Deciphering the Mechanisms of Pre-Attachment *Striga* Resistance in Sorghum Using Genome-Wide Association Studies

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Research Article

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Abstract

Witchweeds (*Striga* spp.) greatly limit production of Africa's most staple crops. These parasitic plants use strigolactones (SLs) – chemical germination stimulants, emitted from host's roots to germinate, and locate their hosts for invasion. This information exchange provides opportunities for controlling the parasite by either stimulating parasite seed germination without a host (suicidal germination) or by inhibiting parasite seed germination (pre-attachment resistance). We sought to determine genetic factors that underpin *Striga* pre-attachment resistance in sorghum using the genome wide association study (GWAS) approach. Results revealed that *Striga* germination was associated with genes encoding hormone signaling functions e.g., the Novel interactor of jaz (NINJA) and, Absciscic acid-insensitive 5 (ABI5). This pointed toward abscisic acid (ABA) and gibberellic acid (GA) as probable determinants of *Striga* germination. To test this hypothesis, we conditioned *Striga* using: ABA, ABA + its inhibitor fluridone (FLU), GA or water. Unexpectedly, *Striga* conditioned with FLU germinated after 4 days without SL. Upon germination stimulation using sorghum root exudate or the synthetic SL GR24, we found that ABA conditioned seeds had above 20-fold reduction in germination. Conversely, FLU conditioned seeds recorded above 20-fold increase in germination and conditioning with GA caused at least 1.5-fold reduction in *Striga* seed germination. Germination assays using seeds of a related parasitic plant (*Alectra vogelii*) showed similar degrees of stimulation and reduction of germination by the hormones further affirming the hormonal crosstalk. Our findings have far-reaching implications in the control of some of the most noxious pathogens of crops in Africa.

Main Conclusion

Striga uses root exudate released by hosts to target them for parasitism. The root exudate is in fact, a complex blend of cross-talking biomolecules that could help manage the parasite.

Introduction

Striga (witchweed) is a major constraint of cereal production in sub-Saharan Africa (SSA), predicted to cause 30-100 percent yield losses and USD 7 billion annually (Ejeta 2007). The obligate hemi-parasitic is difficult to control mainly because of its successful lifecycle that is intricately intertwined with that of its host (Runo and Kuria 2018). Specifically, *Striga* seeds germinate in response to germination signals strigolactones (SLs) produced by the host and non-host root exudates (Matusova et al. 2005; Bunsick et al. 2020). Ironically, dependance of the parasite on host cues for germination also provide a weak link that can be exploited for managing the parasite. Two approaches are generally used: suicidal germination and inhibition of germination (pre-attachment resistance).

In suicidal germination, parasite seeds are stimulated to germinate without a host or with a false host (one that does not support parasite growth). The most notable application a *Striga* eradication program in east Africa popularized as “push pull” technology. In this strategy, the forage legumes in the genus *Desmodium* spp. are used as intercrops to stimulate *Striga* seed germination. Because *Striga* cannot

parasitize *Desmodium* spp., its seeds die leading a gradual decline of *Striga* seedbank in soil (Khan et al. 2014). Recent advances of suicidal germination strategy involve synthesis of small molecules and synthetic SLs with ability to mimic natural SLs (Uraguchi et al. 2018; Kountche et al. 2019).

In pre-attachment resistance, natural mutants of crops that do not effectively stimulate *Striga* seeds to germinate are identified and integrated in breeding programs. The most successful application of this technology has been breeding approaches based on the *Striga* resistant sorghum variety SRN39 (Hess and Ejeta 1992). Extensive biochemical and genomic investigations on SRN39 have now determined that the resistance is caused by a mutation on the low germination stimulant loci 1 (LGS1). As a result of the mutation, the variety emit a less potent SL (orobanchol) relative to 5-deoxy strigol, which is produced by most *Striga* susceptible sorghum genotypes (Gobena et al. 2017).

Since the discovery of the *lgs1* mutants in SRN39, pre-attachment resistance in sorghum has been an area of intense investigation. Notably, studies have shown that: (i) *lgs1* mutations are widespread among sorghum genotypes (Mohemed et al. 2018; Bellis et al. 2020; Mallu et al. 2021) and, (ii) some sorghum genotypes have pre-attachment resistance that cannot be attributed to mutations on the LGS1 loci (Mallu et al. 2021). The later finding has pointed to the possibility of other – yet to be described mechanisms of pre-attachment resistance in sorghum.

We hypothesized that we could home in on such potentially novel genomic regions influencing *Striga* germination, by carrying out a genome wide association study (GWAS) using single nucleotide polymorphism markers (SNPs) derived from Diversity Array Technology® sequencing [DArT seq (Sansaloni et al. 2011)] alongside pre-attachment *Striga* resistance measurements (germination stimulation activity and radicle length) of a diverse collection of sorghum accessions. We report on how this analysis led us to serendipitously discover a well-coordinated hormonal crosstalk that involve abscisic acid, (ABA), gibberellic acid (GA) and SL. Further, we use germination assays to provide experimental validation that these hormones interact to influence germination of two parasitic plants seeds – *Striga hermonthica*, and *Alectra vogelii*. Finally, we discuss the far-reaching implications of such a hormonal crosstalk in management of a parasite that devastates production of cereal crops in sub-Saharan Africa.

Materials And Methods

Sorghum genotypes and phenotypic data

One hundred and seventy-three sorghum genotypes consisting of local Kenyan landraces and a sub-set of the global sorghum reference set were used in the current study as already described in Mallu et al. (2021). A map showing the germplasm collection sites is provided in Suppl. Fig. S1. To establish pre-attachment resistance for the 173 genotypes, data was collected for germination frequency and radicle length as already described in the report by Mallu et al. (2021).

DNA extraction and DArT-sequencing

Ten seeds of each sorghum genotype were germinated. Genomic DNA was extracted from 10 days old resultant seedlings of each sorghum genotype using Isolate II Plant DNA Kit (Bioline Pty Ltd, Nottingham, UK) following the manufacturer's instructions. DNA concentration and quality was determined using Qubit® 2 Fluorometer (Thermo Fisher Scientific Inc, USA). Subsequently, 100 µl of DNA with a final dilution of 50 ng/µl was submitted to the Integrated Genotyping Service and Support (IGSS) at the Bioscience eastern and central Africa (Beca) Lab – International Livestock Research Institute (ILRI) hub, Nairobi, Kenya for genotyping using Diversity Arrays Technology sequencing (DARTseq). The resulting reads were mapped to Sorghum reference genome (version 3.0.1) (<https://phytozome-next.jgi.doe.gov/>) and raw SNPs called using DArTsoft14.

SNP quality control and imputation

Raw SNPs were co-analyzed with a total of 4715 sorghum genotypes, some of which had been previously genotyped using DArT sequencing. Thinning to remove duplicate sites was done using the Trait Analysis by Association, Evolution and Linkage (TASSEL) version 5.2.6 software (Glaubitz et al. 2014). The unique set of markers was formatted using customized scripts (<https://github.com/eacooper400/formatConversion>) then imputed using fastPhase (Scheet and Stephens 2006) with default parameters. Finally, imputed SNPs for the 173 genotypes specific to our study were retrieved and filtered using TASSEL 5.2.6 to a minor allele frequency (MAF) ≥ 0.05 , and SNP minimum call rate of 70%.

Population genetic structure analysis

Population structure was firstly inferred using a Nei genetic distance matrix calculated with the APE package of R software using the filtered SNP data. The resulting matrix was plotted as an unrooted NJ tree phytools in R (Revell 2012). Population structuring was further analyzed using the discriminant analysis of principal components (DAPC) (Jombart et al. 2010). This analysis allowed for determination of the likely number of population clusters using the R package Adgenet and its *find clusters* function (Jombart 2008). By using K-means clustering, the number of subpopulations that had the lowest associated Bayesian Information Criterion (BIC) was determined and the resultant clusters plotted in scatter plots using R's package ggplot2 (Wickham 2016). Finally, the population structuring was inferred using Bayesian-based clustering using ADMIXTURE in plink (Alexander et al. 2009). In this analysis, SNP data in Hapmap format were first converted into plink format and resulting .ped file analyzed by running K values from 1 to 15 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. The output was subsequently visualized as bar plots generated using the ggplot2 function in R (Wickham 2016).

Genome wide association studies analysis (GWAS)

GWAS was carried out using 167 genotypes that passed the SNP filtering and quality control step. Calculated averages of phenotypic datasets (germination % and radicle length) for the 167 genotypes

were combined with the genotypic data and marker-trait associations calculated using the multi-locus mixed model [MLMM (Segura et al. 2012)] as well as the Fixed and random model Circulating Probability Unification [FarmCPU (Liu et al. 2016)]. MLMM follows a stepwise approach to incorporate SNPs as covariates in the GWA model while FarmCPU is a multilocus model that controls for false positives without comprising false negatives (Liu et al., 2016). All analysis were carried out in R using the Genome Association and Prediction Integrated Tool (GAPIT) version 2 (Tang et al. 2016) and the resulting associations displayed as Manhattan plots alongside quantile-quantile (Q-Q) plots to demonstrate the model fitness. The significance of marker-trait associations was determined using the false discovery ratio FDR ($\alpha = 0.05$). Candidate genes were identified within 40 Kb distance from the significant marker.

Linkage disequilibrium (LD) analysis and building the haplotype blocks

LD statistics (r^2 and distance between SNPs) were generated in TASSEL 5.2.6 using a full correlation matrix and LD decay was plotted in R as pairwise LD versus distance between SNPs. The r^2 threshold was set to 0.2. To build haplotype blocks, the same hapmap file used for LD analysis was converted into Plink format, imported into PLINK (Purcell et al. 2007) and confidence intervals calculated as described by (Gabriel et al. 2002). The resulting blocks were visualized in Haploview (Barrett et al. 2005) while the corresponding plots of SNPs were displayed using LocusZoom (Pruim et al. 2010).

***Striga* germination assays**

Striga plant heads containing mature seeds collected from Alupe Kenya (0.45°, 34.13°) in 2018 were firstly threshed and sieved as described in (Kavuluko et al. 2021). Afterwards, 25 mg of *Striga* seeds were surface sterilized by washing with 25 ml of 10 % (v/v) commercial sodium hypochlorite for 10 minutes with vigorous agitation. Seeds were then rinsed thoroughly with distilled water through a Buchner funnel. Surface sterilized *Striga* seeds were further cleaned by centrifugation at 3 0000 rpm for 10 minutes in a 60 % - 40 % sucrose gradient. Following centrifugation, the middle layer – that contained clean *Striga* seeds – was picked with a dropper. Sequentially, seeds were then rinsed with distilled water and dried in a laminar air flow.

Cleaned *Striga* seeds (25 mg) were placed in 90 mm Petri plates lined with Whatman (GFA) filter paper then conditioned using water, or 0.5mgml⁻¹ ABA, ABA+FLU, and GA all 0.5 . Petri dishes were sealed with Parafilm®, wrapped in aluminum foil and incubated in an incubator set at 30 °C in darkness for 14 days. Five Petri dishes were used per treatment and the experiment replicated 3 times. *Striga* seed germination was monitored and photographed daily for 14 days using a stereomicroscope [Leica, MZ7F stereomicroscope fitted with a DFC320FX Camera (Leica, Germany)]. After 14 days, conditioned seeds were stimulated to germinated by treating with either sorghum root exudate – prepared as described in Mallu et al., (2021) – or GR24 (Chiralix, Nijmegen, Netherlands). Germinated *Striga* seeds were viewed and photographed using a Leica MZ7F stereomicroscope. The resulting images were analyzed for germination frequency and radicle length using ImageJ version 1.45 as described by (Mwakha et al. 2020). Means of germination frequency and radicle length were analyzed using analysis of variance

(ANOVA) in R followed by mean separation using Tukey's LSD test. Results of germination frequency and radicle length were displayed in line graphs and box plots made in R using the ggplot2 package (Wickham 2016).

***Alectra vogelii* germination assays**

Plant heads containing mature seeds of the parasitic plant *Allectra vogelii* (Benth.) collected from cow pea infested fields in Embu, Kenya in 2019 (0.41°, 37.39°) were threshed and sieved to obtain seeds. Then, 25 mg, of *Alectra* seeds were surface sterilized and conditioned with ABA, GA, or FLU in petri dishes as described above. Like for *Striga*, *Alectra* seeds germination was also monitored and recorded daily for 14 days and then conditioned seeds from all treatments treated with GR24 to induce gemination. Germination frequencies and radicle lengths were subsequently analyzed.

Results

SNP Quality control, LD decay and frequency distribution of phenotypic data

Out of the 21,113 SNPs imputed for the 173 genotypes, 8,507 quality SNPs were retained across 167 genotypes for downstream analysis after filtering and removal of SNPs from unmapped contigs. The distribution of the 8,507 SNPs across the sorghum genome is shown in Fig.1a. The overall SNP density was ~12 SNPs/Mbp. Results also showed that LD decayed at 48.1 Kb (Fig. 1b). Regarding trait data, both traits germination frequency (GF) and radicle length (RL) revealed quantitative distribution patterns that were either kurtosis (GF) or slightly skewed to the right (RL) (Fig. 1c).

Sorghum accessions have distinct race structures

To determine appropriate models for GWAS, we carried out population structure analysis of the sorghum accessions using three criterions: (i) Bayesian information criterion (BIC), (ii) dissimilarity matrix-based neighbor joining (NJ) tree and (iii) hierarchical Bayesian clustering (HBC)

Bayesian Information Criterion (BIC) identified 8 clusters (Fig. 2a), indicated by an elbow curve of BIC values as a function of K (Suppl. Fig. S2). Clusters 1 and 8 were clearly differentiated from the rest of the clusters. Cluster 8 consisted of Guinea margaritiferum (Gma) and Guinea. Other clusters were cluster 2, Kafir, cluster 4 Guinea gambicum (Gga), cluster 5 Durra and Durra-caudatum DC), 6 and cluster 8, wild.

Unlike BIC that grouped the populations to 8, the NJ tree assigned 7 groups according to the major sorghum races (Fig. 2b). There were distinct groups of Kafir, Guinea, Gga, Gma and wild races. Other clusters comprised races combined with sub-races. These were: Caudatum and DC, Durra, and DC. Bicolor could not be placed on any cluster, instead, it appeared to cluster within most groups but more so with the Durra, DC cluster. Noteworthy, Gma formed the most divergent sub-group appearing to form a long branch of its own. Lastly, Gga, wild and Guinea accessions – which also included the sub-races – formed respective clades. This clustering supported observations made using DAPC.

Hierarchical Bayesian clustering (HBC) using ADMIXTURE grouped the sorghum accessions into 7 clusters using the K value with the lowest cross validation (CV) error ($K = 7$; $CV=0.35293$) as shown in Fig. 2c. Further ADMIXTURE clustering using K8 ($CV=0.35673$) revealed 6 clusters of Durra, Caudatum/Guinea Caudatum, Kafir, Gma and Gga while clustering with K9 ($CV=0.36229$) showed 5 clusters of Kafir, Durra Caudatum, Guinea-caudtum (GC), Gga and Gma. Combined ADMIXTURE plots are shown in Suppl. Fig. S3.

Overall, groups of NJ, DAPC and ADMIXTURE were, in agreement and provided clear evidence of population structuring based on sorghum races. Therefore, subsequent GWAS analysis were carried out with the confounding genetic structuring considerations.

Marker-trait associations and candidate genes

While there were no significant SNPs associated with RL from both analysis models, FarmCPU and MLM identified 9 and 1 SNPs respectively that were significantly associated with GF (Table 1 and Fig. 3). Among the significant SNP markers identified, 5 were localized within candidate genes including: (i) ADP-glucose phosphatase (S2_4487093; $p = 0.00011$), (ii) Membrane-associated kinase regulator 3 (S9_1847809; $p = 0.00082$), (iii) Marspadin like α/β hydrolase (S1_47702467; $p = 0.002290$), (iv) Absciscic acid-insensitive 5 (ABI5) (S3_68089192; p value = 0.00229) and, (v) a disulphide oxidoreductase (S4_4060034; $p = 0.00229$). A closer look at all the significantly associated SNPs revealed a link to hormone signaling and ABA biosynthesis in the following genes: ABI5, NINJA, RPK1, Marspardin and ADP-glucose phosphatase. These results showed a prevalence of hormone and dormancy regulating factors.

Haplotype blocks suggest further hormone signaling activity during *Striga* seed germination

Following this analysis, we found two markers in significant LD with ABI5 (Fig. 4). One of them was at position S3_68025304 and located in the coding region of a gene that encode ethylene response factor 2 (ERF2) on chromosome 3. The other SNP that was in significant LD with ABI5 was located at position (S3_68035093 and S3_68025207) in the exon of the Arabidopsis' ortholog TRANSPARENT TESTA GLABRA1 (TTG1). Two copies of the gene ADP-Glucose-phosphatase were found within the same haplotype block.

The finding that AP2/ERF2 and TT1 are in LD with ABI5 suggest co-inheritance, a further indication of a hormonal crosstalk in the process of *Striga* germination. And an additional copy of ADP-Glucose phosphatase underscores the importance of the gene in the process of germination.

Germination of parasitic plants seeds (*Striga* and *Alectra*) is influenced by ABA, GA in addition to SL

Intrigued by the prevalence of signaling associated SNPs in our GWAS and LD analysis, we sought to test the hypothesis of a possible hormonal crosstalk using *Striga* germination assays. In the experiments, we determined the effect of conditioning of *Striga* seeds with the phytohormones ABA and GA. We further investigated the effect on *Striga* seed germination when ABA was inhibited using fluridone.

To our surprise we discovered that *Striga* seeds treated with FLU at conditioning, germinated before further treatment with any *Striga* germination stimulants. *Striga* seed germination occurred remarkably early – at 4 days after incubation appearing to bypass the requirement for condition. The germination frequency increased in subsequent days and peaked on the 10th day (Fig. 5a and b). Similarly, the mean radicle length of germinated *Striga* seedlings increased and was longest on the 8th day (Fig. 5a and b). No germination was observed in seeds treated with ABA, ABA + FLU or water. To further determine if any of the hormone treatments had interactions with SL, *Striga* seeds conditioned for 14 days with ABA, GA, or ABA+FLU were treated with root exudates of a high germination inducer accession (IS2730) as well as that of a low germination inducer accession (IS27146) and compared to induction of *Striga* germination using the synthetic SL GR24. Results (Fig. 5c) showed that ABA significantly inhibited germination of *Striga* seeds in all cases. Combination of ABA and FLU marginally increased the *Striga* germination percentages in root exudate treatments but more notably in the GR24 experiment. In treatments conditioned with FLU, *Striga* germination was significantly increased in all cases (root exudates of sorghum accessions and GR24). Intriguingly, treatments conditioned with GA indicated notably lower *Striga* seed germination frequency significantly in root exudates from IS27146 and synthetic SL, GR24.

To corroborate and affirm the findings of hormonal crosstalk in other parasitic plants, we performed germination assays using seeds of *Alectra vogelii*. For these experiments, we conditioned seeds of *Alectra* with water, ABA, FLU and GA and determined their germination frequency (GF) as well as radicle length (RL). Remarkably, *Alectra* seeds conditioned with FLU germinated after 5 days independent of SL induction (Fig. 6 and Table 2). No germination occurred in ABA or GA treated seeds (Table 2). Furthermore, upon treating conditioned seeds (ABA, FLU, and GA), we observed inhibition of germination and radicle growth by ABA (GF = 39.42 ± 1.10 %; RL = 0.57 ± 0.12 mm) as well as GA (GF = 69.13 ± 1.15 %; RL = 0.30 ± 0.12 mm), in the same degree as in the *Striga* germination experiments compared to water conditioned seeds (GF = 74.40 ± 3.28 %; RL = 0.91 ± 0.12 mm). In the case of GA, notably small radicles were observed (Fig. 6b). This appeared to indicate that GA inhibited radicle growth of *Alectra* seedlings.

Taken together, these findings provide unequivocal evidence that: (i) ABA causes inhibition of *Striga* and *Alectra* seed germination and that obliteration of ABA activity is enough to trigger germination of the parasitic plants seeds in a SL independent manner, (ii) GA interacts with SL to negatively regulate *Striga* and *Alectra* seed germination.

Discussion

Genome wide association studies provide an efficient tool for identifying genetic causes of complex traits. In this study, we used GWAS to discover genes that are likely involved in the pre-attachment resistance in the parasitic plant *Striga*. This presented a significant advancement in the studies of *Striga*-host interactions. Our analysis used data derived from a subset of the sorghum's reference set that also included local East African landraces (Morris et al. 2013). The panel was appropriate based on good representation of all major races of sorghum, both wild and cultivated and the fact that the sorghum accessions in the panel was also sourced mainly from sub-Saharan Africa further maximizing the

likelihood of finding resistance genes that have evolved resistance because of co-existence with the parasite (Bellis et al. 2020). Distinct genetic structuring of the sorghum panel was characterized using various criteria – BIC, NJ, as well and HBC. All these methods supported earlier work that showed clear population structures in sorghum (Kavuluko et al. 2021).

Consistent with the known fact that *Striga* seeds undergo dormancy, numerous transcription factors that regulate this process were identified. To highlight a few: (i) the Novel interactor of jaz (Ninja) is an adaptor protein that connects Jazs with the co-repressor, Topless (TPL) to modulate ABI5 – a transcriptional regulator of ABA mediated processes including dormancy imposition (Pauwels et al. 2010). (ii) ABI5 is a basic leucine zipper transcription factor that plays a key role in the regulation of seed germination and early seedling growth in the presence of ABA and abiotic stresses.(Arroyo et al. 2003; Skubacz et al. 2016). (iii) The membrane receptor-like protein kinase 1 (RPK1) is directly involved in ABA signaling. Upon ABA accumulation, RPK1 can form a complex and possibly regulate the ABA-mediated activation of the Open Stomata 1 (OST1) kinase—the major regulator of ABA-induced responsive pathways (Shang et al. 2019). (iv) Maspardin domain belongs to α/β -hydrolase superfamily that enhances salt stress (Liu et al. 2014; Wang et al. 2021) and (v) ADP- a downstream target of ABA signaling that mediate conversion of sugar to starch to negatively regulate dormancy release – a hallmark of ABA accumulation (Wang and Messing 2012).

Haplotype analysis led to the identification of more SNPs associated with hormone regulation. Specially, AP2/ERFs and TTG1. AP2/ERFs respond to the plant hormones ABA and ethylene (ET) to help activate ABA and ET dependent and independent stress-responsive genes (Xie et al. 2019) while TTG1 encodes a WD40 repeat transcription factor that mediates the accumulation of seed storage reserves in Arabidopsis during germination (Chen et al. 2015).

The unanticipated prevalence of dormancy associated factors in our study led us to conduct germination assays with hormones central to dormancy release and germination – ABA, GA and SL. We attempted to answer the following questions:

Firstly, what is the role of ABA in *Striga* seed germination? Our results showed that obliteration of ABA using FLU was sufficient to cause germination of *Striga* and *Alectra* seeds. Literature on this subject – especially in parasitic plants is dearth but one can draw useful inferences from work previously reported by (Kusumoto et al. 2006). The authors showed three effects of germination of *S. asiatica* following treatment with FLU and norfluridon. Firstly, that ABA inhibition reduced the period required to precondition seeds of *S. asiatica*, secondly that the inhibition protected the *S. asiatica* seeds against seed inhibitory germination effects of sub-optimal light and temperature and thirdly that ABA inhibition induced *S. asiatica* seed germination as if to mimic the effect of natural germination stimulants. Results of Kusumoto et al., (2006) on *S. asiatica* are consistent with our findings performed using *S. hermonthica* and *A. vogelii* and bring to focus ABA as an important determinant of seed germination in parasitic plants. Other studies have also shown that simultaneous inhibition of ABA with FLU and exogenous GA application causes germination of *S. hermonthica* seeds in a SL-independent manner (Toh et al. 2012).

Then, secondly, what is the role of GA in *Striga* seed germination? Our results showed that GA negatively regulates germination – and seedling growth of the two parasitic plants studied – in consistent with the recent discoveries that suggest interactions between GA and SL in the regulation of plant development processes. Other studies have provided proof to suggest that SL biosynthesis is regulated by GAs (Ito et al. 2017; Marzec 2017). The study by Ito et al., (2017) further showed that GA can regulate pre-attachment *Striga* resistance in rice by suppressing germination of parasite seeds.

In the end, one question remained unanswered. What are the actual mechanisms underpinning *Striga* germination following ABA inhibition by FLU or negative regulation of SLs by GA? Our studies could not resolve this question, so it remains a subject of future research. Ideally, such investigations should consider the stages of *Striga* dormancy release and germination separately – as it is plausible that levels and interactions of hormones vary in these two stages. Curiously, no obvious candidate SNPs related to the SL signaling pathway were identified our study. It is plausible that the markers used did not provide the resolution required to span these regions. Future studies can overcome this limitation by using a larger population of accessions as well as more markers.

Conclusion

Our study has demonstrated the power of GWAS to pinpoint new factors that determine *Striga* germination. Germination assays showed that in addition to SLs, *Striga* germination is influenced by ABA and GA. These findings offer exciting opportunities towards a *Striga* management using a multidimensional resistance breeding approach.

Abbreviations

ABI5: Absciscic acid-insensitive 5

GA: Gibberellic acid (GA)

GWAS: Genome wide association study

NINJA: Novel interactor of jaz

SL: Strigolactones

Declarations

Author contribution statement SR and DAO conceived and designed the study. TSM, GI and SM performed *Striga* and *Alectra* germination and GWAS analysis guided by SR, DAO and SMG. EO carried out analysis of sequence data, quality control and participated in the GWAS analysis. All authors read and approved the final manuscript.

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Conflicts of interest The authors declare that they have no competing interests.

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Ethics declarations

Conflicts of interest

The authors declare that they have no competing interests.

Data availability The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 Significant SNPs identified using based on germination frequencies of *Striga* induced by root exudates of sorghum accessions used in the GWAS analysis.

^a Chr	Position	^b AF	AdjP	Gene ID	Annotation
1	47702467	0.43	2.29E-03	Sobic.001G263200	α/β hydrolase/Maspardin
1	61289239	0.16	6.05E-03	Sobic.001G325850	Uncharacterized
1	*62402188	0.22	7.50E-03	Sobic.001G335400	Novel interactor of Jaz (Ninja)
2	4487093	0.35	1.10E-04	Sobic.002G047700	ADP-glucose phosphorylase
3	58520367	0.09	2.61E-03	Sobic.003G246200	N/A
3	68089192	0.43	2.29E-03	Sobic.003G363400	Absciscic acid-insensitive 5 (ABI5)
4	4060034	0.35	2.29E-03	Sobic.004G050300	Disulphide oxido reductase/ uncharacterized
5	69483209	0.05	4.42E-02	Sobic.005G208275	N/A
8	2263153	0.27	1.71E-02	Sobic.008G025300	N/A
9	1847809	0.45	8.20E-04	Sobic.009G020600	Membrane-associated kinase regulator 3

^aChromosome location

^bAllele frequency

^cAdjusted *P* value after FDR adjustment at $\alpha = 0.05$

*Represent SNP identified using MLMM

Table 2 SL independent germination of *Alectra vogelii* seeds induced by fluridon.

^a DAC	Treatment	Germination Frequency (%)	Radical length (mm)
1	FLU	0.00	0.00
2	FLU	0.00	0.00
3	FLU	0.00	0.00
4	FLU	0.00	0.00
5	FLU	3.81±0.61	0.17±0.05
6	FLU	8.75±3.73	0.46±0.08
7	FLU	9.14±2.38	0.91±0.08
8	FLU	12.44±3.61	1.64±0.23
9	FLU	8.86±5.00	1.63±0.37
10	FLU	10.36±2.51	1.52±0.32
11	FLU	9.76±3.47	1.65±0.22
12	FLU	9.88±5.93	1.59±0.46
13	FLU	1.62	1.45±0.10

^aDays after conditioning

Figures

Figure 1

Analysis of SNP Quality, LD decay and frequency distribution of phenotypic data. a. The distribution of quality SNPs used in across the sorghum genome. SNPs from contigs that were not anchored to chromosomes were discarded b. Genome-wide LD plot for sorghum indicating a decay distance of 48.1 Kb. c. Frequency distribution curves showing quantitative distribution of pre-attachment resistance traits based on germination frequency and radicle length. Both traits revealed quantitative distribution patterns that were either kurtosis (germination frequency) or slightly skewed to the right (radicle length).

Figure 2

Population structuring of the sorghum accessions used in the study using: a. Bayesian Information Criterion (BIC) which, identified 8 distinct clusters. Eigen values and the number of PCAs used are shown in the inset. b. A phylogenetic tree of genotypes using Neighbor-joining tree which, assigned 7 groups according to the major sorghum races. c. hierarchical Bayesian clustering plotted with the K that had the lowest CV value (K=7; CV=0.35293). Color codes shown in the legend apply to all figure panels.

Figure 3

Genome-wide association studies of pre-attachment Striga resistance performed using germination frequency data of sorghum's root exudate using a. Manhattan plot and the corresponding Quantile-quantile (Q-Q) plot obtained using the FarmCPU model b. Manhattan plot and the corresponding Q-Q plot obtained using the MLMM model. The green lines on Manhattan plots indicate the p-value threshold obtained using the false discovery rate (adjusted $p < 0.05$). Horizontal and vertical axes on Q-Q plots show $\log_{10}$ -transformed expected P values, and the observed $\log_{10}$ -transformed P values on the vertical and horizontal axes respectively.

Figure 4

Linkage disequilibrium (LD) decay analysis: a Haploview plot of SNPs in LD with ABI5; and b Haploview plot of ADP-glucose phosphatase. The upper panel show LocusZoom plots indicating significance of association as log10-transformed P values on the vertical axis and the chromosomal locations on the vertical axis.

Figure 5

Interactions between ABA, GA, and SL during germination of *S. hermonthica*. a SL free germination of Striga seeds after treatment with ABA's inhibitor (FLU) as measured using Striga germination frequency (upper panel) and Striga radicle length (lower panel). b Representative images showing progressive SL free Striga seeds germination 3, 6 and 10 days after treatment with FLU. The Asterix in the middle panel point to a newly germinated seed after 6 days of conditioning; Scale bars = 0.5 mm. c Effects of

conditioning *Striga* seeds with ABA, ABA+FLU and GA on *Striga* germination after treatment with sorghums root exudate with a high (IS2730) or lower (IS27146) germination induction or with GR24.

Figure 6

Interactions between ABA, GA, and SL during germination of *A. vogelii*. a Strigolactone free germination of *Alectra* seeds after treatment with ABA's inhibitor (FLU) showing representative of progressive SL free germination 5, 10 and 13 days after treatment with FLU. b Effects of conditioning *Alectra* seeds with ABA, FLU and GA. ABA had reduced germination, GA caused less germination and radicle growth while FLU induced rapid and efficient germination. Asterix point to germinated seeds: Scale bars = 1 mm.

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