Mutation in sorghum *LOW GERMINATION STIMULANT 1* alters strigolactones and causes *Striga* resistance

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***Striga* is a major biotic constraint to sorghum production in semiarid tropical Africa and Asia. Genetic resistance to this parasitic weed is the most economically feasible control measure. Mutant alleles at the *LGS1* (*LOW GERMINATION STIMULANT 1*) locus drastically reduce *Striga* germination stimulant activity. We provide evidence that the responsible gene at *LGS1* codes for an enzyme annotated as a sulfotransferase and show that functional loss of this gene results in a change of the dominant strigolactone (SL) in root exudates from 5-deoxystrigol, a highly active *Striga* germination stimulant, to orobanchol, an SL with opposite stereochemistry. Orobanchol, although not previously reported in sorghum, functions in the multiple SL roles required for normal growth and environmental responsiveness but does not stimulate germination of *Striga*. This work describes the identification of a gene regulating *Striga* resistance and the underlying protective chemistry resulting from mutation.**

Striga | strigolactone | gene | sorghum | stereochemistry

Infestation by the parasitic weed *Striga* (*Striga asiatica* and *Striga hermonthica*) is a serious constraint to the production of sorghum (*Sorghum bicolor*), a staple cereal crop grown widely across sub-Saharan Africa and the Indian subcontinent. Global estimates of *Striga*'s human toll are lacking. An earlier report by the Food and Agriculture Organization of the United Nations focused on West Africa estimated that the livelihoods of 300 million people were negatively affected by the pest (1). Conservative extrapolation from a recent report on losses to *Striga* in rice (2) puts the economic impact on cereal production in sub-Saharan Africa at $1.2 billion annually with losses increasing by $177 million per year. Most of these losses are borne by subsistence farmers (3). Genetic resistance to this pest through low *Striga* germination stimulant activity provides control and permits economic production of this crop (4). Because it is an obligate root parasite, *Striga* seed will not germinate unless it receives a chemical cue from a potential host plant (5). Among chemicals identified in sorghum root exudates with *Striga* germination stimulant activity, the most potent are the strigolactones (SLs), a class of related compounds used by most terrestrial plants as hormones to regulate shoot (6) and root (7) branching. Their presence in root exudates is critical to symbiotic colonization by arbuscular mycorrhizal (AM) fungi (8). Associations with AM fungi greatly improve the performance of sorghum under nutrient and water deficits (9). *Striga* seems to have taken advantage of this signaling to detect its proximity to sorghum roots, germinating at the proper time and place to increase its chances of completing its life cycle on this preferred host. Sorghum produces several SLs and exudes them from its roots, particularly under conditions of limited phosphate and nitrogen, probably in attempt to promote mycorrhizal association (10). Among the SLs reported to be present in sorghum root exudates are sorgolactone, strigol, 5-deoxystrigol, and sorgomol (10–13) (Fig. 1). These compounds differ from each other by various substitutions on the A and B rings but share a common stereochemistry with respect to the β-orientation of their C rings (14). *Striga* is quite sensitive to these SLs, able to germinate at concentrations as low as 10^{-11}M (15), depending on the particular SL (16).

To facilitate the identification and characterization of resistance to *Striga*, our laboratory developed bioassays that allow observations of the parasitic association at its earliest stages, normally hidden below ground. Among these is the agar gel assay wherein the *Striga* germination stimulant activity of sorghum accessions can be quantified based on the distance between the sorghum root and germinating *Striga* seed in agar (17). This useful assay has resulted in the development and release of several *Striga*-resistant sorghum varieties with low germination stimulant activity (18). Although not all sorghum lines showing field resistance to *Striga* had low *Striga* germination stimulant activity, all low-stimulant sorghums that were field-tested showed *Striga* resistance (18). Low *Striga* germination stimulant activity has been an important resistance trait in sorghum improvement but less so in other crop hosts of *Striga* such as maize, millet, and rice (4). Genetic studies have shown that inheritance of low *Striga* germination stimulant activity in sorghum is through a mutant allele (*lgs*) expressed in homozygous recessive individuals (19). The *Striga*-resistant sorghum variety SRN39 carrying this mutation was mated with a Chinese landrace Shanqui Red, with high germination stimulant activity, to generate a genetic mapping population of 600 recombinant inbred lines (RILs). In a previous genotypic and phenotypic evaluation of 328 RILs by the agar gel assay we created a genetic map with 428 markers, placing the *LGS1* (*LOW GERMINATION STIMULANT 1*) locus in a region near the tip of chromosome 5 with fine mapping that delimited it to a 30-gene region (20).

Significance

The parasitic weed *Striga* is the greatest biological constraint to production of many crops in Africa and parts of Asia. Genetic control is the most feasible means of crop protection from this pest. We report on identification of a gene regulating *Striga* resistance in sorghum and the associated change in strigolactone chemistry. Knowing this gene and its various natural alleles, sorghum breeders can design markers within it to facilitate its transfer into improved varieties providing farmers effective control of *Striga* in infested fields. The gene could also be used to potentially improve *Striga* resistance through genome editing in crops such as maize that evolved away from *Striga*, and hence have a paucity of *Striga* resistance genes.

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Data deposition: Sequence reads from the *LGS1* region of Shanqui Red, SRN39, 555, IS7777, and Tetron have been deposited with the National Center for Biotechnology Information Short Read Archive (accession no. SRP098704).

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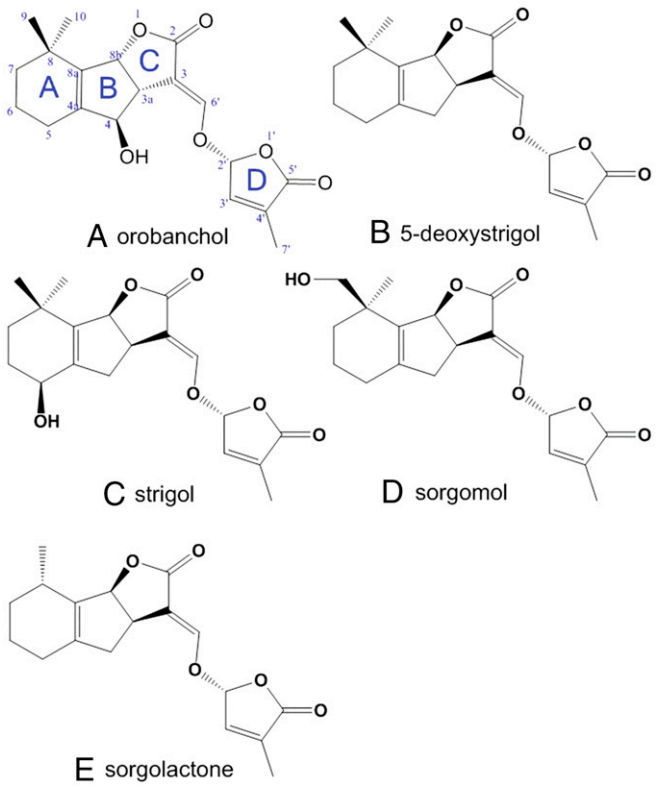

Fig. 1. Chemical structures of SLs found in sorghum root exudates. Orobanchol (*A*) has not been previously reported in sorghum. Note the enantiomeric orientation of its C-ring (α-orientation) with respect to the other SLs (β-orientation), 5-deoxystrigol (*B*), strigol (*C*), sorgomol (*D*), and sorgolactone (*E*), previously reported in sorghum root exudates.

Much has been learned over the past decade about biosynthesis of SLs, particularly since their roles as growth regulators were discovered. The SLs are derived from β-carotene through a series of isomerization, cleavage, oxidation, and cyclization steps to form the four distinctive rings of the SLs (21, 22). Four enzymes have been identified to be involved in these steps: DWARF27 (D27), a carotenoid isomerase that converts all-*trans*-β-carotene to 9-*cis*-β-carotene that can be cleaved by CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7) to form 9-*cis*-β-apo-10′-carotenal, which is converted by CAROTENOID CLEAVAGE DIOXYGENASE 8 (CCD8) to carlactone, which contains the A- and D-rings and is, in rice, subsequently oxidized by an ortholog of the *Arabidopsis* MORE AXILLARY BRANCHES 1 (MAX1), to the first canonical rice SL, 4-deoxyorobanchol (*ent*-2′-*epi*-5-deoxystrigol) (22). Mutant alleles at these loci were identified by plant growth phenotypes that affected shoot branching in model species. Less is known about the later steps of SL biosynthesis, particularly how the additions and/or modifications to functional groups on the member rings occurs. It has been assumed that 5-deoxystrigol is the proto-SL for the strigol-type SLs, having a β-oriented C-ring, and 4-deoxyorobanchol for the orobanchol-type SLs, with the C-ring in α-orientation (22, 23). Both groups have the D-ring in *R* configuration around the chiral center at C-2′ (21). A major SL in rice root exudates is orobanchol (Fig. 1*A*), and all other SLs present in this species share the same stereochemistry with respect to the spatial orientation of the C-ring (14, 24). Other plants, including tobacco (*Nicotiana tabaccum*), exude both types of SLs (14). For sorghum and many other plant species from which SLs have been described, the stereochemistries of their SLs have not always been determined. However, all SLs reported in the root exudates of sorghum (12), including 5-deoxystrigol, strigol, sorgomol, and sorgolactone, are of the strigol type (Fig. 1).

Because mutation at *LGS1* causes a change in *Striga* germination stimulant activity, but without obvious changes to sorghum shoot architecture, we made quantitative and qualitative comparisons of SLs in the root exudates of mutant and WT lines.

Results

***Striga* Germination Stimulation and SLs.** Diverse lines were classified for *Striga* resistance, based on the germination distance of *Striga* embedded in agar from the sorghum root as having high maximum germination distance (MGD ≥10 mm; Shanqui Red, Fig. 2*A*) or low (MGD <10 mm; SRN39, 555, IS7777, SC103, and Tetron) *Striga* germination stimulant activity with four *Striga* sources (Table 1). Low-stimulant genotype SRN39 (Fig. 2*B*), when crossed with high-germination stimulant lines, always result in F_1 hybrids with high *Striga* germination stimulant activity, affirming the recessive nature of the *lgs1* mutation. Complementation tests between SRN39 and all of the low germination stimulant lines in this study indicate that they all carry mutations at a common locus because no complementation occurs in their hybrids, that is, all such hybrid plants produce low *Striga* germination stimulant responses (Table 1). The difference in resistance between low- and high-stimulant varieties is also apparent when lines are cultivated under *Striga* infestation. SRN39 and its derivatives determined in the agar assay to have low *Striga* germination stimulant activity also support fewer parasites in field plots (Fig. 2*C*).

SL profiles of root exudates from *lgs1* variants consistently display reduced 5-deoxystrigol and enhanced orobanchol levels relative to WT *LGS1* root exudates (Fig. 3). Comparison of retention times and mass transitions of dominant SLs in sorghum root exudates with standards of known stereochemistry confirmed the

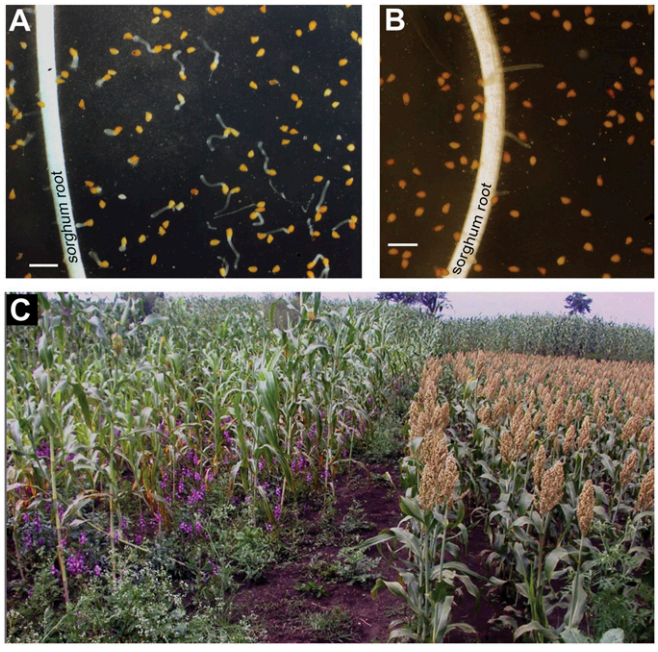

Fig. 2. *Striga* resistance phenotypes of *LGS1* variants. Sorghum seedlings with high *Striga* germination stimulant activity (*A*) will germinate conditioned *S. asiatica* seeds cocultured in agar, a centimeter or more from its root as the germination stimulant, 5-deoxystrigol, diffuses through the medium. Low-stimulant sorghum that exudes orobanchol instead of 5-deoxystrigol will not cause *S. asiatica* seeds to germinate in the agar gel assay, even very near its roots (*B*). (Scale bars, 1 mm.) The photograph (*C*) shows an *LGS1* WT high-stimulant sorghum (left) growing next to a line (right) carrying the *lgs1-1* allele in a field infested with *S. hermonthica* (purple flowers) in Ethiopia.

Table 1. Measures of the *Striga* germination stimulant activity of sorghum lines and hybrids used for genetic mapping of *LGS1*

Sorghum line or hybrid	*S. asiatica* (Derashe, Ethiopia)	*S. asiatica* (North Carolina)	*S. hermonthica* (Samanko, Mali)	*S. hermonthica* (Sinnar, Sudan)
Shanqui Red	15.5 ± 5.0	19.8 ± 3.0	19.6 ± 5.2	21.2 ± 3.6
SRN39	0 ± 0	1.9 ± 1.8	0.1 ± 0.2	0.1 ± 0.2
555	0 ± 0	0.5 ± 0.5	1.3 ± 2.2	0 ± 0
IS7777	0 ± 0	0 ± 0	3.8 ± 1.7	2.1 ± 2.1
Tetron	1.2 ± 2.7	4.3 ± 1.5	1.4 ± 1.9	2.5 ± 3.9
SC103				3.4 ± 2.7
(SRN39 × Shanqui Red)F_1				10.9 ± 2.3
(SRN39 × 555)F_1				0.2 ± 0.3
(SRN39 × IS7777)F_1				6.6 ± 2.8
(SRN39 × Tetron)F_1				7.1 ± 2.6
(SRN39 × SC103)F_1				3.1 ± 2.5

MGD (millimeters) as measured in the agar gel assay (discussed in the text); MGD >10 mm indicates high *Striga* germination stimulant activity; MGD <10 mm indicates low *Striga* germination stimulant activity. Values are means of measures from three plates ± one SD.

β-orientation of the C-ring in 5-deoxystrigol of lines carrying *LGS1* and α-orientation in orobanchol of those with *lgs1* (Fig. 4). RILs with low germination stimulant activity have inherited the low 5-deoxystrigol/high orobanchol profile, whereas those with high germination stimulant activity always contain a threshold level of 5-deoxystrigol and do not accumulate orobanchol, confirming the identity of the gene and the link to this profile (Table S1).

Because SLs serve other functions contributing to crop productivity (6–9), selecting for mutations that knock out SL production may have undesirable outcomes such as excessive shoot branching or impairment of mycorrhization. Sorghum lines examined in this study carrying *lgs1* alleles all had similar SL exudation rates, typically around 2,000 pmol per plant over the 48-h collection period. Although the stereochemistry of the major SL in these exudates profoundly affected *Striga* germination stimulant activity, other SL functions seem to be unchanged by the mutation. Adult SRN39 plants on average have the same number of basal tillers (one) as Shanqui Red at 0.5-m spacing in a field row. The two lines also do not greatly differ in the degree to which their roots are colonized by three AM fungal species, *Rhizophagus intraradices*, *Rhizophagus clarus*, and *Rhizophagus custos*, alone or in combination (Fig. S1). Mutation at *LGS1* results in both quantitative and qualitative changes in SL content of root exudates, effectively lowering *Striga* germination stimulant activity without negative productivity side effects.

A search for polymorphisms in PCR products between the parents of the RILs contrasting for *Striga* germination stimulant activity, Shanqui Red and SRN39, allowed genotyping with eight new markers (Table S2) to refine the position of *LGS1* on the sorghum genetic map. Polymorphisms resulting in PCR product size differences were scored by gel electrophoresis. Most (95%) polymorphic markers in the region cosegregated with the respective trait (RILs with Shanqui Red alleles had high *Striga* germination stimulant activity, whereas those with SRN39 alleles had low germination stimulant activity). The informative recombinants allowed us to rule out several gene candidates.

For a cluster of candidate genes from position 69,977,147–70,011,172 on the sorghum chromosome 5 physical map (Phytozome, Sorghum bicolor v3.1, DOE-JGI), a PCR product could not be obtained from SRN39, so the five genes predicted in this region (*Sobic.005G213500* to *Sobic.005G213832*) could not be scored, except as a presence/absence polymorphism. Whole-genome sequencing of the parents revealed that this five-gene region is deleted in SRN39 (Fig. 5 and Table S3). The allele carried by SRN39 is given the designation *lgs1-1*. We also sequenced whole genomes of several unrelated low *Striga* germination stimulant lines in our collection. Examining genomic sequence of this region from these natural variants determined to be allelic to SRN39, we found that the allele in 555, *lgs1-2* also has a large deletion here, but slightly shifted away from the chromosome tip, spanning the position 69,958,403–69,986,951, and therefore missing three predicted genes, *Sobic.005G213400*, *Sobic.005G213500*, and *Sobic.005G213600*. Its deletion overlapped with that of SRN39 for two genes, *Sobic.005G213500* (*Sb05g026540*), coding for an uncharacterized protein with a functional domain similar to an iron/ascorbate oxidoreductase, and *Sobic.005G213600* (*Sb05g026550*), whose uncharacterized product is predicted to have a sulfotransferase domain. A third allele, *lgs1-3*, with overlapping deletion occurs in IS7777, at Chr05:69,981,523..70,011,172, resulting in a loss of four genes, *Sobic.005G213600*, *Sobic.005G213700*, *Sobic.005G213766*, and *Sobic.005G213832*. The common deleted gene for all these alleles

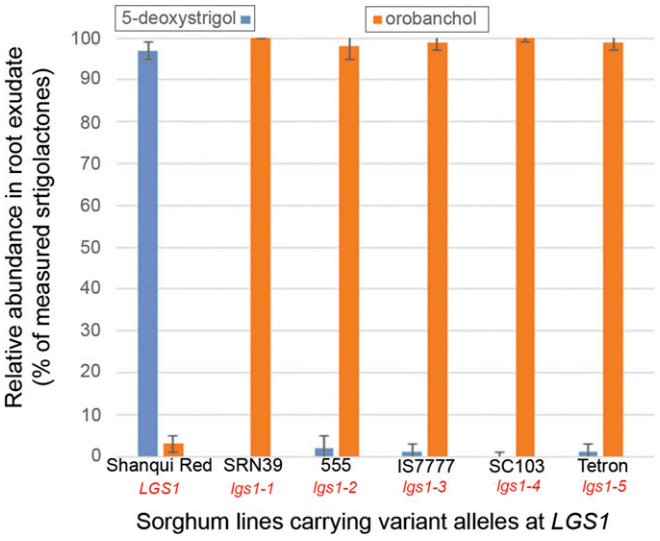

Fig. 3. Chemical phenotypes of *LGS1* variants. SL profiles of root exudates from sorghum Shanqui Red (*LGS1*) with high *Striga* germination stimulant activity, and of five low-stimulant lines with mutant alleles at SRN39 (*lgs1-1*), 555 (*lgs1-2*), IS7777 (*lgs1-3*), SC103 (*lgs1-4*), and Tetron (*lgs1-5*) are shown. Specific SL quantifications are expressed in relative abundance (percent of total measured SLs) in each exudate. Although the absolute amount of the most abundant SL varies from run to run, typical values for 5-deoxystrigol in Shanqui Red or orobanchol in SRN39 are around 2,000 pmol per plant per 48 h. Values are averages of four measures from independent runs ± one SD.

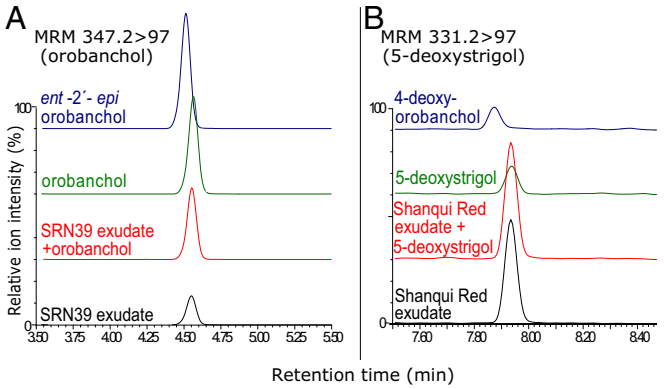

Fig. 4. UPLC-MS-MS determination of identity of major SLs in SRN39 (low *Striga* germination stimulant activity) and Shanqui Red (high *Striga* germination stimulant activity). Channels in the chromatograms monitor the mass transitions associated with loss of the D-ring (*m/z* 97) as SLs come off the UPLC column. The major SL in SRN39 root exudate (*A*) coelutes with authentic orobanchol, not its enantiomer, *ent-2′-epi*-orobanchol. The more typical sorghum SL with a β-orientation, 5-deoxystrigol, is the major one in Shanqui Red root exudate (*B*). This authentic standard is resolved from the α-oriented enantiomer, 4-deoxyorobanchol.

is *Sobic.005G213600*, which codes for the sulfotransferase. Sulfotransferases catalyze the transfer of a sulfate group from the universal donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to a hydroxyl or amide group of its substrate (25). Several PCR primer pairs designed based on the reference genome sequence to amplify portions of *Sobic.005G213600* were used to test a diverse collection of sorghum lines with high and low *Striga* germination stimulant activity. An amplicon was always present for the target locus among accessions with high *Striga* germination stimulant activity but missing in accessions with low *Striga* germination stimulant activity determined to be allelic to SRN39.

An exception to this association was observed in the allelic low-germination stimulant lines, SC103 and Tetron, in which at least some amplicons were obtained from PCR primers targeting this gene. Examining the genomic sequence from *Sobic.005G213600* from these two accessions revealed deletions within the predicted coding region that cause frameshift mutations (Fig. 5, Table S3, and Fig. S2). The more obvious mutation is in SC103, which contains an allele, *lgs1-4*, having a 421-bp deletion in the second exon. This deletion not only results in a 137-aa residue loss in the predicted protein but also introduces a stop codon 46 residues downstream such that the resulting gene product, if it were translated, would be a protein 244 residues shorter than the WT protein. Tetron contains an allele, *lgs1-5*, with a 10-bp deletion 18 bp upstream of the deleted area of SC103 in the second exon, causing a frameshift that would introduce a stop codon after 39 aberrant residues beyond the deletion. A translated product of this mutant allele would therefore be missing 259 residues relative to the WT gene product (Fig. S2). Both of these mutations occur within the annotated sulfotransferase domain of the gene (residues 138–439). The one in Tetron destroys the 5′ PAPS binding motif (PKSGTTW, Fig. S2) highly conserved in all sulfotransferases (26). The conserved PAPS binding residues near the end of the protein (FRKGKVGDWKNYMTPDM) would be missing in both mutant peptides (Fig. S2). Therefore, all described *lgs1* alleles would lack a functional sulfotransferase product from *Sobic.005G213600*.

Expression of *Sobic.005G213600*. Publicly available expression profiles of *Sobic.005G231600* based on ESTs from the sorghum reference, BTx623, from the Morokoshi Sorghum Transcriptome Database (sorghum.riken.jp/morokoshi/Data/Sobic.005G213600) and in the expression track of Phytozome Sorghum bicolor v3.1 (DOE-JGI) indicate that this gene is preferentially expressed in roots and under nitrogen deficiency, two qualities one would expect for genes involved in SL biosynthesis. We monitored the expression of this gene in Shanqui Red by qRT-PCR and confirmed that expression was significantly greater in roots versus shoots (Fig. 6). When seedlings of Shanqui Red were grown in sand for 1 month irrigated with tap water and compared with seedlings irrigated with nutrient solution (12:2:31) in a potting mix (peat and perlite), expression of this gene was approximately fivefold higher under the nutrient-leached conditions. *LGS1* expression was significantly reduced in Tetron relative to Shanqui Red in sand (Fig. 6). The qRT-PCR primers targeting the transcript were nested in the 3′-UTR (Table S2). As expected for a completely deleted gene, no expression of this target was observed in SRN39 in either medium. The severe deletion in SC103 also knocked out expression of this gene.

Motifs identified in silico using the PLACE database search tool (27) of the presumed promoter region of *Sobic.005G213600* (Fig. S3) show some *cis*-acting regulatory elements (CAREs) that match other genes involved in SL biosynthesis, including root-specificity, drought, phytohormone, and nutrient deficiency responsive elements, including a phosphate deficiency response, P1BS. Most CAREs listed in Fig. S3 fall within a few hundred base pairs of the transcription start site, in the presumed core promoter.

Discussion

Mutation at *LGS1* does not eliminate SL biosynthesis, but rather changes the type of SLs present in the root exudates. In a comparison of all possible stereoisomers of the SLs previously reported in sorghum root exudates, strigol, sorgolactone, sorgomol, and 5-deoxystrigol, it was shown that *S. hermonthica* germination was much higher when exposed to these SLs in their natural (β-oriented C-ring) form than when treated with their α-oriented enantiomers (16). Furthermore, Yoneyama et al. (28) predicted that SLs containing a hydroxyl group directly on the A- (e.g., strigol) or B-ring (e.g., orobanchol) would be prone to ring-destroying nucleophilic attack and therefore be less persistent in the soil. Together, these results explain why orobanchol-exuding sorghums, like the *lgs1* mutants, would show low *Striga* germination stimulant activity in our laboratory agar assays, as well as when planted in farm fields infested with *Striga*.

We have presented compelling genetic evidence in the form of multiple mutant alleles at this locus that *LGS1* is *Sobic.005G213600*, an uncharacterized gene with a sulfotransferase domain. Unfortunately, the substrates of sulfotransferases other than a few in *Arabidopsis* are largely unknown and cannot be accurately predicted by in silico modeling based on animal enzyme structures (25). Plant sulfotransferases resemble their better-studied counterparts in animals by the conserved motifs involved in binding PAPS, the universal donor of the sulfate group in the reactions that they catalyze (26). They sulfate a variety of substrates and are generally divided into two main classes: membrane-associated and cytosolic sulfotransferases. Only three of the former have been described in plants (*Arabidopsis*), all sulfating tyrosine residues in relatively small secreted peptides with growth-regulating activities, one that in turn stabilizes transcription factors (29). The larger class of cytosolic plant sulfotransferases sulfate low-molecular-weight substrates including flavonoids, coumarins and phytohormones such as brassinosteroids, salicylic acid, and jasmonates (25).

The *lgs1* mutants preferentially make orobanchol, with an α-oriented C-ring over the common WT SL for sorghum, 5-deoxystrigol, lacking the hydroxyl group at position 4 and having a β-oriented C-ring. The biosynthesis of SLs from carotenoids through carlactone continues to be elucidated in model plants such as rice and likely involves hydroxylation of C-18 and carboxylation at C-19 (22, 23, 30). The orientation of the C-ring with respect to the B-ring must be determined when these rings form during the cyclization that follows oxidation by the sorghum MAX1 ortholog(s). We therefore assume that the sulfotransferase is involved in stereo-control of ring

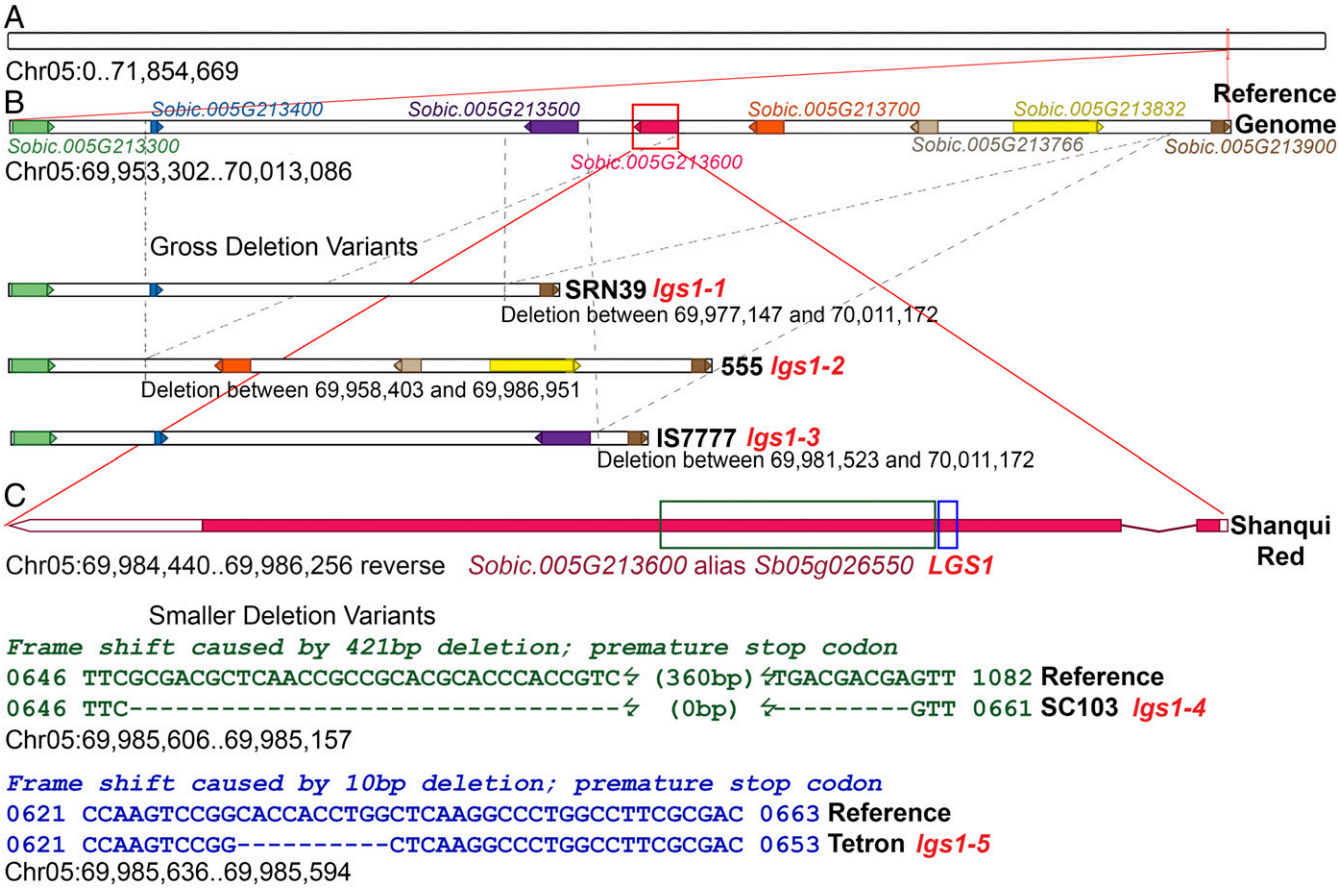

Fig. 5. Schematic representation of the *LGS1* locus and its identity based on mutant analysis. (*A*) Genetic mapping (20) indicated that *LGS1* was near the tip of sorghum chromosome 5. (*B*) Fine mapping based on sequence polymorphisms indicated that the low *Striga* germination stimulant activity was always associated with a deleted region representing a five-gene loss in the low-stimulant parent of the mapping population, SRN39 (carrying allele *lgs1-1*). Comparing this region to other lines with low *Striga* germination stimulant activity determined to be allelic to SRN39, two other gross deletion variants were discovered with overlapping deletions, 555 (carrying allele *lgs1-2*) and IS7777 (carrying allele *lgs1-3*). The common deletion in these three is *Sobic.005G213600*. Further evidence comes from smaller deletion variants of this gene (*C*) in SC103 (*lgs1-4*), missing 421 bp in the second exon, and a 10-bp deletion near there in Tetron (*lgs1-5*), both predicted to cause frameshifts and severely truncated peptides without sulfotransferase function.

closure, perhaps by posttranslationally modifying proteins at the site where this occurs in such a way that it favors closure to β-orientation. Alternatively, the sulfotransferase may regulate, through sulfated phytohormone intermediates, which MAX1 ortholog or other enzymes metabolize carlactone, influencing the degree to which carlactone is oxidized and the catalytic environment in which its oxidized intermediate cyclizes to an SL. As some sulfotransferases do to other phytohormones, LGS1 might even sulfate the SL itself. The hydroxyl of orobanchol, perhaps formed at low levels in sorghum by an alternative pathway, could be sulfated and drive the production of 5-deoxystrigol, whereas accumulation of its unsulfated form, as occurs in *lgs1* mutants, suppresses it. The mutant alleles described at *LGS1* will be useful for further biochemical studies on how stereochemistry of SLs is

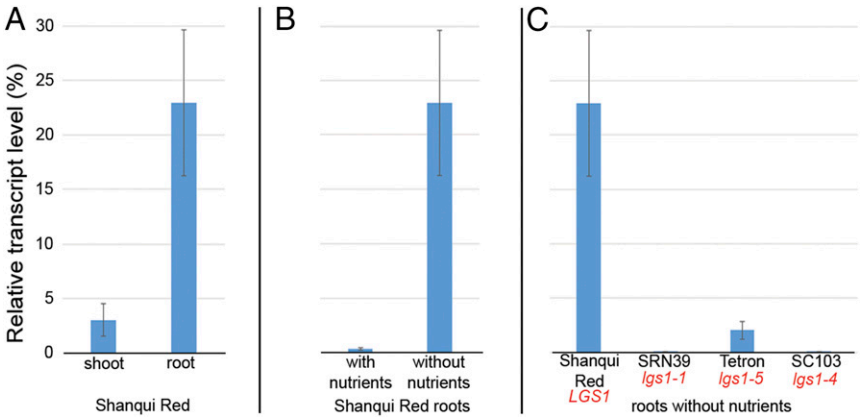

Fig. 6. Expression of sorghum *LGS1*. Expression of this gene is at least fivefold higher in roots than in shoots of Shanqui Red, carrying the WT allele *LGS1* (*A*). RNA was extracted from 4-wk-old seedlings grown in sand without supplemental nutrients. Expression of this gene in roots under these conditions was greatly reduced compared with seedlings of the same age grown in potting mix and irrigated with nutrient solution (*B*). Little or no expression was observed in seedling roots grown in nutrient-leached sand of mutants missing all or part of this gene (*C*). Transcript levels were monitored by qRT-PCR comparing to actin (see *Materials and Methods* for details). Values are averaged from three technical and three biological replicates ± one SD.

determined or favored and help to establish precursor and product relationships among the various types of SLs in sorghum and other plant species.

Striga resistance based on low germination stimulant activity has been long known and successfully exploited in sorghum (17, 18) and improved varieties carrying this trait continue to show resistance to *Striga* populations from both East and West Africa (31). Its simple inheritance (19), particularly with molecular markers within the *LGS1* locus, make it relatively simple to introgress into existing cultivars. Mutation at *LGS1* does not knock out SLs in root exudates; it just changes the relative abundance of certain types, such that the other essential functions of SLs (ability for mycorrhizal colonization, favorable tillering, and root responsiveness to nutritional deficiencies) remain intact. Protection against *Striga* seems to be based on lack of responsiveness to orobanchol of those strains of the weed that parasitize sorghum and the loss of the known chemical cue 5-deoxystrigol. It should be noted, however, that rice, also parasitized by *Striga*, exudes orobanchol-type SLs from its roots (24). The nature of the protection offered by mutation at *LGS1* might be extended to other cereal hosts of *Striga* for which resistance breeding lags behind, such as maize, which exudes a variety of noncanonical SLs (32), among which several of the strigol type have been reported (33).

Materials and Methods

Informative recombinants from the RIL population used to fine-map the *LGS1* locus (20) were further genotyped and their SLs phenotyped by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS-MS) with comparison with standards as previously described (32). In addition to these, four low-stimulant lines (555, IS7777, SC103, and Tetron) with reported *Striga* field resistance and their hybrids with SRN39 were used to establish allelic relationships of *lgs1* mutants and verify the identity of *LGS1* from among the gene candidates. The genotyping ultimately required Illumina sequencing of Shanqui Red, SRN39, 555, IS7777, and Tetron to an average depth of 10–27×. The raw reads from whole genome sequence for SC103 were downloaded from the National Center for Biotechnology Information (NCBI) short read archive (SRA). Sequence reads from the *LGS1* region of Shanqui Red, SRN39, 555, IS7777, and Tetron have been deposited with NCBI-SRA under study accession no. SRP098704. *Striga* germination stimulant activity of the sorghum lines and hybrids were determined by the agar gel assay (17) using four sources of *S. asiatica* and *S. hermonthica*. Shoot branching of field-grown plants and mycorrhization of controlled-environment potted seedlings of Shanqui Red and SRN39 inoculated with three AM fungal species were compared for effects of variation at *LGS1* on these phenotypes. Expression of *LGS1* was monitored in seedlings of Shanqui Red, SRN39, Tetron, and SC103 grown for 4 wk in either sand without nutrients or potting mix to which nutrients were provided in the irrigation water. RNA was extracted separately from roots and shoots. Quantitative PCR was done with three technical and biological replicates comparing *LGS1* with actin transcripts to determine their relative levels. Full details of all protocols used in this study are provided in *SI Materials and Methods*.

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