase and the housekeeping gene, ubiquitin. Analysis of 373 genotypes, using *LGS1/lgs1* maker identified twelve genotypes that had a no amplification product for the sulfotransferase gene. Wild type (*LGS1*) sorghum showed two amplicons, Ubiquitin (223 bp) and sulfotransferase (628 bp) signifying an intact gene consistent with the *Striga* susceptible Shanqui Red, used in this study as a susceptible check while *lgs1* showed a single band at 223bp resulting from Ubiquitin amplification but not sulfotransferase (Figure 2a). Absence of the 628 band is consistent with a deletion in the LGS1 loci previously described in the *Striga* resistant SRN39 used in this study as the resistant check. In total, we identified 12 *lgs1* genotypes from the screened 373 SAP. Two of these genotypes (PI 656040) and (PI 533976) had been previously described in Bellis *et al.* 2020.

The hallmark of the *lgs1* mutations is the ability to direct the SL biosynthetic pathway towards orobanchol instead of 5-deoxystrigol. We therefore sought to determine if the observed *Striga* resistance in the SAP *lgs1*

genotypes was associated with production of orobanchol. LC-MS-MS analysis of SLs from the root exudates of SAP *lgs1* revealed a predominant peak that corresponded to orobanchol (RT=10.86 mins) while SAP LGS1 lines showed a peak identical with that of 5-deoxystrigol (RT =14.59 mins) (Figure 2b). Consistently, mutant SAP *lgs1* genotypes alongside the resistant check SRN 39 had high levels of orobanchol (Figure 2d) while wild type SAP *LGS1* and Shanqui Red, a well characterized *LGS1* genotype, predominantly produced 5-deoxystrigol (Figure 2d). These findings affirmed that mutations in *LGS1* lead to changes in the types and amounts of SL emitted and that *Striga* resistance is associated with production of orobanchol as the major SL in sorghum.

### ***Striga* resistance in SAP low germination stimulant 1 sorghum**

Following on knowledge that sorghum genotypes that exude primarily orobanchol are poor inducers of *Striga* germination, and therefore *Striga* resistant, we performed two bioassays routinely used to measure *Striga* resistance. In the first assay, we used root exudates extracted from roots of sorghum to stimulate germination of *Striga* seeds and determined the germination frequency (Figure 3a). Our analysis showed that most of the SAP *lgs1* accessions were ineffective in stimulating *Striga* germination as displayed by their low *Striga* seed germination frequencies (Figure 3b). Most notably, PI585295, PI533976 and PI655979 had germination frequencies of less than 40 %, two-fold lower than that of the resistant control SRN 39. Expectedly, wild type LGS1 and the susceptible check Shanqui Red had high germination frequencies (Shanqui Red, 62% and PI533839, 73.6%). Surprisingly, PI656054, a wild type LGS1, and a 5-deoxystrigol producer had a notably low germination frequency (31.1 %) but high MGD alluding to the presence of other pre-attachment resistance mechanisms independent of the *LGS1* region. There is a growing body of circumstantial evidence that implicates other hormones, particularly abscisic acid (ABA) in regulation of *Striga* seed germination. It is hypothesized that; some sorghum genotypes emit large amounts of ABA and this could inhibit germination of the *Striga* seeds (Mallu *et al.* 2022). To explore if ABA was responsible for the low germination activity of SAP LGS1, we quantified the concentration of ABA in the root exudates of SAP accessions (Figure S3). However, our results did not show any correlation between germination stimulation and ABA levels in the root exudates, leaving the pre-attachment resistance mechanism of SAP LGS1 PI656054 unclear.

The second assay, agar gel assay involves co-cultivation of sorghum with preconditioned *Striga* embedded in agar. In this assay, resistant host roots stimulate less germination of parasite seeds and only do so for seeds closest to them (Supplementary Figure 2). This germination ability is measured using the metric of maximum germination distance (MGD) that computes the maximum germination distance between sorghum rootlets and the three most distant germinated *Striga* seeds. Resistant hosts therefore have low MGD while susceptible hosts have high MGDs. We found that all 12 *lgs1* SAP sorghum had notably lower MGDs relative to the wild type *LGS1* controls and the susceptible check Shanqui Red (Figure 3c). Notably, the MGDs of 6 SAP *lgs1* genotypes were comparable to that of the resistant control, SRN39.

Considering that the overall resistance of a host is a function of its ability to stimulate germination (pre-attachment resistance) and to block host attachment (post-attachment resistance), we performed pot experiments to determine *Striga* emergence for the SAP *lgs1* genotypes (Figure 3e). Results showed that 5 SAP *lgs1* genotypes had lower or comparable number of emerging *Striga* as shown in Figure 3d (PI655979, count =11.75, PI656040, count =6, PI533576, count= 5.75) with the resistant check SRN39 (count = 16). Additionally, SAP LGS1 accession PI645054 had low *Striga* infestation (14.5), further supporting germination assays described above. Notably, some SAP *lgs1* genotypes had high *Striga* emergence, an indication of weak post-attachment resistance in those genotypes.

### **Performance of SAP low germination stimulant 1 sorghum under natural *Striga* infestation**

The ultimate test for *Striga* resistance performance under natural pest infestations. We, therefore, evaluated the resistance response of the SAP *lgs1* accessions in a *Striga*-infested field in western Kenya by measuring *Striga* emergence using the metric of Area Under *Striga* Number Progressive Curve (AUSNPC); a modification of the area under the disease progress curve (AUDPC) developed for plant pathology studies (Simko & Piepho 2012). In this approach, a quantitative measurement of *Striga* infestation is determined

over time. For our case, we determined *Striga* infestation using data collected at 42, 56, 70, 84 and 98 days after planting.

Resistance measured by AUSNPC showed that most SAP *lgs1* had high *Striga* resistance (Figure 4a). We would like to point out SAP *lgs1* PI533976 which had remarkably lower emergence (p value = 0.00017) compared to the base mean of all accessions. Furthermore, the resistance of PI533976 was higher than that of known *Striga* resistant varieties including SRN39, Framida and IS9830. Also striking was the field resistance displayed by the SAP *LGS1* PI655054 accession, described earlier in germination bioassays and pot experiments. Field resistance of this genotype affirmed its importance as a *Striga* resistant accession. Expectedly, some SAP *lgs1* accessions for example PI656040, PI585295, and PI656010 showed low resistance, even comparable to the susceptible Shanqui Red and *LGS1* PI533839. These same genotypes had displayed low resistance in pot experiments. The low resistance could be attributed to low post-attachment resistance. Two of these accessions (PI585295, PI656010) that were assayed biochemically were also shown to produce relatively large quantities of orobanchol (Figure 2c). Even though orobanchol is a less potent stimulator of germination compared to 5-deoxystrigol, *Striga* populations from western Kenya are particularly sensitive to high concentrations of orobanchol relative to populations from other locations (Haussmann *et al.* 2004; Bellis *et al.* 2020).

In addition to *Striga* resistance screening, field evaluation allowed us to determine the suitability of SAP *lgs1* accessions for growth in western Kenya as well as evaluate other desirable agronomic traits (Table 1). Considering that the SAP accessions were selected for photoperiodism sensitivity, we sought to determine days to flowering by measuring maximum days to 50 % flowering (DFL50 %). We found that DFL50 % of the latest and earliest genotypes averaged between 76.67 in PI5610710 and 55 in PI533976 indicating that the SAP accessions are in general early maturing and do not have variation in flowering time. We also measured the associated trait of plant height under field conditions. Results showed that the average plant height ranged from 65 cm to 235 cm. The plant height of the SAP *lgs1* accessions are comparable to those of sorghum genotypes grown in Kenya such as SRN39 (125.33.73cm), Framida (155.33 cm) and IS9830 (173.67 cm). Lastly, we compared the average yield of SAP *lgs1* lines to that of popular *Striga* -resistant varieties in Kenya. The top 3 highest yielding varieties were SAP *lgs1* PI656094 (2.9667g), PI656096 (3.0333g), and SRN39 while the poorest yielding varieties were PI655979, PI561071 and PI533839. The yields are comparable to varieties Framida (2.8667g), IS9830 (2.6333g) grown in Kenya indicating that the lines are suitable for adoption in Kenyan agro ecological regions.

The other aspect of our field experiments was to determine the effect of *Striga* infestation on growth of the sorghum plants – given that *Striga* infection is associated with severe growth retardation (Fujioka *et al.* 2019a). An effective way to determine the extent to which *Striga* infection affects growth, and how best an infected host copes with the infection is to correlate host growth and *Striga* emergence. Hosts that cope well with infection – described as tolerant genotypes – show less severe effects of parasite infection (Mwangangi *et al.* 2021). We found that *Striga* infection reduced yield by up to 27 %. The most severe reduction in growth was in PI576385 (26 %) while SRN39 and Shanqui Red incurred losses of 6 % and 13 %. Remarkably, PI561071 and PI585295 did not suffer significant yield losses. When we correlated sorghum's yield with *Striga* emergence (AUSNPC) we found a weak positive correlation between yield loss and AUSNP ( $R = 0.33$ ,  $p = 0.25$ ) indicating that most genotypes were tolerant (Figure 4b). PI561071, PI656096, PI656054 maintained high yields even with some *Striga* infestation.

To summarize our field experiments, we ranked *Striga* tolerance, field resistance and other desirable agronomic traits in SAP *lgs1* lines to find the best performers. To achieve this, we used the Rank summation index (RSI) method proposed by (Mulamba & Mock) where a genotype is assigned a rank based on performance for each trait (Figure 4c). A final score (RSI) is then assigned based on all traits. Using this analysis, the best 3 performing genotypes were SRN39, SAP *lgs1* PI533976 (caudatum, USA), and SAP *LGS1* PI656054 (Kafir, South Africa).

Although further field evaluation is required, our results show that some SAP *lgs1* lines are well suited to environments in Kenya and can potentially be used in *Striga* management programs. A representative

photograph depicting the performance of the *Striga*resistant SAP *lgs1* PI561071 against the susceptible LGS1PI533839 and Shanqui Red is shown in Figure 4d.

## Discussion

Expanding the genetic basis of sorghum to cope with parasitism by *Striga*, one of the biggest constraints to cereal production in SSA, could have far-reaching impact towards alleviating food insecurity. An efficient way to achieve *Striga* resistance is to harness advances in genomics and exploit the vast genetic diversity of sorghum. Building on previous successful identification of *Striga*resistant sorghum from global collections (Kavuluko *et al.* 2021; Mallu *et al.* 2021, 2022), we sought to identify and characterize new sources of resistance. Our motivation for selecting the SAP collection was obtaining sorghum with increased resistance to *Striga* parasitism combined with additional traits that accrue benefits to smallholder farmers in Africa. The SAP is also extensively sequenced providing vital data for downstream resistance breeding. Based on our analysis and previous work (Boatwright *et al.* 2022), the SAP comprises all major botanical races of sorghum (caudatum, kafir, guinea, and durra) and a mixed population believed to have originated from bicolor – one of the earliest races to undergo domestication (Harlan & Stemler, 1976).

We adopted a simple methodology for discovery of *Striga*resistance alleles in sorghum based on PCR screening using two sets of primers – making it applicable in basic laboratories without the need for sophisticated equipment and resources for advanced molecular screening. Our approach hinged on identifying sorghum genotypes harboring a chromosomal deletion in the sorghum *LOW GERMINATION LOCI 1* (*LGS1*) region which makes some genotypes inefficient in stimulating the germination of the parasitic plant *Striga* (Gobena *et al.* 2017). Mutants devoid of this region, termed *lgs1* genotypes are *Striga* resistant because they primarily exude the low potent SL, orobanchol, compared to their susceptible wild type counterparts (*LGS1* genotypes) that exude 5-deoxystrigol which is more potent. We found 12 SAP *lgs1* accessions; among them two had been previously described (Bellis *et al.* 2020). This represented 3.5 % of our screened population and importantly, 67 % of the *lgs1* -like genotypes originated from Africa. This finding is important and significant because it points to increased prevalence of *Striga* resistance in sorghum domesticated in Africa, which can be attributed to its adaptation to the parasite as both *Striga* and sorghum have their natural distribution ranges in SSA.

SAP *lgs1* accessions bore the expected phenotype of the reference *lgs1*-like sorghum SRN39 whose hallmark is the production of high proportions of the SL orobanchol relative to 5-deoxystrigol (Gobena *et al.* 2017). Germination assays further affirmed the expected low germination stimulation of *lgs1* -like sorghum genotypes. Our results were consistent with previous work that have reported germination efficiencies of 38 % in *lgs1* -like genotypes (Mallu *et al.* 2021). Unexpectedly, PI656054, an *LGS1* accession, showed notably low germination induction. Our hypothesis of involvement of ABA in mediating the low germination of this genotype was inconclusive as we did not find any correlation between germination and ABA content. One possibility is the production of another less potent SL that was not tested in this study. Determination of the actual mechanisms will be an interesting subject of further investigation. *Striga* seed germination stimulation assayed by agar gel method that measures MGD, the maximum germination distance which host root can stimulate *Striga* germination *in vitro* agar culture showed concurrence of our results with previous reports. For example, Gobena *et al.* (2017) found the MGD of SRN39 to be 1mm. In pearl millet, the *Striga* resistant 29AW had a MGD of 7.96 mm (Dayou *et al.* 2021).

To further validate the resistance of SAP *lgs1* accessions, we performed pot experiments where sorghum was planted in *Striga*-infested soil and the number of emerging *Striga* counted. Our results showed that most SAP *lgs1* had low emergence. We would like to point out PI655979, PI656040, and PI533576, that consistently showed low germination stimulation in the bioassay and low emergence in pot experiments. We would also like to point out PI 656054, the SAP *LGS1* that we described earlier as having low *Striga* germination induction. Because *Striga* emergence in pot experiments is a function of both pre- and post-attachment resistance, all these genotypes represent good candidates for further field evaluation.

The final aspect of our study was to evaluate SAP *lgs1* lines under field infestations. Consistently, SAP *lgs1*

lines that showed low germination stimulation and emergence in the laboratory bioassays endured low *Striga* infestation under field conditions. The genotypes PI533976, PI656094 and PI655979, had *Striga* emergence numbers comparable to the resistant check SRN39. A closer look at the number of *Striga* emergence in SAP *lgs1* showed concurrence with other *Striga* resistant sorghum. For example, the number of emerging *Striga* in Framida and IS9830 were like those of SAP *lgs1* PI533976, PI656094 and PI655979. Serendipitously discovering PI656054, an *LGS1* sorghum with pre-attachment resistance that appears to be independent of the SL signaling pathway, opens new avenues for *Striga* resistance studies. Once validated phenotypically, the accession can be used as donors to incorporate new diversity into breeding lines.

Another important *Striga* management strategy is tolerance – ability of a crop to produce yield even under *Striga* infestation (Mwangangi *et al.* 2021). This trait is well exemplified by N13, a popular *Striga* resistant and tolerant durra sorghum from eastern Africa (Rodenburg, Bastiaans, Weltzien & Hess 2005). Identification of potentially tolerant SAP *lgs1* has important implications because researchers are now advocating for a combination of resistance and tolerance in an integrated *Striga* management approach. We found that most SAP lines could produce yields even under reasonable *Striga* infestation. Particularly, SAP *lgs1* accessions: PI656094 and 656096 are good candidates for deployment as tolerant accessions. Although the mechanism of *Striga* tolerance has not been fully explored, one can extrapolate a hypothesis based on the adverse effects of *Striga* parasitism on growth retardation and yield loss. ABA is a critical hormone for controlling plant responses to water limitation, inducing stomatal closure to limit water loss through transpiration (Mittelheuser & van Steveninck 1969). *Striga* are insensitive to ABA, resulting in higher transpiration rates than their hosts and the maintenance of a water potential gradient favoring nutrient and water transfer to parasites (Fujioka *et al.* 2019b a). One possibility is that host insensitivity to ABA could be extended to imply *Striga* tolerance if host plants control the water potential gradient under *Striga* parasitism via ABA signaling. Because there is no validation data for this hypothesis, for now it remains a subject for further investigation.

Field evaluation provided a further opportunity to study flowering time and yields of SAP *lgs1* lines. Regarding flowering time, all the accessions were within the time considered as early maturity in sorghum. This is important because early maturity helps cope with *Striga* by reducing infestation. This is also important as a drought coping mechanism, another major constraint for sorghum production in SSA.

To conclude, we: (i) describe a simple *Striga* resistance allele mining assay that could be adapted for allele discovery in other sorghum collections or populations, particularly in Africa where sorghum is prone to be enriched for *Striga* resistance, (ii) identify 12 new *Striga* resistant SAP *lgs1* accessions that can be integrated in breeding programs in SSA, and (iii) demonstrate that identified sorghum accessions provide the additional advantage of early maturity and tolerance to *Striga* parasitism.

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## Author Contribution

Conceptualization: SR, SM, EA, EB, SA-B. Methodology: SM. Allele mining, resistance assays. MJ, JYW, LB resistance assays, biochemical characterization. Data curation and analysis: SR, SM, MJ, JYW. Writing original draft: SM, SR, EB. Manuscript Editing: SM, SR, EB, EA, SA-B, JYW, LB. All authors reviewed and approved the final Manuscript.

## Conflict of Interest

Authors have no conflict of interest to declare.

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**Table1:** Days to 50% flowering, plant height (cm) and yield (Hundred grain weight) at maturity of sixteen sorghum genotypes evaluated in a field infested with *Striga* at Alupe.

Genotype	Days to 50 % flowering	Plant height (cm)	HGW (Non infested)	HGW (Infested)
PI656094	58±2.65 <sup>h</sup>	146.33 ±8.08 <sup>cd</sup>	3.0667±0.0577 <sup>a</sup>	2.9667±0.0577 <sup>ab</sup>
PI656096	62.67±0.58 <sup>ef</sup>	130 ±15.39 <sup>de</sup>	3.0667±0.2517 <sup>a</sup>	3.0333 ±0.0577 <sup>a</sup>
PI655979	70.67±0.58 <sup>b</sup>	101.67±5.69 <sup>gh</sup>	2.0667±0.2082 <sup>e</sup>	2±8.01 <sup>h</sup>
PI656054	64±3.46 <sup>de</sup>	235 ±8.89 <sup>a</sup>	2.3333±0.1528 <sup>d</sup>	2.2667±0.2517 <sup>efg</sup>
PI533976	55±21 <sup>h</sup>	65.67 ±13.58 <sup>i</sup>	2.3333±0.1155 <sup>de</sup>	2.2±0.1 <sup>fgh</sup>
PI533839	62±1 <sup>ef</sup>	125.33 ±2.53 <sup>def</sup>	1.7±0.10 <sup>f</sup>	1.5333±0.0577 <sup>i</sup>
PI597960	66±1 <sup>d</sup>	105.67 ±11.02 <sup>fgh</sup>	2.6667±0.1155 <sup>bc</sup>	2.4667±0.2517 <sup>de</sup>
PI585295	61.67±1.15 <sup>f</sup>	88±3 <sup>hi</sup>	2.2667±0.0577 <sup>de</sup>	2.3333±0.0577 <sup>ef</sup>
PI576385	62.67±0.58 <sup>ef</sup>	99.67 ±8.14 <sup>gh</sup>	2.6333±0.0577 <sup>c</sup>	2.3667±0.0577 <sup>ef</sup>
PI561071	76.67±0.58 <sup>a</sup>	116.67±6.81 <sup>efg</sup>	1.7±0 <sup>f</sup>	1.7±0 <sup>i</sup>
PI656010	68.33±0.58 <sup>c</sup>	102 ±2.65 <sup>gh</sup>	2.2333±0.1528 <sup>de</sup>	2.1667±0.1528 <sup>fgh</sup>
PI656040	69.33±0.58 <sup>bc</sup>	93 ±2.65 <sup>h</sup>	2.3±0.1 <sup>d</sup>	2.1±0.1 <sup>gh</sup>
ShanquiRed	63.33±0.58 <sup>ef</sup>	145.67 ±7.51 <sup>cd</sup>	2.4±0.1 <sup>d</sup>	2.2667±0.1155 <sup>efg</sup>
SRN39	69±1 <sup>bc</sup>	125.33 ±3.21 <sup>def</sup>	2.8333±0.1155 <sup>bc</sup>	2.7667±0.1528 <sup>bc</sup>
Framida	62.1± <sup>ef</sup>	155.33 ±9.07 <sup>bc</sup>	2.8667±0.0577 <sup>ab</sup>	2.7667±0.0577 <sup>bc</sup>
IS9830	55±1 <sup>h</sup>	173.67 ±43.36 <sup>b</sup>	2.6333±0.0577 <sup>c</sup>	2.6333±0.0577 <sup>cd</sup>

## Figure Legends

Figure 1: Genetic structure of the sorghum accession panel (SAP): (a) *Striga hermonthica* range in Africa overlapping with sorghum origin (b) A distribution map showing the collection points of sorghum (174 georeferenced samples collection from Africa) genotypes, races are represented by color-coded dots (c) Phylogenetic relatedness of 373 sorghum accessions measured by Neighbor-joining technique. Phylogenetic tree nodes are color coded according to sorghum races.

Figure 2: Identification and characterization of *new low germination stimulant 1 (lgs1)* sorghum. (a) Sulfoltransferase marker analysis of the *lgs1* loci. Presence of a single band characterized by a 223bp PCR product indicates the presence of the resistance allele (*lgs1*) (b) Liquid Chromatography Tandem-Mass Spectrometry (LC-MS/MS) quantification of strigolactones: (upper panel) orobanchol and 5-deoxystrigol peaks of some representative sorghum lines in LC-MS/MS analysis (lower panel) Orobanchol and 5-deoxystrigol mass transitions. (c) Orobanchol quantification (pg per sample) in various sorghum lines. (d) 5-Deoxystrigol quan-



tification (pg per sample) in sorghum lines. The strigolactones collected from the root exudates of various sorghum lines quantified through LC-MS analysis. Data means $\pm$ SE (n=6). The treatments with various letters denote significance (one-way ANOVA, Tukey's post hoc test, P <0.05). (e) Strigolactone proportion of SAP lines. *LGS1* genotypes had high proportions of 5-deoxystrigol. In contrast, *lgs1* genotypes had high proportions of orobanchol.

Figure 3: *Striga* resistance in SAP *low germination stimulant 1* sorghum (a) Bioassay to measure *Striga* resistance by germination frequency induced by root exudates of resistant (blue, SAP *lgs1-1*) and susceptible (orange, SAP LGS1). (b) Measurement of *Striga* germination using germination frequency showing low germination stimulation in most SAP *lgs1* -1 accessions. (c) Agar gel assay to measure *Striga* stimulation activity by sorghum roots *in vitro*. (d) Measurement of *Striga* emergence using pot experiments. Most *lgs1* genotypes had a low number of *Striga* attachments. (e) *Striga* emergence pot experiments. Notable low emergence was observed in most *lgs1* lines and in the LGS1 line PI6506054.

Figure 4: Performance of SAP *low germination stimulant 1* sorghum under natural *Striga* infestation. (a) *Striga* emergence measured as area under *Striga* number progressive curve (AUSNPC) at 98 after planting (b) Correlation of *Striga* emergence (AUSNPC) at 98 days after planting with relative yield loss as a measure of sorghum tolerance (c) Heatmap showing *Striga* resistance levels of sorghum genotypes based on ranked summation index (RSI) of yield, days to 50 % flowering and emergence counts. (d) *Striga* resistance phenotypes. Wild type LGS1 sorghum with high number of emerged *Striga* seedlings growing next to *lgs1* mutant in a *Striga hermonthica* infested field in Alupe, western Kenya. *Striga* resistance evaluation of SAP *lgs1* genotypes showed that here was notable infestation on SAP *LGS1* PI533839 but not in one of the *Striga* resistant SAP *lgs1* genotype PI561071.







