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Characterization of growth and development of sorghum genotypes with differential susceptibility to *Striga hermonthica*

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Abstract

Two sorghum varieties, Shanqui Red (SQR) and SRN39, have distinct levels of susceptibility to the parasitic weed *Striga hermonthica*, which have been attributed to different strigolactone composition within their root exudates. Root exudates of the Striga-susceptible variety Shanqui Red (SQR) contain primarily 5-deoxystrigol, which has a high efficiency for inducing Striga germination. SRN39 roots primarily exude orobanchol, leading to reduced Striga germination and making this variety resistant to Striga. The structural diversity in exuded strigolactones is determined by a polymorphism in the *LOW GERMINATION STIMULANT 1* (*LGS1*) locus. Yet, the genetic diversity between SQR and SRN39 is broad and has not been addressed in terms of growth and development. Here, we demonstrate additional differences between SQR and SRN39 by phenotypic and molecular characterization. A suite of genes related to metabolism was differentially expressed between SQR and SRN39. Increased levels of gibberellin precursors in SRN39 were accompanied by slower growth rate and developmental delay and we observed an overall increased SRN39 biomass. The slow-down in growth and differences in transcriptome profiles of SRN39 were strongly associated with plant age. Additionally, enhanced lateral root growth was observed in SRN39 and three additional genotypes exuding primarily orobanchol. In summary, we demonstrate that the differences between SQR and SRN39 reach further than the changes in strigolactone profile in the root exudate and translate into alterations in growth and development.

Keywords: Developmental delay, gibberellins, lateral root, metabolism, root system architecture, sorghum, *Striga hermonthica*, strigolactone.
Introduction

*Sorghum bicolor* is one of the five most important cereal crops globally (Paterson et al., 2009). Its adaptations to drought, heat, and low nutrient availability make sorghum especially important for agriculture in sub-Saharan Africa, where it is a staple food, feed, and forage (Mace et al., 2013). Among sorghum strategies to withstand abiotic stresses, establishing interactions with arbuscular mycorrhiza (AM) fungi improves phosphate acquisition (Neumann and George, 2004). To recruit AM fungi, plants secrete strigolactones into the soil, carotenoid-derived compounds that induce AM hyphal branching (Akiyama et al., 2005). Low nutrient concentration, primarily phosphate and nitrogen, induces strigolactone exudation (Jamil et al., 2013), which ensures AM fungal recruitment (Bouwmeester et al., 2007; Yoneyama et al., 2007). While this plant–fungus communication system is beneficial for sorghum's performance in nutrient-depleted soils, the strigolactone signal can be hijacked by seeds of the parasitic ‘witchweed’, *Striga hermonthica* (Bouwmeester et al., 2007). Striga is an obligate parasite and strigolactones are essential for its seed germination (Spallek et al., 2013; Bouwmeester et al., 2020). Co-option of strigolactone signaling by Striga ensures it germinates only in the presence of a host plant. The ability to sense the presence of strigolactones allows Striga seeds to stay dormant for years and makes Striga an extraordinarily successful parasite. After strigolactone perception, germinated Striga forms a haustorium that penetrates the roots of a host plant and establishes a connection between its own xylem and the xylem of the host plant, to deprive the host of water and nutrients (Spallek et al., 2013). Striga infestation has a detrimental effect on a host plant and around 20% of sorghum yield is lost annually (Ejeta and Gressel, 2007).

Since strigolactones are essential for Striga parasitism, understanding their biosynthesis and functions is essential to combat Striga parasitism. More than 35 different strigolactones have been discovered across plant species, yet not all have the potential to induce Striga germination to the same extent (Wang et al., 2020). To date, it is not known whether the strigolactone composition of the root and shoot tissue of *LGS1* loss-of-function genotypes is one of the potential costs of Striga resistance (Bellis et al., 2020). Moreover, elevated expression of genes involved in strigolactone biosynthesis in SRN39 roots indicates additional alterations of strigolactone profiles within this genotype, which were not previously captured in root exudate profiles (Bellis et al., 2020). To date, it is not known whether the strigolactone composition of the root and shoot tissue reflects that of the root exudate. Strigolactones do pleiotropically regulate root and shoot architecture (reviewed in Aquino et al., 2021), although it remains unknown whether the influence of strigolactones on plant development and metabolism varies with respect to different strigolactone structural variants.

Given the pleiotropic effect of strigolactones and the influence of LGS1 on strigolactone profiles and photosynthetic efficiency, one might expect that the *lgs1* genotypes would show differences in growth and plant architecture. However, only a minor reduction in leaf area was observed in the *lgs1* mutant (Bellis et al., 2020), and the consequences of *LGS1* loss-of-function on sorghum growth and development have never been characterized in detail. Here, we use transcriptome profiling to identify biological processes that differentiate SQR and the *lgs1* genotype, SRN39. We show that genes involved in metabolism have altered expression in SRN39 as compared with SQR. Furthermore, we explore the phenotypic variation between these two varieties. Our results suggest that the consequences of the genetic differences between SQR and SRN39 for growth rate depend on plant developmental stage. We associate the reduced SRN39 shoot growth relative to SQR with increased accumulation of gibberelin precursors. We further characterize the root system architecture of several *lgs1* genotypes and speculate that various strigolactone structural variants affect root system architecture. Our results suggest that the differences between SQR and SRN39 go beyond distinct susceptibility to Striga, and include differences in growth, development, and root system architecture.

Materials and methods

Plant material

Seeds of *Sorghum bicolor* var. Shanqui Red (SQR) were obtained from GRIN (https://www.ars-grin.gov). Birhan, Framada, Gobiye, and SRN39 seeds were kindly donated by the Ethiopian Institute of Agricultural
Sorghum bicolor

$seqAdapt -noqc -dust-suffix 6/ACTG -dust-suffix-late 6/ACTG -nnn-
trimming and quality filtering was performed with reaper from Kraken
edu/fastx_toolkit/index.html) with parameters: -v -f 12 -Q33. Adaptor
libraries were pooled before reads were processed. Barcodes were
ejected before and after read processing. Technical replicates of
Quality control of resulting transcriptome sequencing data was ac-
RNA-seq data processing and differential expression analysis
Quality control of resulting transcriptome sequencing data was ac-
accessed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) before and after read processing. Technical replicates of
libraries were pooled before reads were processed. Barcodes were
trimmed from raw reads with fastx-trimmer (https://hannonlab.cshl.
d.edu/fastx_toolkit/index.html) with parameters: -v -f -t 12 -Q33. Adaptor
trimming and quality filtering was performed with reaper from Kraken
Suite (Davis et al., 2013) with options: -geon no-bc -tabu $tabu -3pa
seqAdapt -noqc -dust-suffix 6/ACTG -dust-suffix-late 6/ACTG -nnn-
check 1/1 -qqq-check 35/10 -clean-length 30 -polya 5. Trimmed
reads were mapped to the reference genome of Sorghum bicolor BTx623
(McCormick et al., 2018) using STAR (Dobin et al., 2013) with options:
--outFilterMultimapNmax 20 --alignSJoverhangMin 8 --alignIntronMin
20 --alignIntronMinMax 100000 --outFilterMismatchNmax 5 --outSAMtype
BAM SortedByCoordinate --quantMode TranscriptomeSAM
GeneCounts.

Genes whose raw read counts across all samples equaled zero were
removed. Counts per million (CPM) were calculated with the cpm()
function from the edgeR package (Robinson et al., 2010) and only genes
with a CPM >1 in a minimum of three samples were used for further
analysis. CPM values are found in Supplementary Table S1. Differentially
expressed genes (DEGs) were identified with the R/Bioconductor
limma package (Ritchie et al., 2015). CPM values were normalized with
the voom() function with quantile normalization to account for different
RNA inputs and library sizes. The linear model for each gene was spec-
fied as an interaction of the genotype and the time point: log(counts
per million) of an individual gene ~Genotype×Time. Differentially ex-
pressed genes for each term of linear model were selected based on a
false discovery rate of <0.05. Lists of differentially expressed genes for
each term (genotype, time, genotype-by-time interaction) are found in
Supplementary Tables S2–S4.

Clustering analysis and ontology enrichment
Genes differentially expressed between genotypes in a time–dependent
and time–independent manner were clustered in groups of genes that
have similar expression patterns. The 75% most variable genes were
selected for clustering. The log, CPM mean across biological replicates
was calculated for each gene and expression of each gene was then scaled
to the mean expression across all samples. Hierarchical clustering was per-
formed with heatmap v.1.0.12 R package with the Euclidean distance
measure to quantify similarity. Genes assigned to each cluster are listed in
Supplementary Table S3.

Gene Ontology (GO) enrichment analysis was performed with the
GOseq v.1.34.1 R package with a hypergeometric test (Young et al.,
2010). The odds ratio for each ontology was calculated with the for-
mula: (number of genes in GO category/number of all genes in input)/
(number of genes in GO category/number of genes in all clusters).
Enriched ontology terms were selected based on a P-value <0.05 and an
odds ratio >1. Multiple testing correction is not recommended for GO
enrichment due to the graph structure of GO terms (Mi et al., 2012).
GO categories enriched in each cluster are listed in Supplementary
Table S6.

Metabolomic analysis from 28- and 35-day-old plants
Root tissue was manually ground and 70 mg of pulverized tissue was
used for the extraction with 0.7 ml 80% methanol (v/v) containing
6×10−3 mg ml −1 ribitol (Sigma-Aldrich, St Louis, MO, USA) as an-
ternal standard. The mixture was shaken using a tissue-lyser (Tissuelyzer
II, Qiagen) for 5 min at 30 Hz and subsequently sonicated for 5 min.
Samples were centrifuged at 7600 relative centrifugal force at 10 °C
for 5 min, and the supernatant was collected and filtered through a
0.22 µm-pore filter. Metabolite analysis by LC-MS was carried out as de-
cribed in Melkonian et al. (2021) with slight modifications. Five micro-
liters of root extract was injected in a Nexera ultra-high performance
liquid chromatography (UHPLC) system (Shimadzu, Den Bosch, The
Netherlands) preceded by a guard column (1.7 µm particle size, 5×2.1 mm; Acquity UPLC CSH C18, Waters), a flow rate of
16/18). Compounds were separated on a C18 stationary phase column
(1.7 µm particle size, 150×2.1 mm; Acquity UPLC CSH 18, Waters,
EttenLeur, The Netherlands) for 20 min at 30 °C. Elution started at 90% A
(0.1% v/v acetic acid in water) and eluent B (0.1% v/v acetic acid in
100% acetonitrile) were as follows: 0–1 min (5% B); 1–15 min (linear in-
crease to 100% B); 15–18 min (100% B). Compound ionization was car-
ried out by electrospray ionization operating in the negative mode using
N2 as the ionization gas with the following settings: capillary voltage 3500
V; end plate offset 500 V; nebulizer gas pressure (N2) 1 bar; dry gas (N2)
8 l min⁻¹; dry temperature 200 °C. Settings for MS analysis were: funnel
radio frequency (RF) 200 voltage point to point (Vpp); multipole RF

Growth conditions and tissue collection for transcriptome and
metabolome profiling
Four-day-old seedlings of approximately the same radicle length were
transferred to a soil plug, i.e. a 50 ml falcon tube filled with sterile soil
(soil collected from the Clue Field in the Netherlands; 52° 03′ 37.91″ N
and 5° 45′ 7.074″ E, which was further dried, sieved through 4 mm mesh
and sterilized by gamma irradiation) mixed with 5% sterile water (v/v).
Seedlings were watered with autoclaved water every second day. On day
10, seedlings together with the soil plug were transferred to 40 cm-long
cones (Greenhouse Megastore, cat. no. CN-SS-DF) filled with 700 ml of
0.5–1.0 mm filter sand (filcom.nl/). Plants were organized in a random-
ized manner in a greenhouse compartment with a temperature of 28 °C
during the day (11 hours) and 25 °C at night (13 h), with 70% relative
humidity and light intensity of 450 µmol m⁻² s⁻¹. At days 0, 7, and 14, plants
were watered with 50 ml of modified half-strength Hoagland solution
with 0.05 mM KH₂PO₄. On days 1, 4, 10, 13, and 17, plants were watered
with 50 ml deionized sterile water. Two and three weeks after transfer to
the cones (corresponding to 28- and 35-day-old plants) root material was
harvested 2 hours after the light turned on. Each sorghum plant was gently
taken from the cone and the whole root system was cleaned from the sand
and soil by washing in water, dried with paper towels, and snap frozen in
liquid nitrogen (taking approximately 3 min per plant).

RNA-seq library preparation
Root tissue was manually ground and RNA was extracted with RNAsesy Plus Mini kit (Qiagen) with application of QIAshredder columns
(Qiagen) and on-column DNase I (Qiagen). Total RNA obtained was precipitated with 3 M NaOAc pH 5.2 (Thermo Fisher
Scientific) in 100% ethanol and washed with 70% (v/v) ethanol. RNA-seq libraries were synthesized with QuantiSeq 3’ mRNA-Seq Library
Prep Kit (Lexogen) according to the manufacturer’s protocol. Libraries were sequenced at the UC Davis DNA Technologies Core with Illumina
HiSeq 4000 in SR100 mode with four biological replicates and three technical replicates for each RNA sample.

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Root system architecture quantification from 28- and 35-day-old plants

Sorghum plants were gently taken from the pots, and roots were cleaned from the sand and soil by washing in water. Crown roots were separated from the seminal roots and fresh weight was scored for them separately. Roots were then placed in a water tray and scanned at 800 dpi resolution with Epson Perfection V700 scanner. Next, roots were dried with a paper towel, placed in paper bags, dried for 48 h at 65 °C and weighed to determine their dry weight. Root scans were analysed with the DIRT (Digital Imaging of Root Traits) software v.1.1 (Dass et al., 2018). We used DIRT’s area trait that counts the number of pixels representing the root in the image (Bucksch et al., 2014) as a measurement for total root network area (to simplify we refer to it as total root length). Total network length (to simplify we refer to it as total root length) was computed by modifying the original code. As such, we count the number of pixels belonging to the medial axis of the network area after removing spurious medial branches whose medial circle radius at the tip is 2 pixels or smaller (Bucksch, 2014). Mean root network diameter was then calculated as the ratio of network area over network length. Values for each trait were transformed with natural logarithm transformation. A two-way ANOVA was used to determine the significance of the differences between genotype and genotype-by-time interactions with the following model: lm(trait~genotype×time) followed by a pairwise comparison with the formula: enmeans(model, specs=~Genotype×time, adjust=~sidak), with the enmeans v.1.5.2-1 R package.

Root system architecture quantification from seedlings

Germinated seeds of approximately the same radicle length were transferred to 25 cm-long (big pouches for SQR and SRN39 comparisons) or 18 cm-long (medium pouches for SQR and all lgs1 genotypes) germination pouches (PhytoAb Inc., cat. no. CYG-38LG/CYG-98LB) filled with 17 ml or 50 ml autoclaved water, for medium and big pouches, respectively. Pouches were placed in the greenhouse with a maintained temperature of 26 °C and daylight of approximately 15 h. Pouches were scanned daily from the second to the seventh day after germination, approximately 11 hours after the start of the light period. The root system architecture of SQR and SRN39 was quantified at each time point imaged, while for the other lgs1 genotypes (Framida, Birhan, Gobiye), root system architecture was only quantified in 7-day-old seedlings. Main root angle, main root length, lateral root number, and the length of individual lateral roots were quantified by manual tracing with ImageJ. Lateral root density was calculated as the ratio of lateral root number to main root length; lateral root length as a sum of the lengths of individual lateral roots; average lateral root length as the ratio of lateral root length to the number of lateral roots; and total root size as a sum of main and lateral root lengths. Prior to statistical analysis, data were transformed (square root transformation for the number of lateral roots, natural logarithm transformation for all the other traits, while the main root angle values were not transformed). The differences between genotypes at each individual time point were assessed with ANOVA including individual plant as a random factor with a formula: lm(trait~Genotype+(1|Rep), data=x, REML=TRUE), where ‘Genotime’ indicates the combination of genotype and a time point (linem v.1.1.21 R package) followed with custom contrast comparison for two genotypes per time point with the formula: enmeans(model, specs=~Genotime, adjust=~sidak), ‘Genotime’ indicates the combination of genotype and a time point (enmeans v.1.5.2-1R package). Seven to ten seedlings were used per genotype as biological replicates.

Exudate collection and strigolactone quantification

Seeds were surface sterilized by agitating in a solution containing 4% (v/v) sodium hypochlorite and 0.2% Tween-20 (v/v) for 30 min followed by three alternated washes with 70% ethanol (v/v) and sterile water. The disinfected seeds were thoroughly rinsed five times with sterile water and germinated on wet Whatman paper (grade 1) upon incubation at 28 °C for 48 hours in the dark. Germinated seeds with approximately the same radicle size were transferred to a 50 ml tube filled with washed river sand and perforated at the bottom to allow draining. The Falcon tubes were covered with aluminum foil to prevent the roots from being exposed to light. The plants were grown for 14 days with a temperature of 28 °C during the day (11 hours) and 25 °C at night (13 hours), with 70% relative humidity and light intensity of 450 µmol m −2 s−1. Seedlings were watered with modified Hoagland solution with low phosphate content (0.05 mM KH₂PO₄, 10% of the standard Hoagland content) to induce strigolactone exudation. Root exudate was collected from six plants for each of the sorghum genotypes. Each tube was flushed with 5% ethanol (v/v) in water to collect 35 ml of the flow-through. Each exudate sample was purified using solid phase extraction (SPE) with C18 Discovery cartridges (bed wt 500 mg, volume 6 ml, Merck). Cartridges were activated using 5 ml acetonitrile and washed with 5 ml distilled water. Twenty milliliters of sample was loaded on the cartridge, which was further washed with 5 ml distilled water. Finally, compounds were eluted using 3 ml acetonitrile. The acetonitrile was evaporated using a SpeedVac (ScanVac, Labgene) and residual water was removed using freeze drying (Heto Powerdry LL1500, Thermo Fisher Scientific). The sample was reconstituted in 150 µl 25% (v/v) acetonitrile and filtered using a micropore filter (0.22 µm, 0.75 ml, Thermo Fisher Scientific) prior to UHPLC–tandem mass spectrometry analysis as described in (Floková et al., 2020). Values for each compound abundances were transformed with natural logarithm transformation. One-way ANOVA was used to determine the significance of the differences between genotypes with the following model: lm(trait~genotype) followed by a Tukey post hoc test (adjusted P-value <0.05) with agricolae v.1.3-1 and multcompView v.0.1-8 R packages.

Growth analysis

Germinated seeds with approximately the same radicle length were transferred to pots containing a custom potting mix (one part coarse sand, one part compost, one part peat and 2.24 kg m⁻³ dolomite lime) and placed in the greenhouse with a maintained temperature of 26 °C and day light of approximately 15 hours. For each individual plant, every second day, starting from day 4, the number of leaves was counted and plant height measured (from the soil to the bend of the oldest leaf). The time to reach vegetative growth stages as defined in Vanderlip and Reeves (1972) was
affected by the sorghum genotype, by time and in a time by
type, time and a genotype-by-time interaction; Supplementary
Tables S2–S4). First, we identified genes whose expression was
previously observed by Bellis
2017) compared with SQR (Supplementary Fig. S1), as
expression of several sorghum strigolactone biosynthetic genes
a greater extent than for SQR (Fig. 1A). We confirmed higher
expression in SRN39 in 28-day-old plants but higher expres-
sion in 35-day-old plants (cluster 2) were enriched in ‘defense
response’ and ‘response to auxin’. Upon further inspection of
the genes associated with these clusters, we found several genes
related to auxin synthesis and transport: *Sobic.007G191400*,
*Sobic.002G259100*, and *Sobic.004G156300* with hom-
ology to rice OsSAUR50 (SMALL AUXIN UP RNA 50),
OsSAUR72, and OsSAUR72-like, respectively (Supplementary
Table S5) which could be linked to changes in plant growth
and development. Genes involved in abscisic acid signaling,
*Sobic.003G354000* and *Sobic.002G172000*, both with hom-
ology to Arabidopsis HAI3 (HIGHLY ABA-INDUCED
PP2C PROTEIN, At2g29380, Supplementary Table S5) were
found among those influenced by a complex genotype-by-
time interaction (Fig. 1C, D). SRN39 is grown predominantly
in Sudan, often in non-irrigated fields. Thus, future research
should focus on assessment of SRN39 responses to ABA in
relation to drought responses, with consideration of plant age
in this regulation.

**SRN39 has altered gibberellin and fatty acid
biosynthesis relative to Shanqui Red**

The perturbations in expression of metabolism-related genes
that we observed in SRN39 roots prompted us to profile the
root metabolomes of SQR and SRN39 grown in the same
set-up as for the transcriptome analysis. Similar to the tran-
scriptome landscapes (Fig. 1A), the metabolite profiles of
28– and 35-day-old root of SQR and SRQ39 were clearly

**Results**

**SRN39 has altered expression of genes involved
in metabolism and stress responses relative to
Shanqui Red**

Shanqui Red (SQR) and SRN39 are sorghum varieties com-
only used as *Striga*-susceptible and -resistant models, respect-
ively. Their potential differences in whole plant strigolactone
composition as well as extensive genetic divergence suggest
that additional differences in SRN39 growth and development
should be characterized and considered in the interpretation of
experiments using SRN39 and SQR, as controls.

To gain insight into biological processes that may differ be-
tween these genotypes, we profiled the transcriptomes of 28-
and 35-day-old plants, the approximate age used for sorghum
strigolactone profiling (Gobena et al., 2017; Mohamed et al.,
2018). As anticipated, given the extensive genotypic differences
between these varieties (Gobena et al., 2017), multidimensional
scaling revealed a clear separation of transcriptional landscapes
between the two genotypes, as well as an effect of plant age (Fig.
1A). Plant age influences the transcriptome of SRN39 roots to
a greater extent than for SQR (Fig. 1A). We confirmed higher
expression of several sorghum strigolactone biosynthetic genes
in SRN39 compared with SQR (Supplementary Fig. S1), as
previously observed by Bellis et al. (2020). Given the clear role
of sorghum genotype and developmental age (time) in tran-
scriptome variation, we used an ANOVA to identify genes
whose expression differs depending on these factors (geno-
type, time and a genotype-by-time interaction; Supplementary
Tables S2–S4). First, we identified genes whose expression was
affected by the sorghum genotype, by time and in a time by
genotype interaction (FDR threshold 0.05; Supplementary
Fig. 1. Transcriptome profiles of roots of Shanqui Red (SQR) and SRN39. (A) Multidimensional scaling plot of samples based on their transcript abundances (log2 counts per million, CPM). Gray, SQR; orange, SRN39; circles, 28 d old; triangles, 35 d old. (B, C) Six clusters of genes affected by a
separated and plant age had a more profound effect on metabolites of SRN39 than SQR (Fig. 2A). The metabolic features differentially accumulated between genotypes at both plant ages assessed were enriched with gibberellin biosynthesis (Fig. 2B, C). Several metabolic features predicted to be gibberellin precursors (ent-kaur-16-en-19-oate, ent-7α-hydroxykaur-16-en-19-oate, gibberellin A12-aldehyde, and gibberellin A12) had a higher abundance in roots of SRN39 than in SQR (Fig. 2D–H).

While we observed enrichment of genes associated with fatty acid biosynthesis to be more highly expressed in roots of SRN39 than SQR in both 28- and 35-day-old plants, metabolites involved in poly-hydroxy fatty acid biosynthesis and suberin monomer biosynthesis were perturbed only in 28-day-old roots. We observed higher levels of metabolic features predicted to be ferulate and trans-cinnamate in roots of SRN39 at both plant ages assessed were enriched with ABA signaling. Collectively, our transcriptome and metabolome analysis demonstrate differences in hormonal balance between SQR and SRN39.

Differences in levels of gibberellin precursors and identification of complex gene expression interactions between 28- and 35-day-old SQR and SRN39 plants led to the hypothesis that complex differences in their plant growth may also be observed. Observation of the plants we sampled for transcriptome analysis suggest that such differences may occur. While most of the 28-day-old SQR and SRN39 plants had five leaves, almost half of the 35-day-old SRN39 plant progressed to the six-leaf stage, and two-thirds of SQR plants remained in the five-leaf stage (Supplementary Fig. S2B). The SRN39 plants were shorter than SQR at both plant ages assessed (Supplementary Fig. S2A).

**SRN39 root system architecture is distinct from Shanqui Red**

Given the extensive genotype-by-time interactions in root gene expression between these genotypes, we asked whether this results in different root system architectures between these genotypes. We compared the total root system length and area, mean root network diameter and dry weight of 28- and 35-day-old plants grown in the same experimental set-up as used for transcriptome and metabolite profiling. Since strigolactones also play a role in the growth of shoot-borne roots (Kohlen et al., 2012; Rasmussen et al., 2012), we also quantified the above-mentioned traits separately for crown roots (shoot-borne roots) and seminal roots (roots of embryonic origin). The mean root network diameter of 28-day-old SRN39 plants was smaller than that of SQR plants of the same age, whereas in the case of 35-day-old plants, SQR displayed a higher mean root network diameter (Supplementary Fig. S2F). No significant differences were observed in the length and area of seminal roots, crown roots, and total root system (Supplementary Fig. S2C–N). These subtle time-dependent differences in root system architecture resemble the differences in gene expression observed between SRN39 and SQR occurring between the 28th and 35th day of growth (Supplementary Fig. S2B; Fig. 1A–C). The dry weight of seminal roots, but not crown roots or total root system size, was lower in the case of SRN39 than in SQR, independent of plant age (Supplementary Fig. S2C, G, K).

We further characterized changes in root system development in SRN39 and SQR seedlings from the second to seventh day after germination without nutrient supplementation. The SQR main root length was shorter than SRN39 from the very first days after germination and this difference increased over time (Fig. 3A). The main root of SQR deviated more from the gravity vector than the main root of SRN39 (Fig. 3B). While lateral root density was lower in SRN39 than in SQR from the fifth day after germination onwards (Fig. 3C), lateral root length was greater in SRN39 from the third day after germination (Fig. 3D). Although the increase in lateral root length was stable across multiple experiments, the magnitude of the difference in main root length was more variable (Figs 3A, D, 4A, B). Our observations suggest, therefore, that SRN39 has a longer and steeper main root, and longer lateral roots, compared with SQR at the seedling stage. Together this contributes to a greater total root system size of SRN39 as compared with SQR (Fig. 3E).

**Sorghum genotypes producing orobanchol have increased lateral root growth**

To verify whether the observed increase in root system size of SRN39 could be associated with the LGS1 polymorphisms, or other genetic differences between SQR and SRN39, we evaluated the root system architecture of three other sorghum varieties: Birhan, Framida, and Gobiye. These genotypes carry a deletion of the same genomic region as SRN39, which spans five genes, including LGS1 (Gobena et al., 2017). SRN39, Birhan, Framida, and Gobiye all have the rare lgs1-1 allele (Bellis et al., 2020). The increased main root length was only found in Gobiye (Fig. 4A). All lgs1-1 mutant varieties had an increase in average lateral root length (Supplementary Fig. S4B), total lateral root length (Fig. 4B) and total root system length (Fig. 4C) relative to SQR. We also confirmed
Distinct growth of sorghum cultivars for \textit{Striga} research

that, similar to SRN39, Birhan, Framida, and Gobiye varieties exuded orobanchol as a main strigolactone (Fig. 4D; Supplementary Fig. S5). We suggest that the deletion shared by these four genotypes may be associated with the promotion of lateral root growth, but whether it is the consequence of the \textit{lgs1-1} mutation or other genetic components remains to be verified.

SRN39 is developmentally delayed, but accumulates more biomass

To evaluate whether the observed differences between SRN39 and SQR root transcriptomes and system architecture are concomitant with distinct growth and development of the aboveground tissue, we quantified SRN39 and SQR vegetative
growth in pots under standard greenhouse conditions over time. SRN39 plants were shorter than SQR, with the largest differences observed within the first 30 days of growth, after which the differences in plant height diminished, to increase again around day 70 (Fig. 5A). Most of the SRN39 plants did not reach the height of SQR at their respective boot stage (Fig. 5A). To determine whether the differences in height between SQR and SRN39 observed at the boot stage are caused by a decrease in growth specifically within early developmental stages we calculated the rate of height increase given individual plant height. SQR and SRN39 had a distinct growth rate for plants for heights up to 40 cm and for heights greater than 100 cm indicating that there are two phases of growth slowdown in SRN39 (Fig. 5B).

We next compared the time required for each genotype to reach specific stages of sorghum vegetative growth (Vanderlip and Reeves, 1972). Differences between SRN39 and SQR were observed at stage 1 (three-leaf stage), stage 4 (flag leaf visible) and stage 5 (boot stage). No differences were observed between SQR and SRN39 in the time required to reach stage 2 (five-leaf stage) and stage 3 (growing point differentiation) (Fig. 5C–G).

To verify whether the growth slow-down and developmental delay are coupled in SRN39, we compared the height of SRN39 and SQR relative to the emergence of each leaf. The differences in plant height between SRN39 and SQR were most prominent in the five-leaf stage (stage 2) and stage 3 (Fig. 5H). Given that there is no difference in the time to reach stage 2 and stage 3 between SQR and SRN39, a developmental delay cannot explain these final differences in plant height (Fig. 5D, E, H). Over the entire vegetative growth period monitored, most SRN39 plants developed a maximum of 13 leaves, while most SQR developed a maximum of 15 leaves (Fig. 5H). These collective data suggest that SRN39 is developmentally delayed at stage 1 only, while the later differences in growth between SRN39 and SQR are not due to a developmental delay.

We further assessed the biomass of the below-ground (leaves and stalk) tissue in SRN39 and SQR. Consistent with the observed increase in RSA of SRN39 compared with SQR, the dry weight of the SRN39 root system was higher than that of SQR (Fig. 5I). No differences were observed in the dry weight of the shoot tissue (leaves and stalk together) (Fig. 1J), while the biomass of leaves was higher in SRN39 compared with SQR (Fig. 5K). The root biomass to shoot biomass ratio was also higher in SRN39 plants compared with SQR (Fig. 5L).

To summarize, while differences in early and late stages of vegetative growth (height) are observed between SRN39 and SQR, these are due to a developmental delay only at stage 1. A developmental delay is also observed at stages 4 and 5 in SRN39 relative to SQR. (Fig. 5F, G). Despite being shorter and having fewer leaves (Fig. 5H), SRN39 allocates more biomass to its roots and leaves (Fig. 5I, K).

Discussion

Shanqui Red (SQR) is a Kaoliang-type sorghum variety of the bicolor race originating from China, while SRN39 is a released Sudanese variety of the caudatum race. SQR and SRN39 have been studied mostly in the context of their distinct levels of sensitivity to cold and Striga infection (Ejeta and Knoll, 2007). Despite their vast genetic diversity, the differences between these two genotypes have previously been attributed to a polymorphism in the LGS1 locus leading to changes in composition of their root exudates (Gobena et al., 2017). SRQ exudes 5-deoxyxstrigol as its dominant strigolactone, while SRN39 produces only orobanchol (Gobena et al., 2017) (Fig. 4D). 5-Deoxyxstrigol and orobanchol have distinct activity in terms of their stimulation of Striga germination. 5-Deoxyxstrigol is an efficient germination stimulant, while orobanchol only induces poor germination in most Striga seed populations (Gobena et al., 2017). Consequently, SRN39 shows resistance to Striga in the field as opposed to the highly susceptible SQR (Mohamed et al., 2003; Gobena et al., 2017).

Since the determination of the LGS1 contribution to Striga resistance, SQR, and SRN39 have been routinely used as model genotypes in Striga research (Mohamed et al., 2003; Gobena et al., 2017). However, developmental and molecular consequences of the LGS1 polymorphism as well as the extensive genetic divergence between SRN39 and SQR have never been addressed. Here we show that SRN39 has longer main and lateral roots, and greater overall root length than SQR in young seedlings (Figs 3, 5I). The enhanced growth of the main root and lateral roots in SRN39 seedlings was reflected in the higher total root biomass of mature plants, as compared with SQR (Figs 3A, D, 5I). The increased investment in root biomass accumulation might impair the growth of the above-ground plant organs (Lynch, 2007). We observed that both the growth and the development of SRN39 were affected when compared with SQR. SRN39 was delayed in reaching very early (stage 1, three-leaf stage), and late (stage 4, flag leaf visible; stage 5, boot stage) stages of vegetative growth and the growth rate (expressed as height) of its above-ground organs was also diminished at these stages (Fig. 5A–H). The increased investment in SRN39 root growth was observed at the early seedling stages and at the end of the vegetative growth, thus coinciding with the stages when the growth slow-down of the shoot was the largest and when developmental delay was observed: stage 1 (three-leaf stage), stage 4 (flag leaf visible), and stage 5 (boot stage) (Figs 3, 5B, C, F, G, I). The differences in root system architecture and root biomass were marginal in plants of five to six leaves (28- and 35-day-old plants), and thus at the stage where no developmental delay was observed for SRN39 plants and when its growth rate began to increase (Supplementary Fig. S2B–N; Fig. 5B, D). Together, these data suggest that the increased root growth of SRN39 is linked to both its developmental delay and reduced rate of above-ground growth. These differences between SRN39 and SQR could further be attributed to distinct molecular profiles.
Fig. 3. Root system architecture of Shanqui Red (SQR) and SRN39. In all cases the x-axis denotes days post-germination (dpg). (A–E) SRN39, orange; SQR, gray. (A) Main root length, (B) main root angle, (C) lateral root density, (D) lateral root length, (E) total root size. The boxplots denote data spanning the 25th to the 75th percentile and are centered on the data median. Circles represent individual values. Asterisks denote a significant P-value for each time point between genotypes by the least square method. *P < 0.05, **P < 0.01, ***P < 0.001 (n=10).

Fig. 4. Root system architecture and root exudate strigolactone composition of Shanqui Red (SQR) and sorghum varieties with the lgs1 mutation (Birham, Framida, Gobye, and SRN39). (A) Main root length, (B) lateral root length, (C) total root size of 7-day-old seedlings. The boxplots denote data spanning the 25th to the 75th percentile and are centered on the data median. Circles represent individual values. Asterisks denote a significant P-value for each time point between genotypes by the least square method. *P < 0.05, ***P < 0.001 (n=4). (D) Relative abundance (percentage of total strigolactones (SL) measured) of four strigolactones in root exudates of 14-day-old plants (n=6). The abundance of individual strigolactones is presented in Supplementary Fig. S5.

The complex relationship between shoot and root growth over time in SRN39 was also reflected in the root transcriptomic and metabolic profiles. The effect of plant age on both the transcriptome and metabolome landscape was more profound in SRN39 than SQR. (Figs 1A, 2A). Among the metabolic pathways perturbed in roots of SRN39 as compared with SQR,
Fig. 5. Vegetative growth of SRN39 and Shanqui Red (SQR). (A) Plant height from day 4 to 74. (B) Increase in plant height per plant height calculated with a sliding window of 30 cm. Data presented include the mean (solid line) and the 95% confidence interval (shaded). The bottom panel denotes the $-\log_{10}(P)$-value at each time point (Welch’s $t$-test). The dashed line indicates a $P$-value of 0.05. (C–G) Time required to reach stage 1 (three-leaf stage), stage 2 (five-leaf stage) (D), stage 3 (growing point differentiation) (E), stage 4 (flag leaf visible) (F), stage 5 (boot stage) (G). An asterisk denotes the $P$-value from a Wilcoxon test. (H) Plant height at individual leaf stages. The mean is represented by a solid line, while the 95% confidence interval is shaded. The bottom panel denotes the $-\log_{10}(P)$-value per leaf stage as a result of pairwise genotype comparisons using the least square method. (I–L) Dry weight of root (I), stalk (J), leaves (K), and ratio of root to shoot dry weight (L). Boxplots denote the span from the 25th to the 75th percentile and are centered to the data median. Circles represent individual values. Asterisk denotes $P$-value from Welch $t$-test. *$P<0.05$, **$P<0.01$, ***$P<0.001$ ($n=10$).
gibberellin biosynthesis was observed for 28- and 35-day-old plants (Fig. 2B, C). Several gibberellin precursors accumulated to higher levels in SRN39 as compared with SQR, which may be a reason why SRN39 plants are shorter than SQR (Fig. 2D–F). Recently Bellis et al. (2020) created a CRISPR–edited lgs1 knock-out of LGS1 in the Macia variety and profiled its shoot transcriptome. Two transcription factors involved in floral initiation, Sobic.010G180200 and Sobic.008G168400, were differentially expressed in the shoot of a CRISPR–edited knock-out of LGS1 as compared with the wild type, Macia (Bellis et al., 2020), which could explain the delay in reaching a boot stage by SRN39 (Fig. 5G). Together, these results suggest that the growth slow-down and developmental delay of SRN39 plants may be attributed to decreased biosynthesis of active gibberellins and an increase of its precursors and altered expression of transcription factors controlling floral initiation, respectively.

Genes we identified as differentially expressed between SQR and SRN39 roots were enriched with processes related to metabolism (‘carbohydrate metabolic process’, ‘metabolic process’, ‘fatty acid biosynthesis’) and responses to biotic stimuli (Fig. 1C, D). Similar processes were enriched among genes differentially expressed in the CRISPR–edited lgs1 knock-out and its wild type (Bellis et al., 2020). Genes related to fatty acid biosynthesis had higher expression in roots of SRN39 than in SQR (Fig. 1C, D, cluster 5). Consequently, among the metabolic pathways perturbed in SRN39 roots, we found poly-hydroxy fatty acid biosynthesis and suberin monomer biosynthesis (Fig. 2B, C). Suberin, a long chain fatty acid polymer, forms a protective barrier from pathogens (Thomas et al., 2007; Andersen et al., 2015; Holbein et al., 2019) and may serve as an additional barrier from Striga parasitism in SRN39. Indeed, polymorphisms in genes associated with suberin and wax-ester biosynthesis were recently associated with levels of Striga occurrence (Bellis et al., 2020), suggesting that, in addition to pre-attachment resistance, SRN39 may provide another layer of protection from Striga parasitism. Post-attachment resistance in SRN39 was previously suggested by Amusan et al. (2011). Genes involved in fatty acid biosynthesis were also enriched among genes differentially regulated in shoot tissue between the CRISPR–edited lgs1 knock-out of LGS1 and its wild type (Bellis et al., 2020). While SRN39 plants were shorter than SQR, they also accumulated more leaf biomass, which could be due to the increased production of waxes (Fig. 5A, K).

The increased expression of genes related to fatty acid biosynthesis in both SRN39 and the CRISPR–edited lgs1 knock-out leads to our hypothesis that strigolactones might affect suberin biosynthesis. More research is needed to confirm this hypothesis and elucidate whether it is a direct effect of strigolactone signaling or an indirect effect of their exudation, i.e. on nutrient uptake. We cannot conclude which of the additional phenotypic and molecular differences demonstrated here are related to potential differences in strigolactone biosynthesis or exudation or to the extensive genetic variation between SQR and SRN39. The three other sorghum varieties, Birhan, Framida, and Gobiye, with the same lgs1-1 allele as SRN39 and with orobanchol as the dominant exuded strigolactone, also showed longer lateral roots and larger root systems. Again, the mechanism underlying these differences remains to be determined. SRN39 is the parent of Birhan and Gobiye and therefore it is likely these two genotypes share a substantial part of the SRN39 genome (Hess and Ejeta, 1992). Nevertheless, our root system architecture phenotyping results suggest that these orobanchol-producing genotypes have a greater potential to promote lateral root growth than those producing 5-deoxystrigol. Previously, a synthetic strigolactone, GR24, has been shown to repress lateral root formation under phosphate–sufficient conditions, while enhancing it under conditions of low phosphate availability (Ruyter-Spira et al., 2011). Here we suggest that, in the absence of nutrients, strigolactones might be also involved in the elongation of lateral roots. While more research is needed, we speculate that different stereoisomers of strigolactones may have distinct potential in shaping the sorghum root system.

SRN39 not only has increased resistance to Striga, but it is also Striga tolerant, meaning it has a reduced number of Striga infections and that successful Striga attachment causes minimal damage (Rodenburg et al., 2005; Nasreldin, 2018). Striga tolerance is often measured as the degree of decrease in yield, photosynthesis efficiency, or above-ground tissue biomass (Van Ast et al., 2000; Rodenburg et al., 2008). We observed the dynamic character of the growth slow-down in SRN39, increases in leaf, but not whole shoot biomass and complex gene expression patterns over time that distinguish it from the Striga-sensitive SQR (Figs 1A–C, 5A, B, J, K). This suggests that accounting for the developmental stage and tissue used to access sorghum biomass might be important for assessing the tolerance of sorghum varieties with previously reported resistance to Striga.

Supplementary data
The following supplementary data are available at JXB online. Fig. S1. Expression of strigolactone biosynthesis and signaling pathway genes in roots of 28- and 35-day-old plants of Shanqui Red (SQR) and SRN39. Fig. S2. Phenotypic characterization of 28- and 35-day-old plants of Shanqui Red (SQR) and SRN39. Fig. S3. Root system architecture of Shanqui Red (SQR) and SRN39. Fig. S4. Root system architecture of 7-day-old seedlings of Shanqui Red (SQR) and sorghum varieties with the lgs1 mutation (Birham, Framida, Gobiye, and SRN39). Fig. S5. Strigolactone composition in root exudates of Shanqui Red (SQR) and sorghum varieties with the lgs1 mutation (Birham, Framida, Gobiye, and SRN39).

Table S1. CPM values for root transcriptome profiling. Table S2. Genes detected as differentially expressed between genotypes.
Table S3. Genes detected as differentially expressed between time points.
Table S4. Genes detected as differentially expressed between genotypes in a time-dependent manner.
Table S5. Genes assigned to expression clusters.
Table S6. GO terms enriched in each expression cluster.
Table S7. Intensity values for root metabolite profiling.
Table S8. Abundance of metabolic features of selected enriched categories in roots of 28-day-old plants.
Table S9. Abundance of metabolic features of selected enriched categories in roots of 35-day-old plants.

**Data availability**

Sorghum sequences were deposited in NCBI GEO under the accession number GSE167101. Raw data from the growth measurements, root system architecture analysis, strigolactone measurements, ANOVA tables and P-values for each statistical test can be found in Supplementary Dataset S1. Data analysis scripts are publicly available at https://github.com/DorotaKawa/Sorghum-growth-development.git.

**References**


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