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RESEARCH ARTICLE

Transcriptome atlas of *Striga* germination: Implications for managing an intractable parasitic plant

Gilles Irafasha[1] | Sylvia Mutinda[1,2] | Fredrick Mobegi[3] | Brett Hale[4] | George Omwenga[1] | Asela J. Wijeratne[4,5] | Susann Wicke[6] | Emily S. Bellis[4,7] | Steven Runo[1,6]

[1]Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

[2]Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya

[3]Department of Clinical Immunology, PathWest Laboratory Medicine WA, Fiona Stanley Hospital Network, Murdoch, Western Australia, Australia

[4]Arkansas Biosciences Institute, Arkansas State University, Jonesboro, Arkansas, USA

[5]Department of Biological Sciences, Arkansas State University, Jonesboro, Arkansas, USA

[6]Institute for Biology, Humboldt University, Berlin, Germany

[7]Department of Computer Science, Arkansas State University, Jonesboro, Arkansas, USA

Correspondence
Steven Runo, Institute for Biology, Humboldt University, Berlin, Germany.
Email: runo.steve@ku.ac.ke

Emily S. Bellis, Department of Computer Science, Arkansas State University, Jonesboro, AR, USA.
Email: ebellis@astate.edu

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Societal Impact Statement

Witchweeds, parasitic plants of the genus *Striga*, are nicknamed "cereal killers" because of their devastating destruction of Africa's most staple cereals, including maize, sorghum, millets, and upland rice. The parasite relies on biomolecules emitted from the host roots to germinate and therefore initiate its infectious lifecycle. Some sorghum varieties have evolved to not produce effective germination stimulants, making them resistant to the parasite. Here, the genetic factors that underpin *Striga* germination were assessed, followed by a discussion of how such knowledge can be used to develop new *Striga* management strategies through the disruption of host–parasite communication exchange.

Summary

- Seeds of the parasitic plant *Striga* are dormant. They only germinate in response to biomolecules emitted from the host's root exudate, strigolactones (SL). But it is now emerging that *Striga* germination is a much more complex process regulated by crosstalk of hormone signaling pathways.
- To further understand the genetic basis of the communication exchange between *Striga* and its host sorghum, we performed a comparative transcriptomic analysis. We sought to identify major transcriptomic changes that define the germination process in *Striga* and a set of genes that may contribute to the differences in germination rates.
- Results showed that germination proceeds immediately after SL perception and is marked by a wave of transcriptional reprogramming to allow for metabolic processes of energy mobilization. Cluster analysis using self-organizing maps revealed a time-phased and genotype-differentiated response to germination stimulation. The variation in germination was also a function of hormonal crosstalk. The early germination stage was associated with significant repression of genes in the abscisic acid (ABA) biosynthesis pathway. Other hormones influenced germination as follows: (i) ABA and auxin repressed germination; (ii) brassinosteroid, ethylene, and

jasmonic acid promoted germination; and (iii) cytokinin had a more prominent role post-germination rather than during germination. Perception of SL sets the germination program leading to different rates of germination in sorghum, followed by a complex hormonal regulation network that acts to either repress or enhance germination.

- We discuss the implications of these findings and present new plausible *Striga* management strategies.

KEYWORDS
comparative transcriptomics, hormone crosstalk, seed dormancy, *Striga* resistance, witchweed

1 | INTRODUCTION

Striga is a genus of root parasitic plants belonging to the family Orobanchaceae. Parasitism occurs when the parasite seed germinates and attaches to the roots of the host to extract water and photoassimilates. Soon after attachment, the host wilts and suffers irreversible growth retardation or death (Hearne, 2009). The parasite is most prevalent in Sub-Saharan Africa (SSA), where two of its most destructive species *S. asiatica* (L.) Kuntze and *S. hermonthica* (D.) Benth greatly limit production of the most staple cereals including maize, millets, sorghum, and upland rice, exposing millions to hunger and starvation. *Striga* infests approximately 40 million hectares of arable land and results in 30% to 100% losses (reviewed in Spallek et al., 2013). Most control strategies have achieved low to moderate success as the parasite continues to thrive and expand its host and natural range, becoming one of the most intractable problems of African agriculture (Babiker, 2007).

Striga's highly successful parasitic lifestyle can be largely attributed to its remarkably adapted seeds (Runo & Kuria, 2018). Each *Striga* plant produces hundreds of thousands of tiny dusty seeds that are deposited in the soil every planting season (Berner, 1995). The seeds stay dormant in the soil and germinate after conditioning under warm temperatures and moisture, a cue for suitable environmental conditions. Following conditioning, *Striga* seeds perceive host-derived signals, commonly carotenoid-derived phytohormones called strigolactones (SL), which also shape plant shoot architecture and influence hyphal branching in arbuscular mycorrhizal fungi (Gomez-Roldan et al., 2008; Matusova et al., 2005; Umehara et al., 2008). But germination is in fact a trade-off. On one end, germination in the presence of an appropriate host provides an opportunity to successfully infect a host and complete its parasitic lifecycle. On the other end, germination without an appropriate host leads to certain death after the parasite depletes the limited seed reserves.

Studies of how *Striga* makes this "life or death" decision provides opportunities for managing the parasite and has profound societal implications. To illustrate, (i) the *Striga* resistant sorghum variety SRN39, which is ineffective in stimulating *Striga* seed germination, has been widely integrated in *Striga* resistance breeding programs across SSA, helping improve livelihoods of millions (Hess & Ejeta, 1992; Kapran et al., 2007). For its discovery, Prof. Ejeta Gebisa was awarded the World Food Prize in 2009 (https://www.worldfoodprize.org/en/laureates/20002009_laureates/2009_ejeta/). And (ii) host-free germination of *Striga* seeds was pivotal in eliminating the threat of *Striga* in North Carolina, USA (Eplee, 1992). This strategy, called "suicidal germination" involved using ethylene gas to stimulate seeds of *Striga* to germinate without a host and remains the mainstay of the *Striga* eradication program in the United States (USDA Environmental Assessment, April 2020, website https://www.aphis.usda.gov/plant_health/ea/downloads/2020/witchweed-north-south-carolina.pdf).

Expectedly, *Striga* germination became a subject intense investigation. It is now known that germination starts with the perception of SLs by a diverse group of α/β hydrolase receptors known as KARRIKIN INSENSITIVE 2/HYPOSENSITIVE TO LIGHT (HTL/KAI2) (Nelson, 2021; Toh et al., 2015). And that *Striga* germinates more effectively in response to 5-deoxystrigol class of SL—exuded by most sorghum genotypes relative to a few mutants described as *low germination loci 1* (*lgs1*) that emit orobanchol (Gobena et al., 2017). As it happens, *lgs1* mutation is the cause of *Striga* resistance in SRN39 (Gobena et al., 2017). Still, some aspects of *Striga* germination remain unclear. At the forefront is the growing appreciation that the root exudate is a complex blend of biomolecules that could interact to promote or repress *Striga* germination (Mallu et al., 2021, 2022). To shed light on these biomolecular interactions, we used the *S. hermonthica*-sorghum pathosystem in a comparative transcriptome approach that evaluated RNA profiles of high and low inducers of germination against the synthetic strigolactone (GR24) positive control. We sought to answer the following questions: (i) What are the genetic factors that underpin *Striga* germination after conditioning? (ii) What are the genetic underpinnings of *Striga* germination in response to high germination inducers relative to low inducers? And (iii) are there synergistic and antagonistic regulators of *Striga* germination in the root exudate? We discuss our results in the context of *Striga* management by exploiting the communication exchange between the sorghum host and the parasite.

2 | METHODS

2.1 | Plant material

All studies were conducted using *S. hermonthica* seeds obtained from infested fields in Alupe, Kenya (0.45°, 34.13°) in 2018. Sorghum lines, that is, IS2730, IS27146, IS41724, and SRN39, were originally obtained from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) and are now maintained at the plant transformation laboratory of Kenyatta University, Nairobi, Kenya. Selection of the sorghum lines was based on *Striga* germination induction frequencies reported in Mallu et al. (2021). The study reported IS2730 as a high germination inducer, while IS27146 and IS41724 were among the lowest germination inducers. SRN39 was selected as a low germination induction check because of its well characterized low *Striga* germination induction caused by a mutation on the *LGS1* loci.

2.2 | *Striga* germination assay

Striga germination was done according to Mwakha et al. (2020), using sorghum root exudate. Sorghum seeds were germinated in a 13.5 cm × 11 cm × 11 cm container set in a complete randomized block design in three replicates. Plants were gently plucked 7 days after emergence, and each seedling root was cleansed and transferred into 50 ml glass tubes with Long Ashton Nutrient medium (Hudson, 1967). Cotton wool plugs were used to support seedlings. Tubes were covered subsequently with aluminum foil to block out light.

Seedlings were left to grow hydroponically in a greenhouse at 28°C during the day (16 h) and 24°C at night (8 h) under 450 μMm photoperiod and 60% relative humidity. These seedlings were grown for seven more days and were transferred into clean tubes containing 20 ml sterile distilled water and then wrapped with aluminum foil to be incubated. After 48 h, roots were weighed, and root exudates were collected. Individual root weight was used to normalize the volume of root exudate for each Petri plate.

Root exudate (1 ml) per gram of root weight was used to induce the germination of conditioned *Striga* seeds in each Petri plate. GR24 (Chiralix, Nijmegen, Netherlands) measuring 5 ml at a concentration of 0.1 ppm in double distilled water was used as a positive control, while conditioned untreated *Striga* seeds served as a negative control. Petri plates were subsequently sealed using parafilm, covered with aluminum foil, and incubated in the dark at 30°C for 24 h.

In the end, six treatments (four root exudates, GR24, and water control) were used to induce *Striga* germination and RNA extracted from each treatment at 6, 12, and 18 h after treatment. The experiment had five technical and three biological replicates set in a complete randomized design.

2.3 | Data analysis

Striga seed germination frequency and radical length were measured using ImageJ v. 1.45 (http://rsb.info.nih.gov/ij). The germination frequency was calculated using the equation below:

$$\text{Germination}\,(\%) = \frac{\left[\left(\frac{x}{y}\times 100\right)+\left(\frac{x}{y}\times 100\right)+\left(\frac{x}{y}\times 100\right)\right]}{3}$$

x is the number of germinated *Striga* seeds, *y* is the total number of *Striga* seeds in the petri dish, and 3 is the number of replicates in the experiment. Data on seed germination and radical length assessment were determined, and the resulting means were separated using Turkey's honest significant difference (HSD) test at a 5% level of significance. Data were displayed in box plots produced using the ggplot2 package in R (Wickham, 2016).

2.4 | RNA extraction

Whole *Striga* seedlings were harvested at three-time intervals (6, 12, and 18 hai). At each time point, seeds were frozen using liquid nitrogen, followed by immediate RNA extraction using a Bioline ISOLATE II RNA Plant Kit (London, UK) according to the manufacturer's instructions. The obtained RNA samples from each treatment were treated with Deoxyribonuclease I to remove potential DNA contaminations. RNA quantity and quality were determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific), a Qubit fluorometer (Invitrogen), and an Agilent Bioanalyzer. Samples with an RNA integrity number of ≥7.0 were subjected to sequencing using the 3′Tag-Seq method as described in Mayer et al. (2007).

2.5 | Quality control pre-processing of raw 3′Tag-RNA-Seq reads

The 3-Tag-RNA-Seq-analysis NextFlow pipeline (https://github.com/fmobegi/3-Tag-RNA-Seq-analysis), which wraps up the TagSeq utilities v2.0 (https://github.com/Eli-Meyer/TagSeq_utilities), was used to process the RNA-Seq data. Briefly, the quality of raw sequences was assessed using FastQC v.0.11.9. Raw reads were filtered based on Phred per-base quality score (Q) ≥ 20 and a cumulative per-read low quality score (LQ) ≤ 10 using the *QualFilterFastq.pl* script with the parameters *−m 20* and *−x 10*. Reads that passed this step were then depleted of homo-polymer repeats longer than 30 bp using *HRFilterFastq.pl* (parameters *−n = 30*), adapter sequences using *Bbduk.sh*, part of *BBMap* v.35.85, and PCR duplicates using *RemovePCRDups.pl*. Non-template sequences introduced at the 5′ end of cDNA tags during Tag-Seq libraries preparation were trimmed using *TagTrimmer.pl* (parameters: *−b 1 −e 8*).

Quality-processed reads were re-evaluated using FastQC. For mapping, we obtained the *S. hermonthica* transcriptome (StHeBC4) available at the Parasitic Plants Genome Project (PPGP) website (http://ppgp.huck.psu.edu/download.php; http://bigdata.bx.psu.edu/PPGP_II_data) and then used HISAT2 (Kim et al., 2019), which performs local alignments and is well optimized to handle spliced reads. Subsequently, SAMtools v.1.10 (Li et al., 2009) was used to convert the generated sequence alignment map (SAM) files into binary alignment mapping (BAM) format, as well as sorting and indexing the BAM files. Sorted BAM files were then used as input for transcripts quantification using Kallisto (Bray et al., 2016) to obtain different count tables for differential gene expression analysis. The ensuing raw counts in form of TPM were collated in R using the Bioconductor package tximport (Soneson et al., 2015) (parameters; countsFromAbundance = "lengthScaledTPM") and exported for downstream differential gene expression analysis.

2.6 | Analysis of differential gene expression

Raw counts were transformed into counts per million (CPM) using the *cpm()* function in *edgeR* package (Robinson et al., 2010). Genes that had a CPM of greater than 1 were retained for further analysis. Principle coordinate analysis (PcoA) plots and heatmaps were generated using *ggplot2* to determine the relatedness of the biological replicates.

Differential gene expression analysis was performed using *DESeq2* Bioconductor R package (Love et al., 2014; http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html) by comparing germination treatments with non-treated (ungerminated) seeds following normalization using *DESeq2* with a Wald test. A false discovery rate (FDR) cut-off of 0.05 was applied, and a $\log_2$ fold change cut-off of ≥2 to indicate upregulation and ≤−2 to indicate downregulation. Differentially expressed genes (DEGs) were considered at each time point for each host in relation to the controls and displayed as UpSet plots (Conway et al., 2017).

2.7 | Self-organizing maps analysis

Following *DESeq2* analysis, DEGs were hierarchically clustered using 4 × 2 hexagonal self-organizing maps (SOMs) using the package Kohonen (Wehrens & Kruisselbrink, 2018) and the outcome visualized in a principal component analysis (PCA) with PC values calculated from gene expression across samples (R stats package, prcomp function). Further visualization of scaled gene expressions across treatments was performed using line graphs generated in R (*ggplot2*). To obtain gene ontology (GO) terms, fasta sequences of the clustered gene-sets (SOMs) were analyzed with OmicsBox (Conesa et al., 2005), and then, the resulting terms were subjected to pathway enrichment (biological processes) in ShinyGO (Ge et al., 2019) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

3 | RESULTS

3.1 | *Striga*'s variable germination response to different sorghum genotypes

Germination rate of *Striga* is variable depending on various blends of SL emitted by hosts (Gobena et al., 2017). Therefore, we first measured the germination efficiency and radical lengths of the selected sorghum genotypes: SRN39, IS41724, IS27146, and IS2730 evaluated against a positive control (synthetic SL, GR24).

Germination efficiencies and radical lengths observations were made at 6, 12, and 18 h after induction (hai) as described in Figures 1a,c. We found that germination had occurred at 6 hai in IS 2730 (16.45%), GR24 (15.62%), and IS 27146 (15.36%) but not in SRN39 and IS41724. At 12 hai, germination had occurred in all genotypes, and it was higher in GR24 (24.61%) than in IS2730 (22.49%), IS27146 (18.83%), IS41724 (17.32%), and SRN39 (20.48%). At 18 hai, germination increased in all genotypes. Overall, there were no difference in seeds treated with IS2730 (49.79%) and GR24 (45.76%), but these values were higher than IS27146 (20.50%), SRN39 (24.90%), and IS41724 (20.05%) observed at that time point.

Radical length differences also varied across all treatments at different time points. At 6 hai, radical growth was observed in GR24 (0.09 mm), IS2730 (0.10 mm), and IS27146 (0.08 mm). At 12 hai, Turkey's mean separation using HSD resulted in groups of radical lengths that were significantly different. GR24 (0.25 mm) induced the longest radicals, IS41724 (0.17 mm), SRN39 (0.14 mm), IS2730 (0.13 mm), and IS27146 (0.11 mm). At 18 hai, *Striga* seeds treated with IS2730 (0.42 mm) had longer radicals, GR24 (0.33 mm), SRN39 (0.26 mm), IS41724 (0.20 mm), and IS27146 (0.15 mm). These results underscore significant variations in germination induction kinetics by the genotypes at the different time points.

3.2 | Transcriptional gene activation underlies rates of *Striga* germination induction by sorghum genotypes

To examine the genetic complexity of observed germination rates, we performed a comparative transcriptome analysis of germination induction of the sorghum genotypes at the 6, 12, and 18 hai and compared gene induction profiles to those of imbibed but untreated non-germinated seeds.

Comparing the number of DEGs with germination rates of the different treatments pointed to a positive correlation between germination induction and the number of DEGs, implying that an earlier activation could be responsible for the different germination rates in the treatments (Figures 2 and S1). In support of this hypothesis, there was a notable increase in DEGs for SRN39 and IS41724 at 12 hai (when these genotypes induced germination) and a decrease in DEGs in seeds induced by IS2730 and GR24 treatments at 12 hai except for the number of DEGs in IS27146. At 18 hai, DEGs had decreased in all treatments except in SRN39. Figure S2 further shows common genes

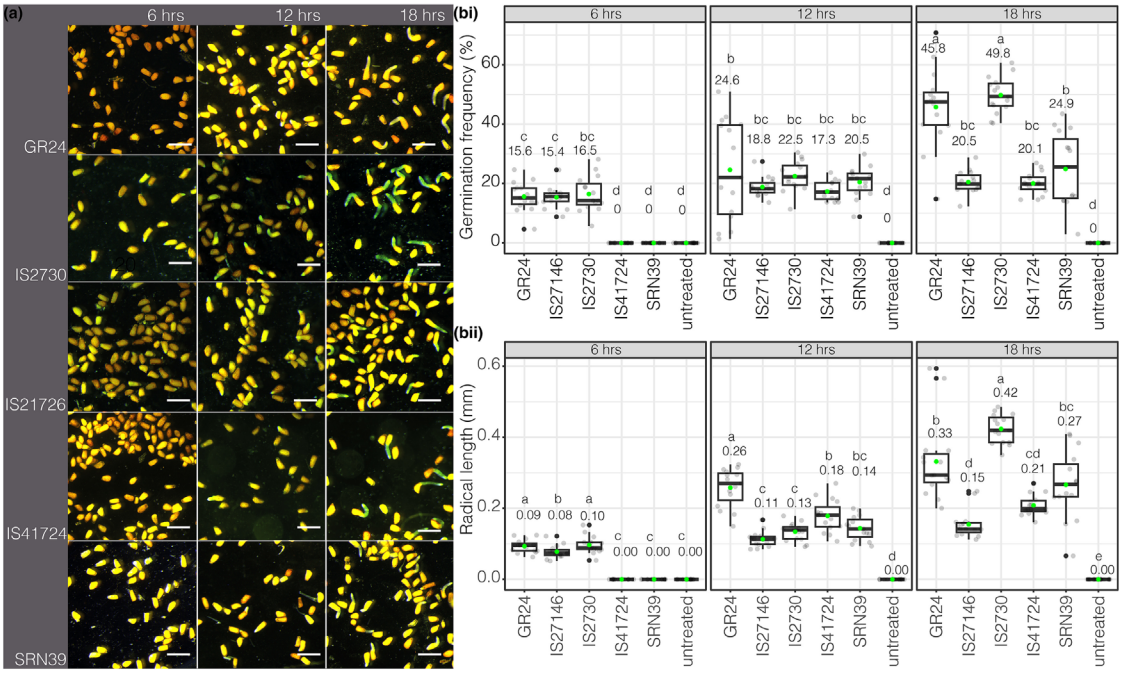

FIGURE 1 Germination response of *Striga* to different root exudate treatments. (a) Illustrative images of *Striga* germination induction using treatments of root exudates from different sorghum genotypes compared with the synthetic strigolactone (GR24) and SRN39 as positive and susceptible checks. Induction was performed after 6, 12, and 18 h. Scale bar = 1 mm. (bi) Boxplots depicting rates of *Striga* germination as induced by various treatments 6, 12, and18 h after induction (hai). At 6 hai, germination was already observed in high germination inducers and the positive control but not in low germination inducers (IS41724 and SRN39). (bii) Boxplots of *Striga* radical lengths after treatments with root exudates of various sorghum genotypes at different time points. Early induction of germination by high inducers of germination led to notably longer radical lengths. Letters indicate significant differences between treatments for the same time point at $p < .05$; $n = 15$. Green dots represent the means for each treatment.

whose expression was in all treatments at 6, at 12, and at 18 hai. GO enrichment (BP) for the intersect of genes at 6 hai (Figure S2) showed that these genes are involved in cell wall loosening, amino acid metabolism, carbohydrate metabolism, and energy metabolism. At 12 hai, (Figure S2) DEGs were enriched for cell wall loosening and catabolism of cell wall material (arabinian and xylan metabolism), while at 18 hai, they were enriched for (polyribonucleotide nucleotidyltransferase) HSP, glucose-repressible protein, oil body-associated protein 2C-like probably involved in seed growth (Dataset S1). We presumed that the genes that were differentially expressed in all treatments were part of core germination processes.

3.3 | *Striga* germination is phased, and genotype is differentiated

To determine the dynamics of germination induction responsible for variability in *Striga* germination rates as induced by various sorghum genotypes, we categorized the treatments by hierarchical clustering using SOMs. In this analysis described by (Kohonen, 1982), gene expression values from the different treatments are mean-centered and variance-scaled separately generating treatment-specific expression patterns. This provides the advantage of focusing on expression patterns, thereby avoiding biases due to differences in the magnitude of gene expression.

Results of SOM analysis displayed as PCA and line graphs for scaled gene expression chats are shown in Figure 3a,b. Additionally, Gene Ids and InterPro and KEGG annotations for the clusters are provided as Datasets S2 and S3. There were eight clusters that differed in the gene expression patterns. Notably, the expression pattern in SOM5 showed a steady increase in gene expression with a clear differentiation between imbibed non-germinated seeds and germination induction treatments at 6 hai. In this cluster, gene expression went up at 12 hai but declined at 18 hai. Other notable SOM clusters were SOM4, SOM7, and SOM8, which showed either genotype-specific or a time-specific gene expression pattern.

Pathway enrichment analysis of these SOM clusters showed specific developmental processes of seed germination. This is well illustrated with SOM8 in relation to SOM4. SOM8 (Figure 3ci,cii) represents the pathways that defined a switch between dormancy break and germination. This SOM was significantly enriched for response to Karrikin—representing response to the germination stimulants, followed by notable DNA repair processes, dormancy release process, and embryo development. Consistent with the progression of the germination process, SOM4 (Figure 3di,dii) was enriched for protein synthesis using extant mRNAs, seed reserve mobilization, further DNA repair, transport of solutes, and, notably, seedling development. The low activity in 12 and 18 hai in the early germinators is consistent with repression of seed maturation to allow a transition to germination (Bewley, 1997). A similar inference (repression of gene activity)

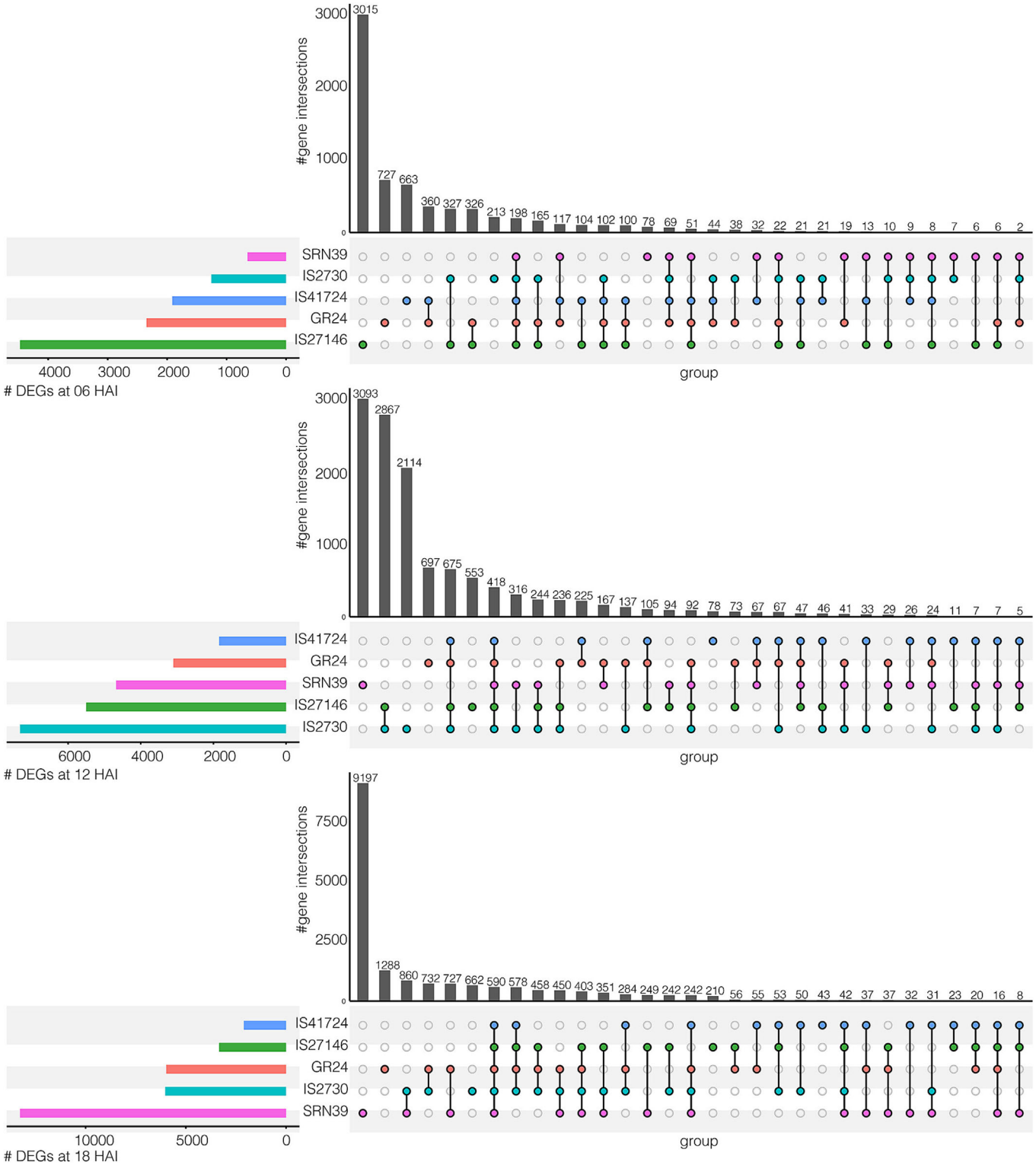

FIGURE 2 Transcriptional reprogramming during *Striga* germination. An UpSet plot showing the overlap of differentially expressed genes (DEGs) between comparisons. Colored horizontal bar graphs indicate the number of DEGs for each comparison, while black vertical bar graphs show the intersection size of DEGs. Colored dots represent contributing comparisons from the DEG analysis. For fast inducers of germination, many genes were induced as early as 6 h (upper panel on IS27146). Late gene induction is observed 12 h after induction (hai) for the genotype IS2730 (middle panel) and at 18 hai for SRN39 (lower panel). Both genotype-specific and conserved (core gene) induction responses were observed.

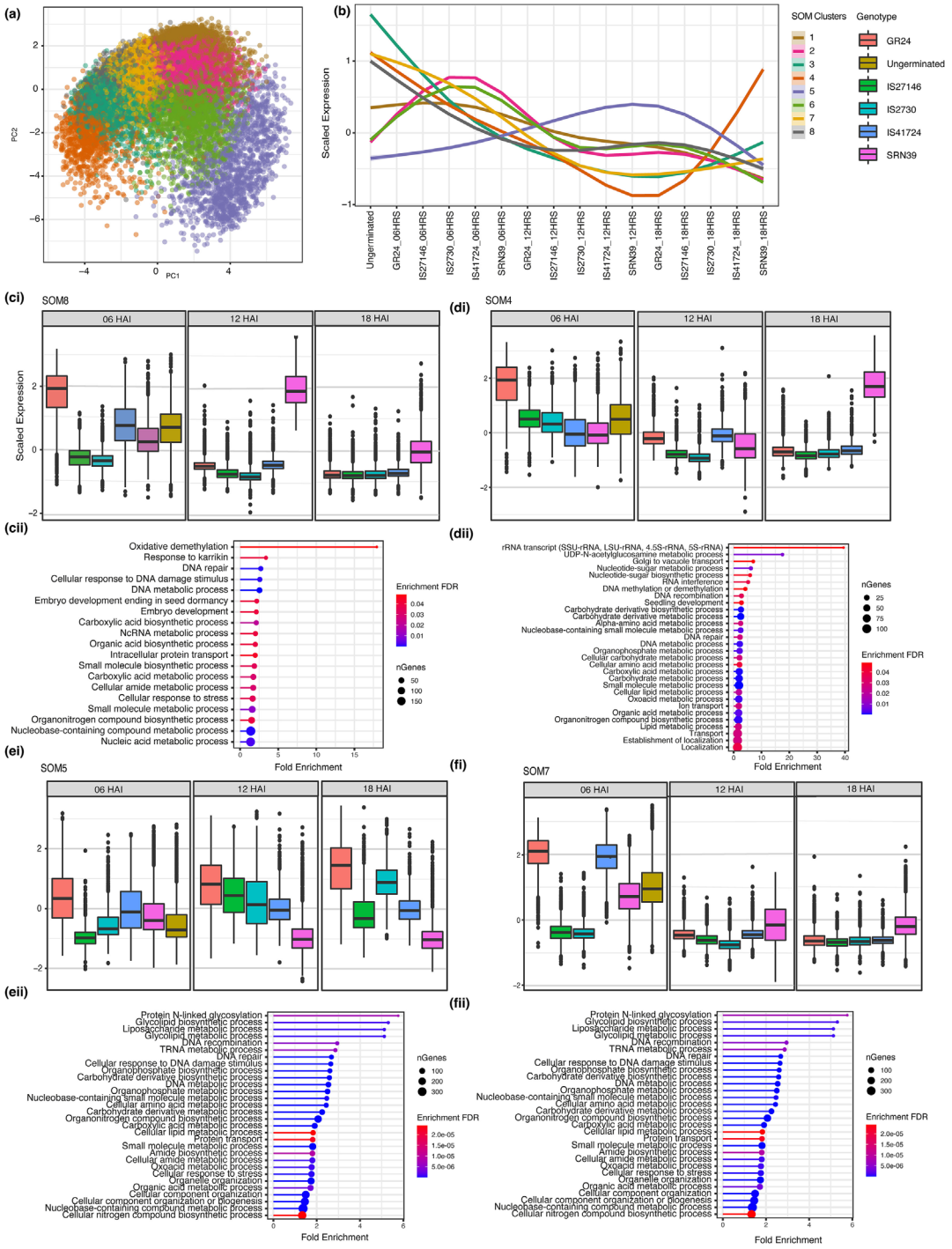

FIGURE 3 *Striga* germination is time and genotype specific. (a) Principal component analysis (PCA) diagram of a 4 × 2 self-organizing map (SOM) for germination induction treatments showing eight distinct clusters. (b) Line graphs depiction of trends in gene induction and repression for various SOMs. (c and d) Time-phased gene expression patterns: (ci) early gene expression patterns in SOM8 and (cii) corresponding biological processes (BP) GO enrichment. (di) Late gene expression in SOM4 and (dii) corresponding pathway enrichment. (e and f) Genotype-specific expression patterns: (ei) SOM 5 showing early induction of gene expression in GR24, IS2730, and IS21746 and late induction in IS41724 and SRN39. (eii) Corresponding BP pathways enrichment showing overlap between early germination and late germination processes. (fi) Similar induction of gene expression in low germination inducers SRN39 and IS41724 and (fii) corresponding BP enrichment.

can be made regarding genotype specificity in germination. Early induction of gene expression was in GR24, IS2730, and IS21746 as indicated by induction of seed maturation pathways in SOM5 (Figure 3ei,eii). Induction of genes for IS41724 and SRN39 was highest at 12 and 18 hai. Pathways induced in this cluster included an overlap between early induction of germination processes and later germination processes. Similarly, SOM7 (Figure 3fi,fii) was specific to induction of germination by SRN39 and IS41724—indicating

similarities in these two genotypes. Because both SRN39 and IS41724 are low inducers of germination, we presumed that the similarities could be associated with the resistant phenotype. Together, these results point to a time-differentiated response to germination stimulation by the different treatments. The rates are likely dependent on the perception of germination signals in the different treatments.

3.4 | *Striga* germination is associated with abscisic acid repression

The switch from dormancy to germination occurred at 6 h upon perception of the germination signal—SL. Studies of seed germination have implicated the hormone abscisic acid (ABA) as the primary growth regulator for dormancy release (Gianinetti & Vernieri, 2007; Nambara et al., 2010; Shu et al., 2016). We therefore explored the hypothesis that germination occurred with repression of ABA against the alternate that ABA has no role in influencing *Striga* germination rates. We analyzed specific gene expression patterns in the ABA biosynthesis pathway mapped in the carotenoid biosynthesis pathway of the Kyoto KEGG. The results are shown in Figure 4.

The first step in carotenoid biosynthesis is the conversion of phytoene lycopene. The enzymes involved in this step PSY, PDS, Z-ISO, ZDS, and CRTISO were all consistently induced in non-germinated seeds, and treatments of resistant sorghum and GR24 treated seeds at 6 hai. Lycopene is then catalyzed into carotene by the cyclases, LCY-B and LCY-E. In our case, LCY-E was significantly induced in the non-germinating seeds, resistant sorghum and GR24. Finally, carotenoids are degraded by carotenoid cleavage dioxygenases (CCD) to form either SLs or ABA. One CCD gene and nine NCED genes were all

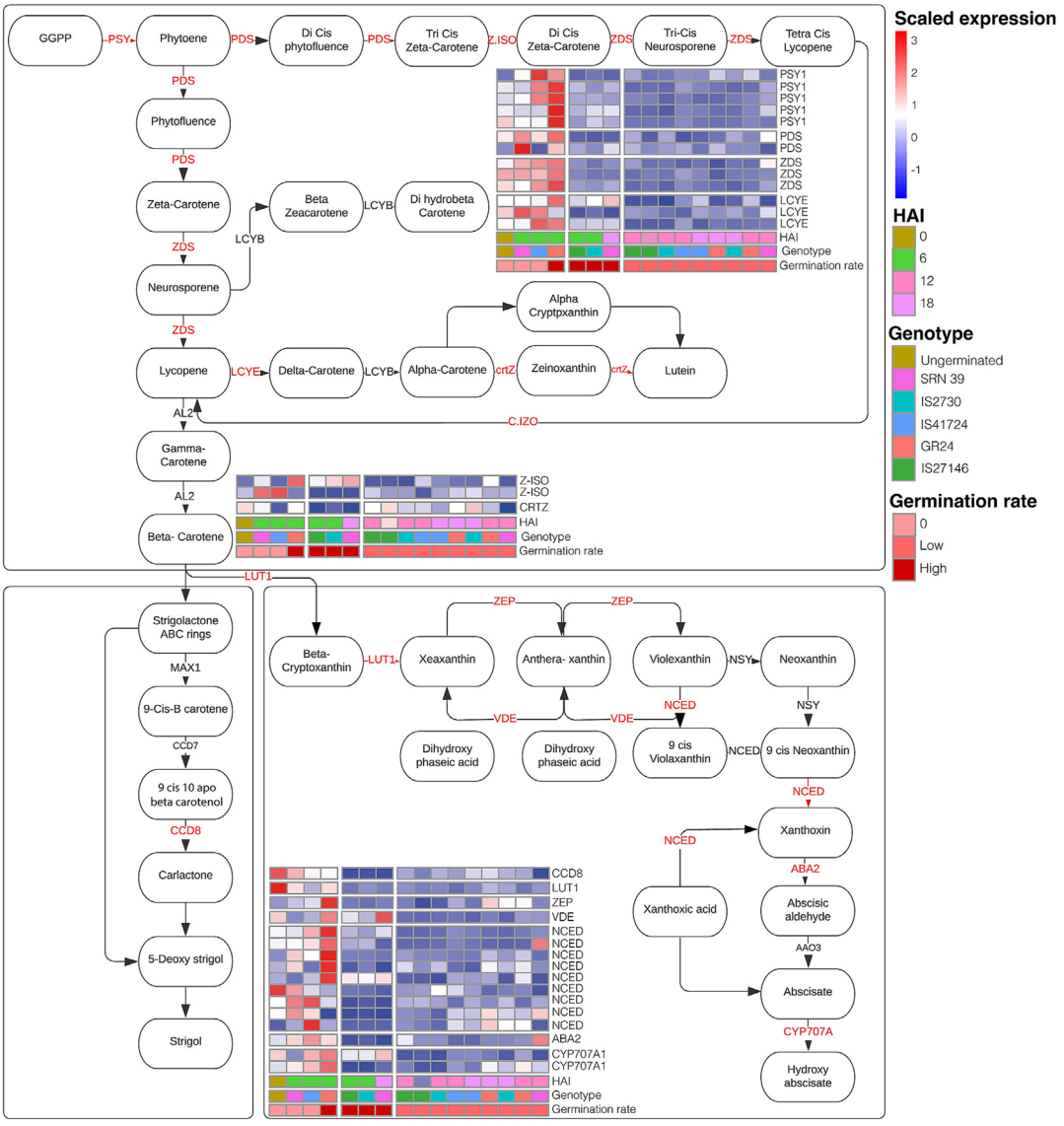

FIGURE 4 A schematic the Kyoto Encyclopedia of Genes and Genomes (KEGG) map for carotenoid biosynthesis pathway. Expression of specific genes is shown as heatmaps. Abscisic acid (ABA) biosynthesis genes were induced 6 h after induction (hai) and repressed at 12 and 18 hai. Repression of ABA corresponded with germination.

induced in non-germinating seeds, resistant treatments, and GR24 at 6 hai. In all cases, germinating seeds showed repression of the carotenoid biosynthesis and degradation genes. Because of the role of the enzymes encoded by these proteins in the biosynthesis and degradation of carotenoids, we conclude that the observed changes in gene expression may cause differences in germination rates. Furthermore, because of inhibitory roles of ABA in seed germination, we surmised that ABA repressive effects must be alleviated for seed germination to occur. But appearing to contradict this hypothesis is notable induction of carotenoid biosynthesis genes in germinating seeds treated with GR24. This could be suggestive of ABA suppression by components in root exudate that are not present in GR24. Equally perplexing is the induction of the SL biosynthesis gene CCD8 that leads to biosynthesis of the intermediate carlactone. Bearing in mind that *Striga* lacks the downstream genes that lead to canonical SL biosynthesis (Abe et al., 2014), it was not clear why this enzyme was induced. One possibility is that CCD8 expression may just be responding to increased traffic down the SL biosynthesis path when carotenoid biosynthesis is high. The observations, however, bring to the fore a possible interaction between SL, ABA, and other hormone pathways.

3.5 | *Striga* germination is modulated by a hormonal crosstalk

To address the question of a plausible hormonal crosstalk modulating *Striga* germination, we mapped the differentially expressed genes during *Striga* germination as induced by different treatments on the hormone signaling pathways in KEGG maps. We considered genes in the ABA, auxin, brassinosteroid (BS), cytokinin (CK), ethylene (ET), gibberellic acid (GA), and jasmonic acid (JA) pathways (Figure 5). Analysis of gene expression patterns showed ABA and auxin repressed germination. ABA genes PPC2, SnKR2, and ABF were repressed in germinating seeds and induced in non-germinating seeds. Similarly, at 12 hai, auxin genes GH3, TIR1, and AUX1 were repressed in seeds that showed higher germination induction than IS41724 and SRN39, and at 18 hai, GH3 and AUX1 were notably induced in the low germination inducer SRN39. The observed gene expression patterns are consistent with mechanistic of germination inhibition by ABA and auxin. ABA germination suppression occurs upon activation of PP2C by ABA, triggering release of SnRK2 kinase and subsequent activation of ABSCISIC ACID INSENSITIVE5 (ABI5) that exerts transcriptional con-

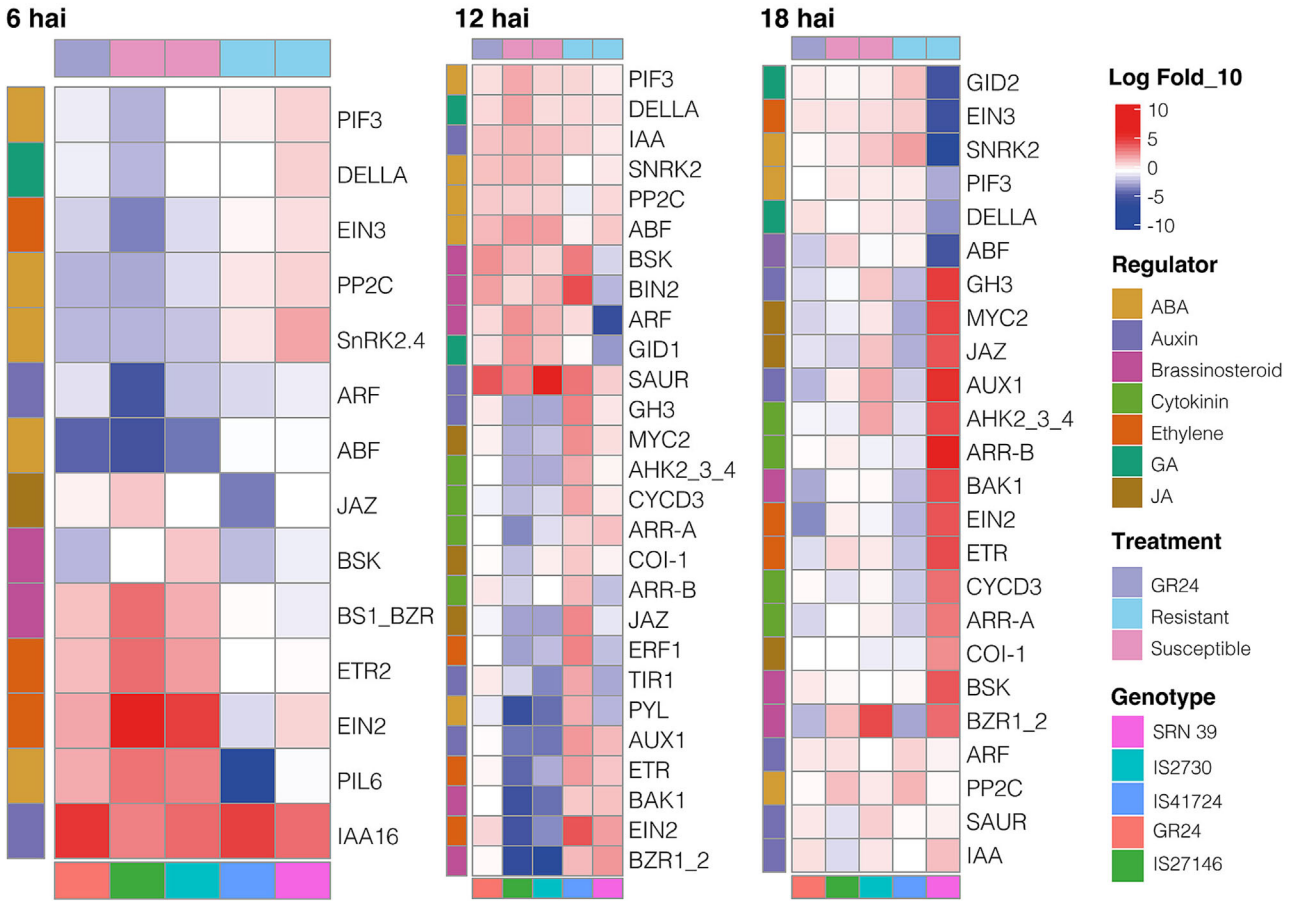

FIGURE 5 The role of hormones in the *Striga* germination temporal patterns. Heatmaps show gene expression patterns of the plant hormone signaling pathways of Kyoto Encyclopedia of Genes and Genomes (KEGG) map 6, 12, and 18 h after induction (hai) for different root exudate and GR24 treatments. ABA, abscisic acid; GA, gibberellic acid; JA, jasmonic acid.

trol over seed dormancy (Fujii et al., 2009). Likewise, auxin represses germination through ARF through stabilization of the ABA transcription factor ABI3 (Liu et al., 2013).

Brassinosteroid, ET, GA, and JA enhanced germination as follows: (i) BS genes BS1, BSK BZR, and BAK1 were induced in germinating seeds pointing to a positive regulatory of seed germination through BSs. It is known that BIN2 interacts with ABI5 to mediate antagonism between ABA and BS (Hu & Yu, 2014) and that SL-dependent *Striga* germination requires functional BS signaling (Bunsick & Lumba, 2020). (ii) Ethylene proteins (ETR, ERF, and EIN2) were consistently induced in germinating seeds reinforcing the extensively studied antagonism of ethylene on ABA reviewed in (Gazzarrini & Tsai, 2015; Linkies & Leubner-Metzger, 2012). Furthermore, PIL6, which promotes ethylene activity in the dark (Khanna et al., 2007), was induced in germinating seeds in a pattern like ethylene proteins. (iii) GA repressor gene DELLA was repressed in germinating seeds at 6 hai. Although this pattern points to a positive regulatory role of GA in *Striga* germination, DELLA was induced in germinating seeds at 12 hai, making it difficult to specifically determine if GA indeed has a positive regulatory role in *Striga* seed germination. Finally, (iv) JA, signaling genes JAZ, MYC, and CO1 were induced in germinating seeds indicating that JAZ is a positive regulator of germination in agreement with previous works demonstrating JAZ's repression of ABI3 and ABI5 (Pan et al., 2020).

We considered that CK has a less prominent role in *Striga* germination on account that there were no cytokine DEGs at early germination stages. Cytokinin genes A-ARR and B-ARR were upregulated in already germinated seeds at 12 and 18 hai, leading us to speculate that these genes had functions after germination. Because of notable expression of PIF3 transcription factors involved in photomorphogenesis (Feng et al., 2008; Kim et al., 2003) and cell cycle genes (CYDC), we deduced that CK gene activation was involved phytochromes interactions of ketomorphogenesis. Interestingly, CK specifically antagonizes ABA-mediated inhibition of cotyledon greening with minimal effects on seed germination in (Guan et al., 2014), further affirming a possible post-germination, rather than a germination role for CK.

In summary, our results suggest that (i) ABA and Auxin repress germination; (ii) BS, ET, and JA promote germination; and (iii) CK and GA may have a more prominent role post-germination rather than during germination. We provide a proposed model in Figure 6.

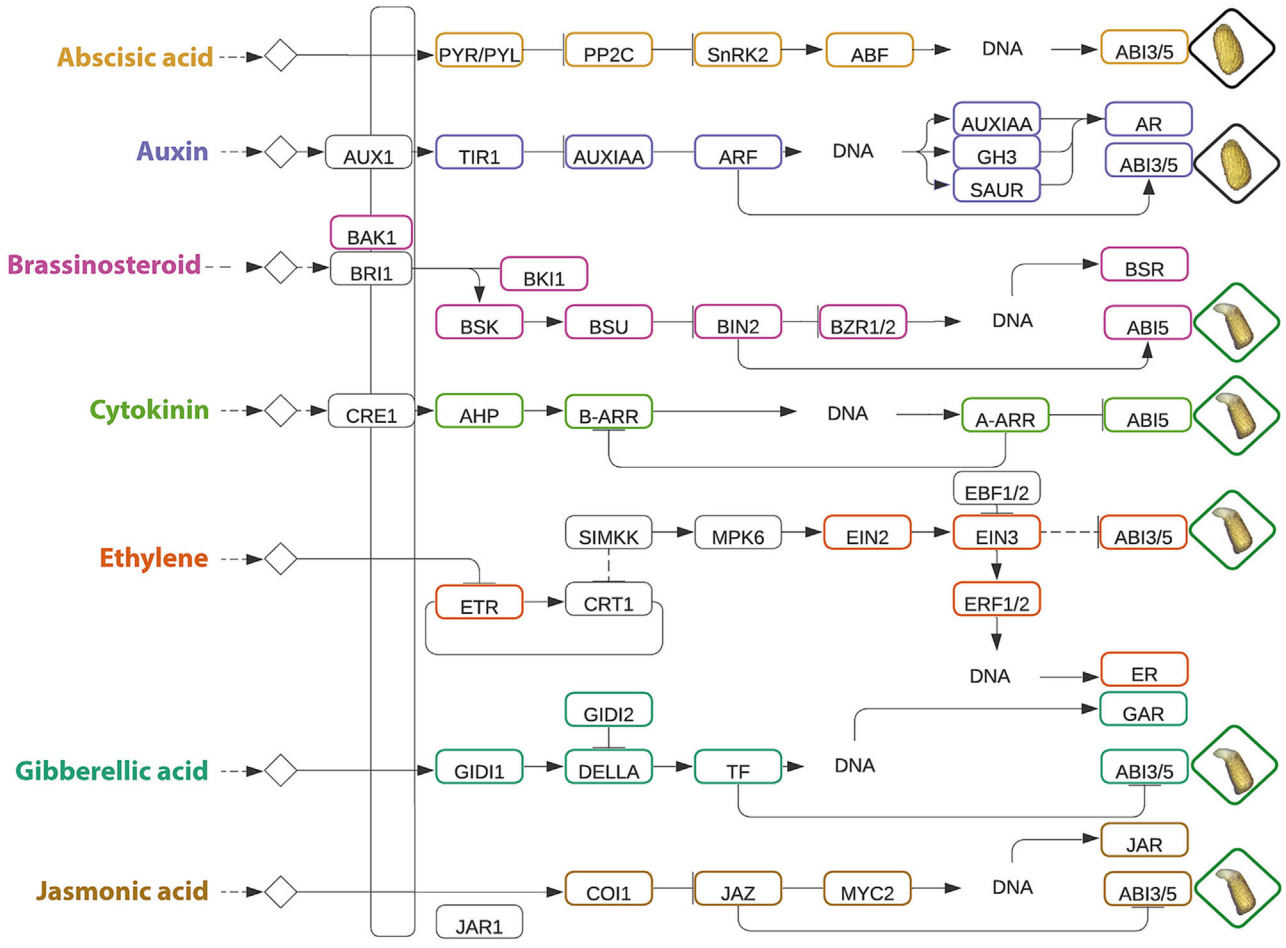

FIGURE 6 Proposed model of hormone signaling moderation based on gene expression patterns of the Kyoto Encyclopedia of Genes and Genomes (KEGG) map for plant hormone signaling highlighting positive and negative regulators of *Striga* seed germination. Differentially expressed genes for each pathway are color-coded. ABA, abscisic acid; GA, gibberellic acid; JA, jasmonic acid.

4 | DISCUSSION

Processes that lead to germination of the parasitic plant *Striga* could provide useful insights into strategies for its management. Principally, *Striga* germinates in response to germination stimulants emitted from the host root exudate. However, germination is highly variable in most *Striga*–host interactions. In sorghum, for example, genotypes that have a mutation on the *lgs1* loci—such as SRN39 emit the less powerful germination inducer SL (orobanchol) unlike their *LGS1* counterparts that emit the powerful inducer 5-deosystrigol. Mutants (*lgs1*) stimulate *Striga* seed germination less efficiently and are advocated for *Striga* resistance breeding in SSA. To unravel the genetic basis of this complexity in sorghum, we did a comparative transcriptomic analysis of the various genotypes of sorghum that exhibit variation in stimulating *Striga* germination. We sought to identify not only the major transcriptomic changes that define the germination process in *Striga* but also a set of genes that may contribute to the differences in germination rates exhibited by these genotypes. For this purpose, we carried out RNA sequencing (RNA-seq) on four selected genotypes (IS2730, IS27146, IS41724, and SRN39) that show varied germination induction and the positive control (synthetic SL, GR24) at different stages of seed germination.

Analysis of differential gene expression of *Striga* seeds in response to stimulation by different root exudates revealed distinct genotypic differences and core genes required for germination. The core DEGs were enriched for amino acid metabolism, carbohydrate metabolism, energy metabolism, biosynthesis of other secondary metabolites, lipid metabolism, and signal transduction. This is consistent with the metabolic processes of seed germination in other seed plants (Boter et al., 2019; Weitbrecht et al., 2011), including seeds of the parasitic plant *Raflesia speciosa* (Molina et al., 2023). Studies have shown that the seed expands rapidly at the initial stage of germination due to water absorption. Genes associated with sugar, lipid, and amino acid metabolism are upregulated. Additionally, cell wall synthesis and degradation genes are upregulated to provide small molecules and materials for cell wall synthesis during seed germination (Boter et al., 2019).

We then used SOMs to cluster differentially expressed genes according to the rates of germination induction by various treatments at different time points. The switch to germination occurred first at 6 hai. This timepoint marked the dormancy release phase and commencement of germination. Germination itself was phased into early and late germination processes. Early germination was characterized by the perception of SL, resource mobilization, DNA repair, dormancy release, and embryo development, while late germination was characterized by protein synthesis and carbon fixation. Germination was also genotype-specific, resulting in early and late inducers of germination. Rapid seed maturation processes were observed in the GR24, IS27146, and IS2730 treatments. Late seed maturation was observed in seeds treated with root exudates of SRN39 and IS41724, and IS27146. SOM analysis showed that early germination (6hai) processes in early inducers were clustered with later treatments of later inducers, implying that these processes were delayed in late inducers. This finding is consistent with what is known about germination induction by IS41724 and SRN39. Previous work has shown that these genotypes support poor *Striga* germination and radical growth (Mallu et al., 2021, 2022). Our findings are also consistent with previous studies of seed germination. Transcriptomic analysis of barley seeds (Leišová-Svobodová et al., 2020) revealed transient upregulation of cell wall synthesis genes. Regulatory components such as transcription factors, signaling, and post-transcriptional components are transiently upregulated during the early germination phase, whereas the late germination phase is characterized by transcriptional activation of genes representing the histone families and many metabolic pathways such as amino acid metabolism, nucleotide metabolism, and those related to cell division and cell cycle. The post-germination phase is characterized by the induction of genes involved in photosynthesis and reserve mobilization.

Because the carotenoid pathway leads to the biosynthesis of *Striga* germination crucial phytohormones, SL and ABA, we examined differential gene expression patterns in the different treatments for a possible modulation of germination because of these hormones. Consistently, ABA was induced in non-germinating, resistant, and GR24 treatments in the early phase of germination induction (6 hai) but repressed in germinating seeds and late stages of germination induction (12 and 18 hai). This observation is reminiscent of an ABA dormancy release phenomena previously observed in studies of seed germination (Kusumoto et al., 2006; Mallu et al., 2022). Our study also pointed to a possible interaction between ABA and other hormones.

Therefore, the last aspect of our study investigated the role of phytohormones in regulating the rates of *Striga* germination. Induction of ABA and auxin genes corresponded with the suppression of germination. This is consistent with physiological germination experiments that show ABA severely limits *Striga* germination, and its repeal using the inhibitor fluridon causes spontaneous germination of *Striga* seeds (Kusumoto et al., 2006; Mallu et al., 2022). Although not shown in *Striga*, the role of auxin in dormancy imposition in an ABI3-dependent manner has been well described in Arabidopsis (Liu et al., 2013). This is also consistent with literature that shows that auxin leads to a release of ARF, which stabilizes the binding of ABI5 and thereby enhances ABA responses. Both processes lead to the inhibition of germination in an ABI5-dependent manner. Enhanced germination in interactions with other hormones such as BS and JAZ could result from antagonizing ABI5. Such a hypothesis is supported by the fact that ABI5 physically interacts with the BRASSINOSTEROID INSENSITIVE2 (BIN2) and brassinosteroid-related protein kinases and that the BIN2-ABI5 cascade mediates the antagonism between BS and ABA during seed germination (Hu & Yu, 2014). Additionally, JAZ proteins directly interact with ABI5 to negatively regulate ABA (Ju et al., 2019). Ethylene is known to play a role in dormancy release (Corbineau et al., 2014) and spontaneous germination of *Striga* seed (Logan & Stewart, 1991). Regarding GA, there are reports on GA being a positive regulator of *Striga* germination (Seo et al., 2008), while others have reported GA as a negative regulator of *Striga* germination (Ito et al., 2017; Mallu et al., 2022). We found that DELLA, a GA repressor, was downregulated in germinating seeds but upregulated in non-

germinating seeds. Unlike seeds of other plants, *Striga* bypasses GA-dependent germination in favor of SL-dependent germination (Bunsick et al., 2020), and this may confound results of exogenous GA application.

Curiously, we observed a notable association of hormones with the phytochrome-interacting transcription factor (PF3) post-germination. Under light conditions, PF3 negatively regulates light responses, including hypocotyl elongation, cotyledon opening, and hypocotyl-negative gravitropism. This brings to focus the remarkable capability of *Striga* and other parasitic plants to suppress shade phenotypes—elongated hypocotyl growth and closed cotyledons. One possibility is that *Striga* maintains hyperactive phytochromes consistent with the phenotypes of PHYA over-expressors (Franklin & Whitelam, 2005). Interestingly, it was recently shown that ABA modulates shade avoidance by inducing hyponasty movement in Arabidopsis (Michaud et al., 2022), adding another piece of circumstantial evidence for ABA involvement in *Striga*'s germination and infection process. This is an interesting subject for future investigation.

5 | CONCLUSIONS AND IMPLICATIONS FOR *STRIGA* MANAGEMENT

We have shown that *Striga* seed germination is controlled by complex signaling and a gene expression regulatory network. Perception of the germination signal (SL) sets the germination program for different rates of germination or abortion for sorghum genotypes. After SL perception, *Striga* appears to share similar molecular mechanisms, such as phytohormone behavior, transcription and translation activation, and the process of radical protrusion, with other plants. Beyond germination, hormones interact with phytochromes to ensure *Striga* seedling growth and development sub-terranean. These findings provide significant insights into *Striga* management.

First, natural host resistance remains a cornerstone in *Striga* management. The LGS1 is now well characterized, making it possible to enrich the sorghum gene pool for *Striga* resistance. This can be achieved through (i) a traditional marker assisted breeding approach (Hess et al., 1992; Hess & Ejeta, 1992) and integration into SSA's sorghum Striga resistance breeding programs, (ii) allele mining from diverse sorghum panels (Mallu et al., 2021) followed by breeding or deployment in *Striga* prone areas, or (iii) gene editing which is now gaining traction in Africa (Tripathi et al., 2022).

Second, *Striga* management can be achieved by exploiting other growth regulators. Modern day and future *Striga* management strategies will heavily rely on "suicidal" germination or inhibiting germination using new biomolecules (Kountche et al., 2019; Uraguchi et al., 2018; Zarban et al., 2021). Significant strides have also been made in identification of microbes that produce *Striga* germination stimulants or inhibitors (Kawa et al., 2023). Because of their versatility, microbes can be expected to be at the nexus of *Striga* management in the short term.

AUTHOR CONTRIBUTIONS

Steven Runo and Emily S. Bellis designed the study. Gilles Irafasha, Sylvia Mutinda, and Brett Hale collected data. Fredrick Mobegi, Steven Runo, and Gilles Irafasha analyzed the data with help from Emily S. Bellis, Asela J. Wijeratne, Susann Wicke, and George Omwenga. Gilles Irafasha wrote the manuscript with help from Fredrick Mobegi, Steven Runo, Susann Wicke, and Emily S. Bellis.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in National Center for Biotechnology Information Sequence Read Archive (at https://www.ncbi.nlm.nih.gov/bioproject/, reference accession number PRJNA904327).

ORCID

Gilles Irafasha https://orcid.org/0000-0002-0900-5196
Sylvia Mutinda https://orcid.org/0000-0001-6887-647X
Fredrick Mobegi https://orcid.org/0000-0003-0554-9919
Brett Hale https://orcid.org/0000-0002-4984-017X
Asela J. Wijeratne https://orcid.org/0000-0002-7817-6351
Susann Wicke https://orcid.org/0000-0001-5785-9500
Emily S. Bellis https://orcid.org/0000-0001-6066-1466
Steven Runo https://orcid.org/0000-0002-9043-2338

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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