Theor Appl Genet (2012) 124:989–1003
DOI 10.1007/s00122-011-1763-9

ORIGINAL PAPER

# Molecular tagging and validation of microsatellite markers linked to the low germination stimulant gene (*lgs*) for *Striga* resistance in sorghum [*Sorghum bicolor* (L.) Moench]

**Kanuganti Satish · Zenbaba Gutema · Cécile Grenier · Patrick J. Rich · Gebisa Ejeta**

Received: 8 September 2011 / Accepted: 23 November 2011 / Published online: 13 December 2011


**Abstract** *Striga* is a devastating parasitic weed in Africa and parts of Asia. Low *Striga* germination stimulant activity, a well-known resistance mechanism in sorghum, is controlled by a single recessive gene (*lgs*). Molecular markers linked to the *lgs* gene can accelerate development of *Striga*-resistant cultivars. Using a high density linkage map constructed with 367 markers (DArT and SSRs) and an in vitro assay for germination stimulant activity towards *Striga asiatica* in 354 recombinant inbred lines derived from SRN39 (low stimulant) × Shanqui Red (high stimulant), we precisely tagged and mapped the *lgs* gene on SBI-05 between two tightly linked microsatellite markers SB3344 and SB3352 at a distance of 0.5 and 1.5 cM, respectively. The fine-mapped *lgs* region was delimited to a 5.8 cM interval with the closest three markers SB3344, SB3346 and SB3343 positioned at 0.5, 0.7 and 0.9 cM, respectively. We validated tightly linked markers in a set of 23 diverse sorghum accessions, most of which were known to be *Striga* resistant, by genotyping and phenotyping for germination stimulant activity towards both *S. asiatica* and *S. hermonthica*. The markers co-segregated with *Striga* germination stimulant activity in 21 of the 23 tested lines. The *lgs* locus similarly affected germination stimulant activity for both *Striga* species. The identified markers would be useful in marker-assisted selection for introgressing this trait into susceptible sorghum cultivars. Examination of the sorghum genome sequence and comparative analysis with the rice genome suggests some candidate genes in the fine-mapped region (400 kb) that may affect strigolactone biosynthesis or exudation. This work should form a foundation for map-based cloning of the *lgs* gene and aid in elucidation of an exact mechanism for resistance based on low *Striga* germination stimulant activity.

Communicated by A. Paterson.

K. Satish · Z. Gutema · C. Grenier · P. J. Rich · G. Ejeta (✉)
Department of Agronomy, Purdue University,
915 W State Street, West Lafayette, IN 47907-2054, USA
e-mail: gejeta@purdue.edu

## Introduction

Witchweeds (*Striga* spp., family Orobanchaceae) are persistent agricultural constraints in sub-Saharan Africa (SSA) and parts of Asia. They are obligate root hemiparasitic weeds, relying on host plant roots for the acquisition of water, minerals and reduced nitrogen. Although they are photosynthetically capable upon emergence, they also require fixed carbon from their hosts for the subterranean phase of their life cycle (Graves et al. 1990). In addition to diverting sustenance from their host, they negatively affect host growth and fitness through what can be generally called toxic effects (Frost et al. 1997). The common name of these parasites (witchweeds) attests to the stunting of shoot growth, often accompanied by chlorosis and, under severe infestations, leaf firing resembling symptoms caused by severe moisture stress. The overall effect of *Striga* on crops is reduced grain yields, even to the point of total loss. The most economically important *Striga* spp. include *S. asiatica* and *S. hermonthica*, both parasites of cereals including sorghum, maize, millets, and upland rice (Ejeta 2007a). There are over 50 other *Striga* spp. (Mohamed et al. 2001) with some, like *S. gesnerioides*, affecting legumes. For the remainder of this paper, however, we will refer collectively only to *S. asiatica* and *S. hermonthica* when we mention the genus name *Striga*.

Recent estimates for crop losses due to *Striga* are scant and unsupported by detailed survey. Experts agree that the problem is worsening in SSA. In 1991, an estimated 21 million hectares were infested with *Striga* (Sauerborn 1991) with the figure rising to 50 million hectares in 2007 causing over a billion USD in losses annually (Ejeta 2007a). *Striga* is now often identified as the greatest biological constraint to food production in Africa (Ejeta and Butler 1993; Scholes and Press 2008). Effective control of *Striga* through conventional agronomic practices alone is difficult as this parasite causes its greatest damage long before its emergence above ground (Frost et al. 1997). The complicated life cycle of *Striga,* its dynamic interactions with the host plant and the tens of thousands of long lived seeds produced by a single parasite further makes control very difficult. Development of resistant cultivars based on host plant resistance has been considered as the best method to control *Striga* parasite (Ejeta 2007b).

Although resistance has now been described in most of *Striga*'s host crops, it is most advanced in sorghum. The process by which *Striga* infects its host (Joel et al. 2007) involves synchrony of weed seed dormancy and conditioning with the cropping season. *Striga* relies on the same rains to break its dormancy as the fields are wetted to support the sown crop seedlings through early establishment. Because *Striga* cannot survive a long period without a host plant root, it responds to chemical germination stimulants present only within the host rhizosphere. If no host is available, seeds will return to a dormant state. Subsequently, yet another chemical cue signals contact with the host root and haustorial formation that allows the parasite to attach, penetrate and acquire from its host the water, minerals and metabolites to support its growth through the subterranean phase to emergence, maturity and seed set. Relatively few plants serve as *Striga* hosts. Such plants give the proper signals in sufficient quantities and offer the compatible anatomical and physiological factors that support the parasitic association. All of these detailed requirements, including production of chemical cues and defense responses, are under genetic control. Defining these host factors and the genes that control them opens the possibility to engineer a crop variety with specific factors to disrupt the intimate relationship that *Striga* achieves with its hosts. To date, we have discovered several *Striga* resistance traits or mechanisms in sorghum, including low germination stimulant activity, low haustorial initiation activity, a hypersensitive response and incompatibility (Ejeta 2007b).

The best exploited of these resistance mechanisms is low germination stimulant activity in sorghum. The *Striga* seeds require host produced germination stimulants to germinate and form an initial contact with the host root. The mutants which produce low quantities of germination stimulants in root exudates were described over 50 years ago (Williams 1959). The sorghum accessions which produce little or no germination stimulants have been shown to be resistant to *Striga* in field experiments (Ramaiah 1987; Hess et al. 1992). Identification of source "low stimulant" sorghums and selection for the trait in breeding lines improved with bioassays like the "double pot technique" (Vasudeva Rao 1985) and the agar gel assay (Hess et al. 1992). The chemical nature of the primary *Striga* germination stimulant in sorghum and other plant root exudates has been identified as a class of related compounds called strigolactones (Siame et al. 1993). These compounds have received much attention in recent years since the discovery that, in addition to stimulating the germination of root parasitic Orobanchaceae, they also promote mycorrhization by acting as branching factors for symbiotic fungal species (Akiyama et al. 2005) and phytohormones that inhibit shoot branching (Gomez-Roldan et al. 2008; Umehara et al. 2008). These latter functions explain why strigolactones are so widespread among terrestrial plants. The first described strigolactone germination stimulant, strigol, was isolated from cotton, a non-host to *Striga* (Cook et al. 1972).

Genetic studies on low *Striga* germination stimulant activity suggested single gene (*lgs*: low germination stimulant) control with recessive inheritance (Ramaiah et al. 1990). Further studies involving a highly resistant low germination stimulant line, SRN39, confirmed the recessive nature of the *lgs* allele responsible for the trait and its nuclear location (Vogler et al. 1996; Mutengwa et al. 2005). However, the actual gene and the type of functional mutation responsible for the loss or low germination stimulant activity have not been elucidated. Mutations in genes causing changes in *Striga* germination stimulant activity are likely to involve changes in strigolactones (either type or amount) present in root exudates. These mutant genes are not only useful in *Striga* resistance breeding but also in biochemical and molecular studies to elucidate the metabolic origin of the various strigolactones produced by plants (Rani et al. 2008).

The efficiency of *Striga* resistance breeding could greatly improve with the use of marker-assisted selection (MAS) because, once established, specific resistance traits could be monitored by simple and more widely accessible genotyping methods. Moreover, *Striga* resistance traits could be more quickly combined and introgressed into vulnerable cultivars. Identification of tightly linked and validated molecular markers is an essential step for MAS of any trait which is controlled by either single gene or quantitative inheritance in a resistance breeding program. As low *Striga* germination stimulant activity is found to be associated with *Striga* field resistance, controlled by a single recessive gene (*lgs*) and screening using the

laboratory assay is laborious and time consuming, a molecular tag for the trait is highly desirable. Tightly linked PCR-based molecular markers could be used in a large-scale MAS program and eventually to identify candidate genes involved in its expression to isolate and characterize the gene, and manipulate the strigolactone biosynthesis pathway for improving *Striga* resistance in sorghum.

The objectives of the present study were to precisely localize the *lgs* gene on the genetic map, identify DNA markers tightly linked to the *lgs* gene and validate the tightly linked markers in a set of diverse sorghum accessions for their potential and utility in MAS, and ability to distinguish the low and high *Striga* germination stimulant activities. Additional objectives were to locate probable candidate genes in the fine-mapped *lgs* region using the available sorghum genome sequence and to conduct comparative analysis of the sorghum *lgs* region with the syntenous region of the rice genome.

## Materials and methods

### Genetic material

A recombinant inbred line population (RILs) consisting of 354 lines, developed by crossing SRN39 (low stimulant), an African caudatum and Shanqui Red (high stimulant), a Chinese kaoliang line, was utilized in this study. The parental lines differ significantly in field resistance to *Striga* as well as in *Striga* germination stimulant activity. SRN39 is a low germination stimulant producing line which exhibits excellent field resistance (Hess et al. 1992; Rodenburg et al. 2006). In addition to low germination stimulant activity, SRN39 shows an incompatible response to *Striga* at the post-attachment stage (Amusan et al. 2011). Shanqui Red has high *Striga* germination stimulant activity and is highly susceptible to *Striga* in the field (Ejeta and Butler 1993). A set of nine advanced inbred lines derived from SRN39 and 14 sorghum cultivars from different geographic regions and contrasting for *Striga* germination stimulant activity were used to validate the markers tightly linked to the *lgs* gene. The details of the accessions used for marker validation are provided in Table 3.

*Striga asiatica* seed was collected from various cereal hosts (maize, sorghum and rice) from the USDA station in Oxford, NC, USA. *Striga hermonthica* seed was collected from a sorghum host in Samanko, Mali. *Striga* seeds were received under quarantine conditions prescribed by USDA APHIS and the Indiana Department of Natural Resources for our parasitic *Striga* containment laboratory. Both seed sources were stored in the Purdue University Parasitic Weed Containment Facility under desiccation at 20°C.

### Phenotyping

The two parental lines of the RIL population, 354 RILs and a total of 23 diverse sorghum accessions including nine advanced inbred lines derived from SRN39 were evaluated for *S. asiatica* germination stimulant activity using the agar gel assay (AGA) originally developed by Hess et al. (1992) and modified by Rich et al. (2004) in a simple lattice design blocked over batches and time with six replications. The 23 sorghum genotypes used for marker validation were also phenotyped for *S. hermonthica* germination stimulant activity in addition to *S. asiatica*.

Conditioning *Striga* seed was accomplished by first surface sterilizing the seeds by sonicating for 2 min in 75% ethanol followed by 1 min in 50% bleach (2.1% NaOCl) and 10% Metricide 28 (Metrex Research Corporation, Romulus, MI, 0.25% glutaraldehyde) followed by two water washes. Sand and debris were removed during surface sterilization. The seeds were soaked for 2 days in a fungicide solution containing Benomyl (Dragon Corporation, Roanoke, VA; 0.0008% methyl 1-[butylcarbamoyl]-2-benzimid-azolecarbamate). Approximately, 3,000 surfaced sterilized imbibed seeds were then dispersed in 25 ml 0.7% (w/v) agar–water in a 85 mm Petri dish to a density of about 50 *Striga* seeds per $cm^2$. The seeds were conditioned in this agar in the dark at 29°C for an additional week. The root of a surface sterilized pre-germinated, 24-h old sorghum seedling (average root length of 3 cm) was gently inserted into the agar after the 9-day conditioning period and returned to the dark 29°C incubator for an additional 4 days. Blank plates containing only *Striga* seed in agar were included in the experiment as a check for germination potential for each batch of conditioned *Striga* seed by spraying 0.25 ml of a $10^{-5}$M solution of the synthetic strigolactone GR24 (final concentration $10^{-7}$M).

Germination stimulant activity was estimated by measuring in millimeters (mm) the maximum germination distance (MGD) in agar between the sorghum root and the furthest three germinated *Striga* seeds under a stereomicroscope at 10× magnification. All entries were replicated six times and MGD values for each line were averaged across the six replications. Sorghum accessions were classified as having low *Striga* germination stimulant activity if the MGD was below 5 mm and high if the MGD was above 10 mm, following the classification used by Haussmann et al. (2004). This separation was confirmed by mean comparison using the PDIFF function of SAS and converting the output using the PDMIX612 macro (Saxton 1998).

### PCR amplification, electrophoresis, and genotyping

Genomic DNA from the RILs was extracted using the CTAB method (Saghai-Maroof et al. 1984). DNA from the

accessions used for the marker validation portion of this study was extracted using the high throughput method described by Xin et al. (2003). The RIL population was genotyped by both the Diversity Array Technology® (DArT) and simple sequence repeat markers (SSRs or microsatellite markers). The genomic DNA of 354 RILs was sent to DArT P/Lc, Yarralmula, Australia for DArT marker genotyping. Genotyping of additional SSR markers was done in our laboratory to aid in identification of the chromosomes following the PCR conditions described by Knoll et al. (2008). PCRs for the accessions used in the validation test were set up using MangoMix (Bioline USA, Inc.), a ready to use 2× pre-optimized reaction mix containing MangoTaq® DNA polymerase, dNTPs, tracking dyes and $Mg^{2+}$. PCRs were carried out in 96-well plates in a PTC-100 Thermal Controller (MJ Research, USA) and programmed as follows: initial denaturation at 94°C for 60 s, followed by 33 cycles of 94°C (denaturation) for 10 s, 55°C (annealing) for 35 s, and 72°C (extension) for 45 s, followed by a final extension at 72°C for 3 min. In the case of 'SB' series SSR markers, an annealing temperature of 60°C was used for better amplification. The amplicons of all SSR markers were separated on 3% high-resolution agarose gel (Genepure HiRes, ISC Bioexpress, USA) in 0.5× TBE buffer ran at 70 V overnight and visualized by a nonhazardous GelRed® nucleic acid gel stain (Biotum Inc., USA). The SSR alleles in RILs were manually scored either as A (SRN39 allele) or B (Shanqui Red allele). For the DArT markers, the dominant scores generated by the DArT system (1 present; 0 absent) were translated to A (SRN39 allele) or B (Shanqui Red allele). All DArT markers genotyped and mapped in this study were abbreviated as DM (e.g., DM277). The mapped SSR markers with the prefix 'Pustri' are newly developed markers in our study. The 'Xtxp' series markers are SSR markers developed by Bhattramakki et al. (2000) and 'SB' series are SSR markers developed by Yonemaru et al. (2009). For bulk segregant analysis or BSA (Michelmore et al. 1991), equal amounts of genomic DNA from 12 low stimulating and 12 high stimulating lines randomly chosen from the RIL population were pooled to make the bulks. Both the bulks along with the two parents of the population were screened to identify markers showing polymorphisms clearly among the four groups ($P_1$, $P_2$, low bulk, and high bulk). The polymorphic markers identified in BSA were subsequently genotyped in the full RIL population for further linkage analysis.

### Linkage analysis and genetic mapping

A Chi-square test was applied to test for the significance of the expected 1:1 ratio for single gene inheritance for *Striga* germination stimulant activity in the RILs. Segregation of RILs for the low and high *striga* germination stimulant activity was scored as SRN39 type allele (A type) if the MGD was below 5 mm and as Shanqui Red type allele (B type) if the MGD was above 10 mm (Haussmann et al. 2004) to test for goodness of fit against proposed single gene Mendelian ratio and in linkage analysis with other genotyped markers to integrate and determine the map position on the genetic map. Detailed linkage analysis was conducted and a genetic linkage map constructed using the Joinmap 3.0 software (Van Ooijen and Voorrips 2001). The Kosambi function was used to convert recombination frequency into the genetic distance (centimorgans, cM). The graphical representation of the genetic map was drawn using the Mapchart software (Voorrips 2002) and chromosomal nomenclature was followed according to Kim et al. (2005). To further confirm the mapped position of the *lgs* gene based on binary data, actual MGD data were subjected to QTL analysis using multiple QTL mapping (MQM) mapping method to identify the major genomic region associated with *Striga* germination stimulant activity. MQM analysis was done both with and without mapping of the *lgs* phenotype score (high or low) using the MapQTL 5 software (Van Ooijen 2005), according to the parameters used by Satish et al. (2009). Probable candidate genes were searched within the fine-mapped *lgs* region using the closest linked sequence-based markers and the published sorghum genome sequence (Paterson et al. 2009) available in the Phytozome genome database (http://www.phytozome.net/sorghum). The sequence-based SSR markers and gene sequence information in the fine-mapped sorghum *lgs* region, rice genome sequence (http://www.phytozome.net/rice.php), and cMap tool (http://www.gramene.org/cmap) available in the Gramene genome database were utilized for synteny analysis.

## Results

### Genetic analysis

The parental lines of the mapping population differed significantly for germination stimulant activity toward both *Striga* species. The low stimulant parent SRN39 showed an average MGD value of 1.9 contrasting to a MGD value of 19.8 of high stimulant parent Shanqui Red with *S. asiatica*. The frequency distribution for this trait in the RILs showed a bimodal type of distribution with distinct classes, high and low, supporting single genic inheritance of the trait (Fig. 1). Out of 354 RILs screened with *S. asiatica*, 187 were classified as having low germination stimulant activity (MGD $<$ 5 mm) and 167 had high germination stimulant activity (MGD $>$ 10 mm). The segregation pattern of *Striga* germination stimulant activity in the RILs fit

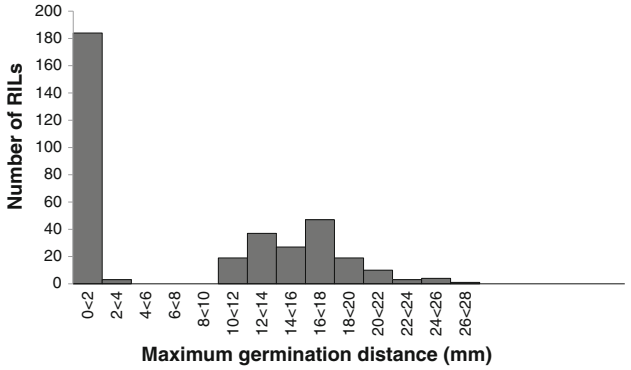


**Fig. 1** Frequency distribution in RILs of the cross SRN39 × Shanqui Red for maximum germination distance (MGD) data

**Table 1** Segregation analysis and Chi-square test for low *Striga* germination stimulant activity trait in the RIL population

| Accession | MGD (mm) | Frequency | | $\chi 2$ | *p* value |
|---|---|---|---|---|---|
| | | Observed | Expected | | |
| SRN39 | 1.9 | | | | |
| Shanqui Red | 19.8 | | | | |
| RILs | <5 | 187 | 1:1 ratio | 1.130 | 0.28 |
| | >10 | 167 | | | |

the 1:1 ratio expected for single gene inheritance as determined by the Chi-square test (Table 1). These results are in agreement with those reported earlier on the segregation analysis of this trait in different crosses (Ramaiah et al. 1990; Vogler et al. 1996; Mutengwa et al. 2005).

## Linkage analysis and fine-mapping of the *lgs* gene

Prior to linkage analysis and mapping, low-quality DArT markers with a large number of missing data points and segregation distortion were removed. Genotyping data on a total of 343 DArT markers, 15 SSR markers and *Striga* germination stimulant activity binary score were subjected to linkage analysis with the Joinmap 3.0® software. Initial linkage analysis mapped the *lgs* gene on to the tip of SBI-05 chromosome. The constructed linkage map consisted of 12 linkage groups for ten sorghum chromosomes, including two linkage groups for chromosome SBI-01 and one unknown linkage group. The total map length was 1,387 cM with an average distance of 3.9 cM between each mapped marker.

To further fine-map the *lgs* region and tag with tightly linked microsatellite markers, a targeted BSA was conducted using new SSR markers specific to the SBI-05 chromosomal tip developed by Yonemaru et al. (2009) and some newly developed SSR markers in our study (Table 2). Out of 29 markers tested in the BSA, ten markers were identified as polymorphic among the four groups ($P_1$, $P_2$, low bulk and high bulk). Of these ten, four markers (SB3344, SB3346, SB3343 and SB3352) precisely distinguished both the low and high bulks from the parents. A gel image showing segregation of one of the tightly linked markers SB3344 to the *lgs* gene identified in the BSA is provided in Fig. 2. Subsequently, further linkage analysis of these four markers in the full RIL population resulted in the fine-mapping of the *lgs* region with three markers (SB3344, SB3346 and SB3343) flanking on the same side with genetic distances of 0.5, 0.7 and 1.5 cM, respectively, from the *lgs* gene. The marker SB3352 flanked the *lgs* gene on the opposite side with a genetic distance of 1.5 cM. Additionally, mapping of four other polymorphic markers delimited the entire fine-mapped region to a total interval of 5.8 cM with a total of eight mapped markers. A partial genetic map of sorghum with the fine-mapped *lgs* region on SBI-05 is provided in Fig. 3. Of the eight mapped markers in the *lgs* region, one marker (SB3343) was dominantly scored and all others were co-dominant in the RIL population. The genetic map positions of some "SB" series markers, which were previously developed by Yonemaru et al. (2009) but genetically unmapped, are reported in our study.

The mapping position of *lgs* based on binary phenotypic data was further confirmed by two rounds of initial QTL analysis with MQM mapping using the genetic maps with and without presence of the *lgs* locus. The two rounds of QTL analysis showed the same major QTL peak on the tip of SBI-05 (70% $R^2$ and 82 LOD), confirming the presence of major gene in this genomic region. A third round of

**Table 2** Primer sequence information of new microsatellite markers developed in this study

| Marker name[a] | SSR motif | Forward primer (5′→3′) | Reverse primer (5′→3′) | Ann. Temp. (°C) | Expected size |
|---|---|---|---|---|---|
| Pustri4 | (AG)14 | ATTTGGGCTCTTCCTTCCTC | AACTAACTCCGTGCGTCGAG | 55 | 223 |
| Pustri15 | (GC)4 | ACTACTTCCGCGTCACCAAC | CTCATCGAGCACGAAGATCA | 55 | 941 |
| Pustri24 | (TG)25 | CTTTGGGCATGCAAGTTTCT | TGTTGTTGCTGGTTCAAGGT | 55 | 171 |
| Pustri26 | (GT)4 (GC)6 | AACCATGTAAGCGACTGCAC | TAATGCACACTCCCGTTTCA | 54 | 295 |

[a] Pustri4 and Pustri26 were developed for the sorghum ortholog of *DWARF27* [Phytozome sorghum gene ID Sb05g022855]. Pustri15 was developed from the sorghum *ARS1* gene [Phytozome sorghum gene ID Sb05g022500] targeting the SSR motif and most of the single exon

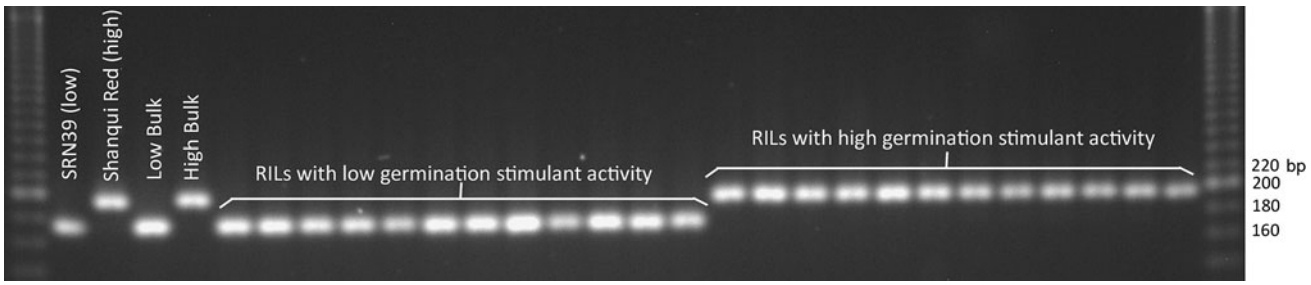


**Fig. 2** Segregation of the SB3344 marker tightly linked to the *lgs* gene among the low and high stimulating RILs used in the BSA

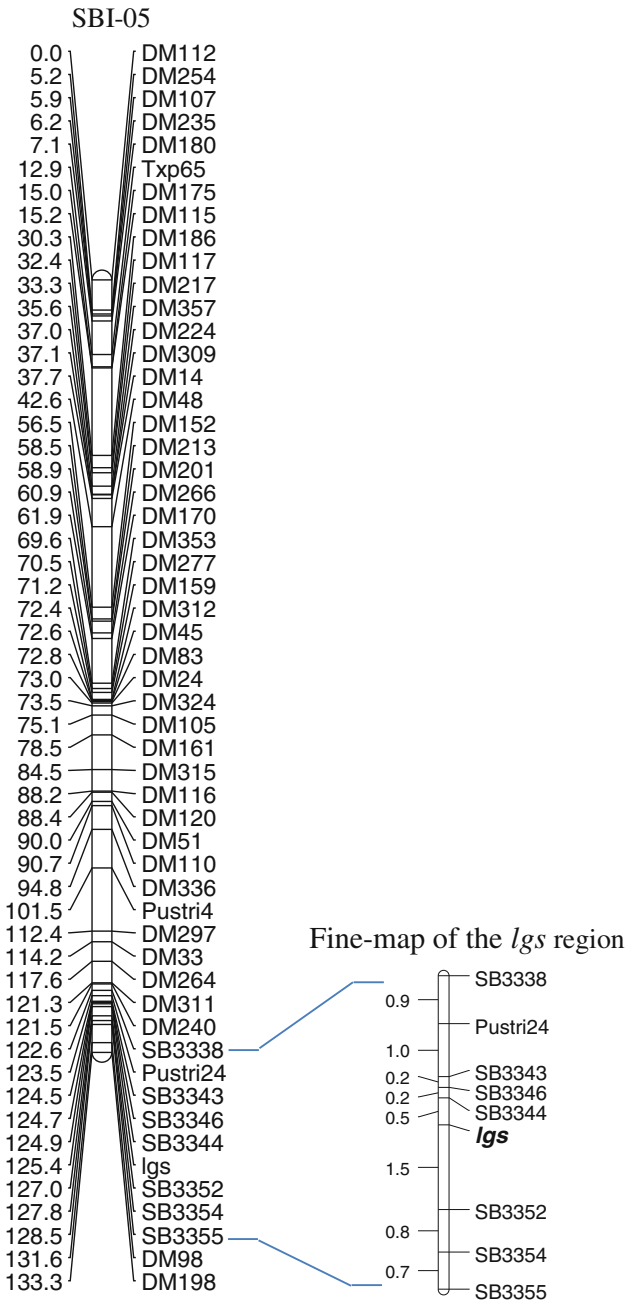


**Fig. 3** A partial genetic map of the sorghum showing the fine-mapped *lgs* gene on SBI-05 chromosome

QTL analysis including the fine-mapped *lgs* region with eight additional markers showed again only one major QTL peak exactly on the *lgs* locus with the $R^2$ of 90% and 191 LOD value. No additional minor QTLs were identified for this trait.

## Validation of tightly linked markers

The potential utility of the three tightly linked markers (SB3344, SB3346 and SB3352) that we identified for the *lgs* gene was further validated using a set of 23 diverse sorghum accessions, including nine SRN39-derived advanced *Striga*-resistant lines developed at Purdue and released in various African countries. Eight of these (P9401-P9408) are progenies of the cross SRN39 (low) × P954063 (high) and one accession, named 'Brhan', was derived from SRN39 and Framida (another line with low *Striga* germination stimulant activity). In addition to these 11 accessions and the parents of the RIL population used to map *lgs*, ten diverse accessions with previously reported *Striga* resistance and germination stimulant activity were included in the validation test (Table 3). Although the 354 RILs used to map *lgs* were not screened with *S. hermonthica*, we found the parents of this population (Shanqui Red and SRN39) and the other accessions included in the validation study to have similar germination stimulant activity toward both *Striga* spp. An accession showing low germination stimulant activity toward the *S. asiatica* seed source used in the AGA also showed low germination stimulant activity toward the *S. hermonthica* seed source. The tightly linked markers SB3344 and SB3346 clearly separated all accessions with low *Striga* germination stimulant activity from high stimulant activity accessions, with the exception of two, 555 and the wild sorghum line PQ-434 (*S. bicolor drummondii*). Among the SRN39 derivatives, the markers were always associated with the trait; those with low *Striga* germination stimulant activity in all cases showed the SRN39 type allele. The third marker, SB3352, was monomorphic between SRN39 and the other two parents of the derived lines (P954063 and Framida) so in these lines this marker did not distinguish between high and low types. However, the SB3352 marker was associated with the low stimulant

**Table 3** Phenotyping and validation of the microsatellite markers associated with the *lgs* gene in diverse sorghum accessions

| Accession | Pedigree of developed lines or origin of source lines | Phenotypic data (MGD)[a] | | *Striga* germination stimulant activity | Field reaction to *Striga* (reference) | Genotyping[b] | | |
|---|---|---|---|---|---|---|---|---|
| | | *S. asiatica* | *S. hermonthica* | | | SB3344 | SB3346 | SB3352 |
| Shanqui Red | China | 19.8 ± 3.0 | 19.6 ± 5.2 | High | Susceptible (Ejeta and Butler 1993) | + | + | + |
| SRN39 | Sudan | 1.9 ± 1.8 | 0.1 ± 0.2 | Low | Resistant (Rodenburg et al. 2006) | − | − | − |
| P954063 | United States | 13.6 ± 1.3 | 11.8 ± 2.0 | High | Susceptible (Ejeta and Butler 1993) | + | × | − |
| P9401 | SRN39 × P954063 | 2.0 ± 1.1 | 0 ± 0 | Low | Resistant (Tesso et al. 2007) | − | − | − |
| P9402 | SRN39 × P954063 | 0.4 ± 0.7 | 1.0 ± 1.5 | Low | Resistant (Mangombe et al. 2000) | − | − | − |
| P9403 | SRN39 × P954063 | 14.7 ± 2.7 | 13.1 ± 2.5 | High | Resistant (Tesso et al. 2007) | + | × | − |
| P9404 | SRN39 × P954063 | 18.3 ± 4.4 | 11.2 ± 3.4 | High | Resistant (Mangombe et al. 2000) | + | × | − |
| P9405 | SRN39 × P954063 | 0.7 ± 1.0 | 0 ± 0 | Low | Resistant (Mbwaga et al. 2007) | − | − | − |
| P9406 | SRN39 × P954063 | 13.2 ± 2.3 | 14.0 ± 2.3 | High | Resistant (Mbwaga et al. 2007) | + | × | − |
| P9407 | SRN39 × P954063 | 17.6 ± 3.1 | 12.9 ± 2.6 | High | Resistant (Mangombe et al. 2000) | + | × | − |
| P9408 | SRN39 × P954063 | 24.8 ± 3.6 | 23.0 ± 7.0 | High | Resistant (Unpublished) | + | × | − |
| Brhan | Framida × SRN39 | 4.0 ± 2.7 | 1.2 ± 1.7 | Low | Resistant (Tesso et al. 2007) | − | − | − |
| Framida | South Africa | 0.2 ± 0.4 | 1.1 ± 1.4 | Low | Resistant (Ramaiah 1987) | − | − | − |
| 555 | India | 0.5 ± 0.5 | 1.3 ± 2.2 | Low | Resistant (Ramaiah 1987) | + | × | + |
| SAR33 | GPR148 × Framida | 0.7 ± 0.7 | 4.2 ± 3.2 | Low | Resistant (Mangombe et al. 2000) | − | − | − |
| IS9830 | Sudan | 1.0 ± 1.6 | 0.3 ± 1.0 | Low | Resistant (Rodenburg et al. 2006) | − | − | − |
| N13 | India | 17.1 ± 1.9 | 12.8 ± 1.0 | High | Resistant (Rodenburg et al. 2006) | × | × | + |
| ICSV1006 | India | 0.3 ± 0.4 | 0 ± 0 | Low | Resistant (Ramaiah 1987) | − | − | − |
| CK60A | United States | 11.8 ± 2.2 | 14.3 ± 3.1 | High | Susceptible (Rodenburg et al. 2006) | × | + | + |
| Tetron | Sudan | 4.3 ± 1.5 | 1.4 ± 1.9 | Low | Resistant (Ramaiah 1987) | − | − | − |
| IS4225 | China | 18.8 ± 0.7 | 12.7 ± 2.6 | High | Susceptible (Ejeta and Butler 1993) | × | × | + |
| ICSV1007 | Burkina Faso | 1.2 ± 0.6 | 0.9 ± 1.7 | Low | Resistant (Ramaiah 1987) | − | − | + |
| PQ-434 | Sudan | 0 ± 0 | 1.2 ± 1.2 | Low | Not tested (Rich et al. 2004) | × | × | + |

[a] MGD = maximum germination distance (mm) as measured in the agar gel assay (see text); MGD >10 mm indicates high *Striga* germination stimulant activity; MGD < 5 mm indicates low germination stimulant activity. Values are means of measures (mm) ± 1 standard deviation

[b] Allele symbols: + indicates the Shanqui Red (wildtype) allele; − indicates the SRN39 (mutant) allele; × indicates a third allele. Approximate allele sizes for SB3344: + = 190 bp, − = 170 bp, × = 195 bp; SB3346: + = 285 bp, − = 300 bp, × = 290 bp; SB3352: + = 700 bp, − = 230 bp

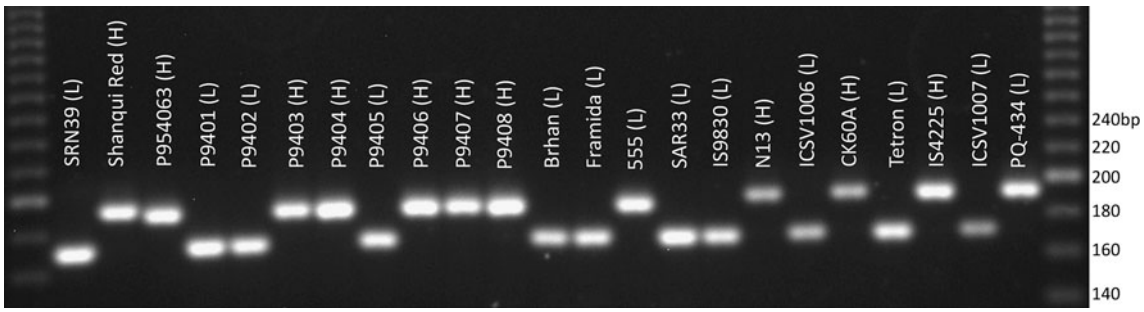


**Fig. 4** Validation of one of the tightly linked marker SB3344 in the diverse sorghum accessions differing for *Striga* germination stimulant activity (*L* low and *H* high)

phenotype in the other accessions, except in those previously mentioned and in ICSV1007. A gel image showing the validation of tightly linked marker SB3344 for *Striga* germination stimulant activity in the 23 sorghum accessions is presented in Fig. 4. The complete details on genotyping and phenotyping of these lines for both *Striga* spp. are provided in Table 3.

### Identification of probable candidate genes and comparative analysis of the *lgs* region with the rice genome

The sequence-based microsatellite markers in the fine-mapped *lgs* region were used to identify probable candidate genes between the tightly linked flanking markers SB3344 and SB3352, using the sorghum genome sequence available from the Phytozome database. The physical distance between the two flanking markers in the sorghum genome is approximately 400 kb and showed 30 annotated genes. The genes coding for NBS-LRR protein, NB-ARC protein, Glutathione-S-transferase, Serine-threonine protein kinase, Epoxide hydrolase, Laccase15, and several Cytochrome P450s are very closely located probable candidate genes to the tightly linked markers. A complete list of annotated genes for the *lgs* fine-map is provided in the Table 4.

An initial comparative analysis of the sorghum SBI-05 with rice chromosomes using the Gramene cMap analysis tool, based on the commonly mapped RFLP markers between the two crops, identified syntenic rice chromosome 11. Further detailed comparative analysis of the specific sorghum *lgs* region with the rice chromosome 11 by BLASTn analysis of the sequence-based sorghum markers and genes in the *lgs* region on to the rice genome (http://www.gramene.org/Multi/blastview; http://www.phytozome.net/rice.php) identified the syntenic rice region between 21.7 and 27.2 Mbp (Fig. 5). Interestingly, this syntenic rice region contains a gene named *DWARF27* reported to be involved in the strigolactone biosynthetic pathway and inhibition of shoot branching (Lin et al. 2009). To know whether the *DWARF27* gene is associated with low *Striga* germination stimulant activity in sorghum, we obtained the published *DWARF27* gene sequence from the NCBI database (http://www.ncbi.nlm.nih.gov/; clone ID-FJ641055) and identified the corresponding sorghum orthologous gene (Phytozome gene ID Sb05g022855) on SBI-05 based on BLASTn analysis. Subsequently, two microsatellite marker tags were developed for the sorghum orthologous *DWARF27* region (Table 2). Only one of these, Pustri4, (within 350 bp of the orthologous gene) was found to be polymorphic between the parents of the mapping population (SRN39 and Shanqui Red). Linkage analysis of Pustri4 mapped the ortholog to SBI-05 at 21 cM from the *lgs* locus, indicating that this is not the actual gene responsible for the observed *Striga* germination stimulant activity in this sorghum population.

Two QTLs for *S. hermonthica* field resistance traits were reported on syntenic rice chromosome 11 (Kaewchumnong and Price 2008), however, detailed comparative analysis using QTL flanking RFLP marker sequences (Rz141, R642 and G320; sequences obtained from the Gramene database; http://www.gramene.org), revealed the syntenic QTL position on the sorghum chromosome completely away from the fine-mapped *lgs* region.

## Discussion

Low *Striga* germination stimulant activity, encoded by a single recessive gene (*lgs*), is one of several resistance traits we have identified in sorghum. The mutant *lgs* gene results in low *Striga* germination stimulant activity. It is the best characterized and most widely exploited resistance character. By a detailed linkage analysis of this trait in a large RIL population using a high density genetic linkage map, we precisely tagged and mapped the gene (*lgs*) to the short arm of sorghum SBI-05. Through fine-mapping, the region containing the *lgs* gene was delimited to a 5.8 cM interval with a total of eight mapped microsatellite markers (Fig. 3). Three tightly linked microsatellite markers, SB3344, SB3346 and SB3343, were spaced with genetic

**Table 4** List of sorghum annotated genes in the fine-mapped *lgs* region (~400 kb)

| No. | Gene ID[a] | Description | Functional annotation |
|---|---|---|---|
| 1 | Sb05g026420 | DNA-directed RNA polymerase | RNA polymerase II subunit 9 |
| 2 | Sb05g026430 | Uncharacterized protein | Uncharacterized |
| 3 | Sb05g026450 | Zinc finger POZ domain protein | Zinc finger POZ domain |
| 4 | Sb05g026460 | Predicted protein | Unknown |
| 5 | Sb05g026470 | NB-ARC domain containing protein | Apoptotic ATPase |
| 6 | Sb05g026480 | NBS-LRR like protein | Apoptotic ATPase |
| 7 | Sb05g026490 | Glutathione-S-transferase GST 22 | GTS domain containing |
| 8 | Sb05g026510 | Uncharacterized protein | Unknown |
| 9 | **Sb05g026520** | Predicted protein | Plant invertase/pectin methylesterase inhibitor |
| 10 | Sb05g026530 | Os11g0678000 protein | Serine-threonine protein kinase |
| 11 | Sb05g026540 | Uncharacterized protein | Iron/ascorbate family oxidoreductases |
| 12 | Sb05g026550 | Os09g0555100 protein | Sulfotransferase |
| 13 | **Sb05g026560** | Epoxide hydrolase | Alpha/beta hydrolase related |
| 14 | Sb05g026570 | Leucine Rich Repeat family protein | Serine/threonine protein kinase |
| 15 | **Sb05g026580** | Acyltransferase family protein, | Cytochrome P450 CYP2 subfamily |
| 16 | **Sb05g026600** | Os10g0171300 protein | Cytochrome P450 CYP2 subfamily |
| 17 | **Sb05g026610** | Uncharacterized protein | Cytochrome P450 |
| 18 | Sb05g026620 | Predicted protein | Multi-copper oxidase |
| 19 | Sb05g026630 | Laccase15 precursor | Multicopper oxidases |
| 20 | Sb05g026650 | Bowman-Birk serine protease inhibitor | BBS protease inhibitor |
| 21 | Sb05g026660 | Uncharacterized protein | Unknown |
| 22 | Sb05g026670 | Bowman-Birk serine protease inhibitor | BBS protease inhibitor |
| 23 | Sb05g026680 | Bowman-Birk serine protease inhibitor | BBS protease inhibitor |
| 24 | Sb05g026690 | Bowman-Birk serine protease inhibitor | BBS protease inhibitor |
| 25 | Sb05g026700 | Bowman-Birk serine protease inhibitor | BBS protease inhibitor |
| 26 | Sb05g026710 | O-methyltransferase family protein | Hydroxyindole-O-methyltransferase |
| 27 | Sb05g026730 | O-methyltransferase family protein | Hydroxyindole-O-methyltransferase |
| 28 | Sb05g026740 | Transposon protein, CACTA, En/Spm subaclass | Splicing factor U2AF |
| 29 | Sb05g026750 | Predicted protein | Unknown |
| 30 | Sb05g026760 | Predicted protein | Unknown |

[a] Sorghum gene ID in the Phytozome genome database [http://www.phytozome.net/sorghum]. The genes in bold font are strong candidates assuming that the *lgs* gene is involved in biosynthesis or exudation of strigolactones

distances of 0.5, 0.7 and 0.9 cM, respectively, from the *lgs* gene on the same side. On the opposite side of the gene, the nearest co-segregating flanking marker was SB3352 with a genetic distance of 1.5 cM. Furthermore, the validation of tightly linked markers in a set of diverse sorghum accessions showed their potential utility in MAS and in identification of low germination stimulant activity in source materials. Generally, markers linked at a distance <5 cM to the target gene and validated in diverse accessions, as those obtained in the present study, can be effectively utilized for indirect selection (Weber and Wrickle 1994). Moreover, the efficiency of MAS can be increased by employing the markers flanking the gene of interest for recombinant selection. Thus, a set of eight microsatellite markers associated with the fine-mapped *lgs* gene in the present study in a total interval of 5.8 cM could be effectively used for foreground and recombinant selection in a MAS program for efficient transfer of the *lgs* gene into new and existing cultivars targeted for *Striga* endemic areas.

In the present study, genetic analysis affirmed that low germination stimulant activity is controlled by a single major gene, supporting earlier genetic studies that were conducted using different segregating populations (Ramaiah et al. 1990; Vogler et al. 1996; Mutengwa et al. 2005). The single genic inheritance of low *Striga* germination stimulant activity in the SRN39-derived RIL population in our study was also in agreement with previous pot-based and in vitro studies (Hess and Ejeta 1992; Vogler et al. 1996). The frequency distribution for this trait in our RIL population was bimodal, clearly separating the RILs

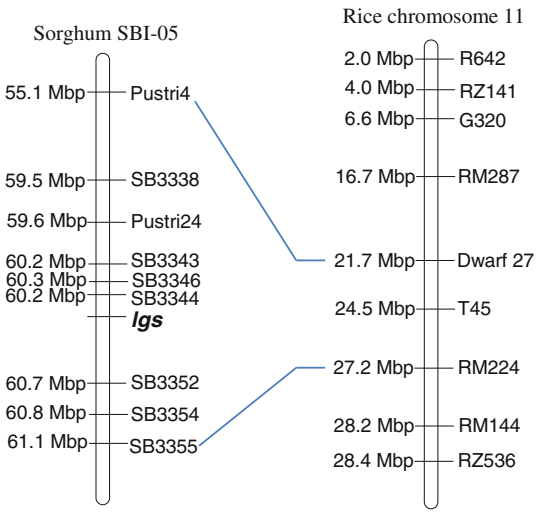


**Fig. 5** Synteny analysis of the sorghum *lgs* fine-mapped region on SBI-05 with rice chromosome 11

and other pure breeding accessions into distinct classes, high (MGD > 10 mm) and low (MGD < 5), although some variation in the MGD was observed among the high stimulant accessions. The high values may be influenced by artifacts of the assay such as position of the sorghum root on the plate or uneven *Striga* seed distribution. Variation among high-stimulating lines for *S. hermonthica* was reported in the genetic experiments of Haussmann et al. (2001), which they attributed to possible minor or modifier genes. To identify any additional genes influencing the trait, the actual MGD data of the RILs measured in the AGA were subjected to MQM analysis. MQM identified only one QTL (LOD 191) near the *lgs* gene explaining 90% of $R^2$, supporting the hypothesis of a single major gene influencing the trait. However, influence of unknown modifier genes on the MGD data cannot be ruled out.

At least 15 different strigolactone structures have been determined from plant root exudates (Xie et al. 2010), with most plants producing a mixture of strigolactones. Sorghum produces at least five different strigolactones, including 5-deoxystrigol, sorgolactone, strigol, strigyl acetate, and sorgomol (Hauck et al. 1992; Siame et al. 1993; Awad et al. 2006; Xie et al. 2008). Strigolactone biosynthesis and exudation in sorghum are reported to increase under conditions of nitrogen and phosphorous deficiencies (Yoneyama et al. 2007) and can also differ in terms of relative abundance of specific strigolactones between sorghum varieties (Awad et al. 2006; Yoneyama et al. 2010). The *lgs* gene mapped in this study likely has a major influence on one or more of these stigolactones either through production or exudation. The resistant sorghum variety SRN39, which is one of the parents of our RIL population, exudes 35-fold less 5-deoxystrigol than susceptible sorghum Tabat (Yoneyama et al. 2010). The amount of 5-deoxystrigol in the high stimulant sorghum variety Swarna (Ramaiah et al. 1990), collected and measured in a similar manner, was more than 1000-fold the level measured in SRN39 (Awad et al. 2006; Yoneyama et al. 2010). Since the mutation in *lgs* is recessive, it probably represents a loss of function, resulting in deficiencies of 5-deoxystrigol in the root exudate. However, SRN39 is known to have other strigolactones in its root exudates, including sorgolactone and sorgomol, and possibly others not detected through the methods of extraction and analysis in the reported investigation of this line (Yoneyama et al. 2010). If the *lgs* mutant allele caused a complete or partial block in 5-deoxystrigol production, one would expect a general reduction of all strigolactones in lines homozygous for the mutation since the current model for strigolactone biosynthesis places 5-deoxystrigol as the precursor to all other strigolactones (Rani et al. 2008). However, SRN39 actually has more sorgolactone than the susceptible sorghum that has wildtype alleles at the *lgs* locus (Yoneyama et al. 2010). More detailed analysis of the strigolactone profiles of root exudates of SRN39 and the other accessions of this study should reveal much more about the involvement of the *lgs* gene in strigolactone biosynthesis.

It should be noted that there is a critical difference between how *Striga* germination stimulant activity was phenotyped in our study and in the strigolactone biochemical studies conducted by Yoneyama et al. (2010). In the Yoneyama et al. (2010) study, comparison of SRN39 and Tetron with susceptible sorghum cultivars revealed very little difference (less than fourfold) in germination stimulant activity towards *S. hermonthica*. This markedly contrasts with the roughly 20-fold difference we observed in MGD between these low and the high stimulant accessions in this and other studies that we have conducted (Hess et al. 1992; Rich et al. 2004). Some strigolactones (like strigol) contain an allylic hydroxyl group which make them less stable than the non-hydroxy strigolactones (like 5-deoxystrigol and sorgolactone). Measuring germination stimulant activity by direct exposure of the exudates to *Striga* seed preserved the structural activity of the component stigolactones, whereas in the AGA used in our study, contact between the exuded stigolactones and *Striga* seed occurred after days of diffusion through the agar medium, allowing ample time for degradation and loss of germination stimulant activity. Therefore, exudates of low stimulant activity accessions, like SRN39, may contain mostly unstable hydroxy-strigolactones, like orobanchol (not measured in the Yoneyama study). Exudates rich in hydroxy-strigolactones would show germination stimulant activity if exposed relatively quickly to *Striga* seed, but show little or no germination stimulant activity when

measured in the AGA (Yoneyama et al. 2010). The AGA is therefore a 'truer', more direct measure of *Striga* germination stimulant activity because it better reflects what occurs in the soil where unstable hydroxy-strigolactones would be short lived.

Using a RIL population, Haussmann et al. (2004) mapped the low germination stimulant gene for *S. hermonthica* initially on the tip of chromosome I (new SBI-06). However, the positioning of *lgs* was considered as preliminary in their study due to inconsistent *lgs* map positions based on different LOD and REC parameter settings. In our study, we determined a precise map position of *lgs* using different LOD parameters (*lgs* position was stable between LOD 4–10). The QTL analysis of the MGD data using a genetic map with and without the *lgs* locus both identified a common major QTL on the chromosomal tip of SBI-05, which further confirms our mapped location of the *lgs*. In our study, the low stimulant parent IS9830 used by the Haussmann et al. (2004) was phenotyped for both *Striga* species and genotyped for tightly linked markers to the *lgs*. IS9830 showed the same alleles as SRN39 for the tightly linked markers, supporting the presence of a common gene in both accessions for *Striga* germination stimulant activity for both species. Moreover, allelism tests for the *lgs* gene between IS9830 and SRN39 also indicates mutation at a common locus since (SRN39 × IS9830)$F_1$ retains the low stimulant phenotype (MGD = 1.1, unpublished results). Several studies have reported the association of low germination stimulant activity with *Striga* field resistance in sorghum (Ramaiah 1987; Hess and Ejeta 1992; Hess et al. 1992; Haussmann et al. 2004). The most consistent and major *S. hermonthica* field resistance QTL corresponded to the *lgs* locus in the latter study. Analysis of QTL regions with multiple field evaluations under *Striga* infestation using a sub-set of the RIL population that we used for mapping in the present study showed that one of the major and consistent QTLs common to both *S. asiatica* and *S. hermonthica* field resistance is associated again with the *lgs* locus (Ibrahim 1999). However, the identification of additional QTLs in both the QTL experiments with field measurements of *Striga* resistance strongly suggests the involvement of some additional resistance mechanisms controlled by other genes.

Validation of markers associated with a gene or QTL is an essential requirement for MAS in resistance breeding programs. In the present study, we validated three co-segregating markers (SB3344, SB3346 and SB3352) tightly linked to the *lgs* gene in a set of 23 diverse sorghum accessions phenotyped for reaction to two *Striga* spp. We did not include SB3343 marker in the validation test, although it was tightly linked, due to dominant scoring of the marker in the RILs. Based on the marker genotyping, we found that the alleles corresponding to low *Striga* germination stimulant activity in the donor parent SRN39 were shared by nearly all other low stimulant lines. We were able to distinguish 21 out of 23 lines based on the marker allelic banding pattern (Table 3; Fig. 4). Nine lines used in the validation were SRN39 derivatives (P940_series and Brahan) which were released as *Striga*-resistant varieties in several African countries (Mangombe et al. 2000; Mbwaga et al. 2007; Tesso et al. 2007). Some of these lines have inherited the low *Striga* germination stimulant activity, while others possess only the other resistance trait from SRN39, incompatibility (Amusan et al. 2011), independently inherited from the low stimulant trait (Grenier et al. 2001). Among these nine SRN39 derivatives, all four with low *Striga* germination stimulant activity carry the SRN39 type alleles. This suggests that these tags could be successfully used to introgress the low stimulant resistance trait into new and existing sorghum varieties.

Among the sources of low *Striga* germination stimulant activity, Framida, 555, SAR33, IS9830, ICSV1006, ICSV1007, Tetron, and the wild sorghum accession PQ-434, all carried the SRN39 marker alleles for the *lgs* gene except 555 and PQ-434. ICSV1007 did not share the SRN39 allele at SB3352, possibly due to a recombination event between this marker and *lgs* which uncoupled the two. The accessions 555 and Framida have been used by ICRISAT as sources of low germination stimulant-based resistance in its 'SAR' (*Striga asiatica* resistant) lines (Vasudeva Rao 1985). We determined through genetic tests that the low *Striga* germination stimulant activity of 555 is recessive and allelic to SRN39, based on the high stimulant activity of the $F_1$ hybrid of the cross Shanqui Red × 555 (MGD = 10.3). A cross between both low stimulant parents [(SRN39 × 555)$F_1$] has a low stimulant phenotype, showing an MGD of 2.0 (unpublished results), indicating that no complementation has occurred and so the mutations must affect the same gene. But SRN39 clearly has different alleles at the *lgs*-associated markers than 555 (Table 3; Fig. 4). Hence, we hypothesize that 555 contains an independent mutation in *lgs* which occurred in a background coupled with different alleles at the marker micro-satellites. Similar to 555, the low *Striga* germination stimulant accession PQ-434 did not share the SRN39 type alleles for any of the associated markers. Genetic tests confirmed that this mutant *lgs* phenotype, like SRN39, is recessive since a high germination stimulant activity is restored in hybrids of this line with high stimulant lines. The MGD of $F_1$ hybrid between CK60 (high stimulating) and PQ-434 (low stimulating), for instance, is 10.1 (unpublished results). In crosses of PQ-434 with lines carrying mutant (SRN39 type) alleles at *lgs*, however, wildtype function is restored, [the MGD of (SRN39 × PQ-434)$F_1$ = 10.2 (unpublished results)]. Because complementation occurs in the $F_1$, the

mutations likely involve different genes in both SRN39 and PQ-434 genotypes.

It appears that the recessive alleles at *lgs*, and those at the mutated gene in PQ-434, result in low germination stimulant activity toward both *S. asiatica* and *S. hermonthica*. The phenotyping and genotyping of the 23 diverse accessions of the validation portion of this study showed similar MGD values in the AGA, regardless of which *Striga* spp. was used. This suggests that the markers would be broadly applicable in improving resistance to both *Striga* spp.

Synteny between the genomes of major cereal crops is often used as a base for molecular marker saturation, identification of candidate genes, and for positional cloning of target genes (Li et al. 2010; Vu et al. 2010). Different QTL studies on *Striga* field resistance in rice reported both major and minor QTLs (Kaewchumnong and Price 2008; Swarbrick et al. 2009). However, genetics of *Striga* germination stimulant activity is not well described in rice. Comparative analysis of the sorghum *lgs* region on SBI-05 with the rice genome determined that it is syntenic to rice chromosome 11. Although, this rice chromosome harbored some *S. hermonthica* field resistance QTLs, the position of the QTLs is away from the syntenic region. Furthermore, the mapping and QTL analysis with a sorghum *DWARF27* ortholog, which we identified based on syntenic rice chromosomal position, showed no association with the sorghum *lgs* gene, indicating that *DWARF27* is likely not the actual gene involved in *Striga* germination stimulant activity in sorghum. Though *DWARF27* in rice is reported to be involved in strigolactone biosynthesis, the exact role of the orthologous gene in sorghum is unknown. It is likely involved in strigolactone biosynthesis in sorghum but the mutation responsible for low *Striga* germination stimulant activity among the sorghum accessions we have examined does not appear to involve this locus. As with many strigolactone mutants, the rice *DWARF27* mutant has a typical hypertillering phenotype because of the phytohormone role of strigolactones in inhibition of shoot branching (Lin et al. 2009). Among all the sorghum cultivars examined in this study, none possess a hypertillering phenotype. However, PQ-434 (*S. bicolor drummondii*), as is typical of wild sorghums, does tiller significantly more than the cultivated *S. bicolor* accessions. Whether the non-allelic mutation, which we identified based on allelism studies, carried in this accession is associated with the *DWARF27* ortholog is being examined. Detailed studies are needed to confirm the exact role of *DWARF27* in the strigolactone biosynthetic pathway and its contribution to *Striga* germination stimulant activity in both the sorghum and rice. Our results on *lgs* mapping would form a base for further understanding and exploitation of the trait in sorghum, rice and other crops that serve as hosts for *Striga*.

Dihydrosorgoleone, chemically distinct from the strigolactones, is another compound present in the sorghum rhizosphere with *Striga* germination stimulant activity (Netzly et al. 1988). It is produced in the oily coating of sorghum root hairs and upon exudation is quickly converted by autoxidation to sorgoleone, sorghum's notorious allelopathic agent. Although very little variation in the levels of dihydrosorgoleone (measured as sorgoleone) was found between many of the high and low germination stimulant accessions (N13, P954063, SRN39, Framida and IS9830) used by Hess et al. (1992) study, others reported a nearly 30-fold variation among 25 sorghum varieties (Nimbal et al. 1996). Some have also suggested that variations in the levels of resorcinol (methoxy-dihydrosorgoleone), a biochemically related metabolite, could stabilize dihydrosorgoleone, preserving its function and thereby modulating the *Striga* germination stimulant activity in the rhizosphere (Erickson et al. 2001). *Alkylresorcinol synthase 1* (*ARS1*), a sorghum gene involved in biosynthesis of dihydrosorgoleone, was recently identified on SBI-05 and cloned (Cook et al. 2010; Phytozome database sorghum gene ID is Sb05g022500). We developed two primer pairs (Pustri 15 and 16) targeting the *ARS1* and tested polymorphism between the parents of our RIL population (Table 2). However, we were not able to resolve polymorphism on the agarose gel between the parents, hence we mapped the gene through in silico mapping based on the available sequence. We identified the physical location of the *ARS1* gene on the sorghum SBI-05 at 54.4 Mbp, very close to the sorghum ortholog of *DWARF27* (at 55.1 Mbp). Our QTL analysis for *Striga* germination stimulant activity identified the *lgs* gene between the markers SB3344-SB3352 located on the physical map between 60.2 and 60.7 Mbp, completely away from the region containing both the *ARS1* and *DWARF27* genes. This indicates that neither is responsible for the variation we observed in *Striga* germination stimulant activity in our mapping population. This is supported by earlier observations of a lack of correlation between measured sorgoleone levels in both resistant and susceptible accessions for *Striga* germination stimulant activity measured in the AGA (Hess et al. 1992).

The ultimate use of molecular markers is to enhance the efficiency of breeding via MAS and to identify genes involved in the trait expression through map-based cloning. The recent availability of the sorghum genome sequence facilitates identification of probable candidate genes underlying any genomic region on the genetic map. The *lgs* gene in this study was flanked by two microsatellite markers at a distance of 0.5 and 1.5 cM with a physical sequence distance of approximately 400 kb, harboring 30 genes. Unfortunately, the details of strigolactone biosynthesis are still rather sketchy, especially in the details of precursor–product relationships between individual strigolactones.

It is generally agreed that the strigolactones as a group are derived from the carotenoid biosynthetic pathway in plants (Matusova et al. 2005) and that 5-deoxystrigol is the proto-strigolactone, with addition of hydroxyl groups and other modifications giving rise to the other known strigolactones (Rani et al. 2008). The characterized genes closest to the tightly linked markers are those coding for NBS-LRR protein, NB-ARC protein, Glutathione-S-transferase, Serine–threonine protein kinase, Epoxide hydrolase, Laccase15, and several Cytochrome P450s (Table 4). Although some laccases and NB-LRR proteins are involved in parasitic plant interactions with their hosts (Yoder and Scholes 2010), they affect steps in the parasitic association beyond germination stimulation.

It is likely that the *lgs* gene is involved in strigolactone biosynthesis or exudation. None of the characterized genes in the *lgs* fine-mapped region (Table 4) is known to be directly involved in strigolactone biosynthesis, but then very few genes specific to this pathway have been identified. No annotated genes in the region match any of the few described strigolactone biosynthetic genes, the *MAX1*, *MAX3* and *MAX4* genes from *Arabidopsis* or their orthologs in pea, petunia or rice (Xie et al. 2010), although orthologs of these are present at distal locations of the sorghum genome. Strong candidates in the fine-mapped interval include an Epoxide hydrolase (Sb05g026560) which could be involved in production of some strigolactone intermediates in the proposed pathway (Rani et al. 2008). The region also contains several genes coding for enzymes with cytochrome P450 functional domains (Sb05g026580, Sb05g026600 and Sb05g026610) which could catalyze any of the several proposed oxidation steps in the strigolactone biosynthetic pathway (Rani et al. 2008). Of the few described genes involved in strigolactone biosynthesis, *MAX1* codes for a cytochrome P450 (Booker et al. 2005). We observed no aberrant tillering habits among the cultivars with low germination stimulant activity. If the common mutation in *lgs* we find among the low stimulant sorghum cultivars affects strigolactone content of the root exudates, either qualitatively or quantitatively, the alteration is not sufficient to obviously affect shoot morphology. Perhaps, the deficiencies reported in 5-deoxystrigol levels in the root exudates of SRN39 (Yoneyama et al. 2010) are compensated by other strigolactones that can substitute for the inhibition of shoot branching.

Alternatively, the mutation may specifically affect exudation of the strigolactones, without critical effect on their levels in the shoot meristems. Among the uncharacterized genes in the region is a gene encoding a pectin methylesterase (PME) inhibitor (Sb05g026520). These proteins regulate the pectin content of cell walls, including those of roots. It is known, for instance that PME activity is associated with aluminum tolerance because their activity modulates exudation of organic acids at the root tip that can prevent absorption of aluminum (Mimmo et al. 2009). Mutations in a PME inhibitor may alter strigolactone exudation as well.

Further metabolic characterization of the *lgs* mutants may provide clues to narrow down the candidate genes. If it involves strigolactones, then this mutation will be very useful in revealing the biochemical details of these broad acting compounds. By analyzing a range of published QTL studies in plants, Price (2006) showed that, in many cases, the gene was located within 1–2 cM of the QTL peak. It is, therefore, likely that *lgs* is among those 30 annotated genes in the region (Table 4). In this case, rather than saturation mapping with additional molecular markers in a high-resolution mapping population, a combination of QTL analysis with gene expression data would be advantageous as there are only a few genes available in the region. This approach has been well demonstrated for a *Striga* resistance major QTL region in rice (Swarbrick et al. 2008).

This study shows that *Striga* germination stimulant activity in sorghum is determined by a single major gene, with low germination stimulant activity inherited through recessive alleles. The tagging and validation of microsatellite markers linked to the *lgs* gene in diverse sorghum accessions indicated that they could be effectively used for marker-assisted breeding to enhance *Striga* resistance in sorghum. The molecular marker tags for the *lgs* gene identified in this study could be useful for indirect assaying of the sorghum accessions for their *Striga* germination stimulant activity without any laborious laboratory assay. Additionally, the fine-mapping of the *lgs* region provided clues to the possible candidate genes forming a foundation for map-based cloning, isolation of the gene and elucidation of the exact molecular basis of low germination stimulant *Striga* resistance.

**Acknowledgments** We gratefully acknowledge financial support from the United States Agency for International Development (USAID) INTSORMIL grant #EPP-A-0006-00016-00.

## References

Akiyama K, Matsuzaki KI, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Amusan I, Rich P, Housley T, Ejeta G (2011) An in vitro method for identifying post-attachment *Striga* resistance in maize and sorghum. Agron J 103:1472–1478

Awad A, Sato D, Kusumoto D, Kamioka H, Takeuchi Y, Yoneyama K (2006) Characterization of strigolactones, germination stimulants for the root parasitic plants *Striga* and *Orobanche*, produced by maize, millet and sorghum. Plant Growth Regul 48:221–227

Bhattramakki D, Dong J, Chabra AK, Hart GE (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Booker J, Sieberer T, Wright W, Williamson L, Willett B, Stirnberg P, Turnbull C, Srinivasan M, Goddard P, Leyser O (2005) *MAX1* encodes a Cytochrome P450 family member that acts downstream of *MAX3/4* to produce a carotenoid-derived branch-inhibiting hormone. Dev Cell 8:443–449

Cook CE, Whichard LP, Wall ME, Egley GH, Coggan P, Luhan PA, McPhail AT (1972) Germination stimulants II. The structure of strigol, a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). J Am Chem Soc 94:6198–6199

Cook D, Rimando AM, Clemente TE, Schroder J, Dayan FE, Nanayakkara NPD, Pan Z, Noonan BP, Fishbein M, Abe I, Duke SO, Baerson SR (2010) Alkylresorcinol synthases expressed in *Sorghum bicolor* root hairs play an essential role in the biosynthesis of the allelopathic benzoquinone sorgoleone. Plant Cell 22:867–887

Ejeta G (2007a) The *Striga* scourge in Africa: a growing pandemic. In: Ejeta G, Gressel J (eds) Integrating new technologies for *Striga* control: towards ending the witch-hunt. World Scientific Publishing Company PTE LTD, Singapore, pp 3–16

Ejeta G (2007b) Breeding for *Striga* resistance in sorghum: exploitation of an intricate host-parasite biology. Crop Sci 47(S3): S216–S227

Ejeta G, Butler LG (1993) Host plant resistance to *Striga*. In: Buxton DR, Shilbes R, Forsburg RA, Blad BL, Asay KH, Paulsen GM, Wilson RF (eds) International crop science I, vol 1. Crop Science Society of America, Madison, pp 561–569

Erickson J, Schott D, Reverri T, Muhsin W, Ruttledge T (2001) GC–MS analysis of hydrophobic root exudates of *Sorghum* and implications on the parasitic plant *Striga asiatica*. J Agric Food Chem 49:5537–5542

Frost DL, Gurney AL, Press MC, Scholes JD (1997) *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA. Plant Cell Env 20:492–4873

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194

Graves JD, Wylde A, Press MC, Stewart GR (1990) Growth and carbon allocation in *Pennisetum typhoides* infected with the parasitic angiosperm *Striga hermonthica*. Plant Cell Env 13:367–373

Grenier C, Rich PJ, Mohamed A, Ellicott A, Shaner C, Ejeta G (2001) Independent inheritance of *lgs* and *IR* genes in sorghum. In: 7th international parasitic weed symposium. Nantes, France, pp 220–223

Hauck C, Muller S, Schilknecht H (1992) A germination stimulant for parasitic flowering plants from *Sorghum bicolor*, a genuine host plant. J Plant Physiol 139:474–478

Haussmann BIG, Hess DE, Omanya GO, Reddy BVS, Welz HG, Geiger HH (2001) Major and minor genes for stimulation of *Striga hermonthica* seed germination in sorghum, and interaction with different *Striga* populations. Crop Sci 41:1507–1512

Haussmann BIG, Hess DE, Omanya GO, Folkertsma RT, Reddy BV, Kayentao M, Welz HG, Geiger HH (2004) Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. Theor Appl Genet 109:1005–1016

Hess DE, Ejeta G (1992) Inheritance of resistance to *Striga* in sorghum genotype SRN39. Plant Breed 109:233–241

Hess DE, Ejeta G, Butler LG (1992) Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to *Striga*. Phytochem 31:493–497

Ibrahim YH (1999) A sorghum linkage map and predicted response to phenotypic and markers selection for resistance to *Striga* in sorghum. Dissertation, Purdue University

Joel DM, Hershenhorn J, Eizenberg H, Aly R, Ejeta G, Rich PJ, Ransom JK, Sauerborn J, Rubiales D (2007) Biology and management of weedy root parasites. In: Janick J (ed) Horticultural reviews. John Wiley & Sons, Inc., New York, pp 267–349

Kaewchumnong K, Price AH (2008) A study on the susceptibility of rice cultivars to *Striga hermonthica* and mapping of *Striga* tolerance quantitative trait loci in rice. New Phytol 180:206–216

Kim JS, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM (2005) Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics 169:1169–1173

Knoll J, Gunaratna N, Ejeta G (2008) QTL analysis of early-season cold tolerance in sorghum. Theor Appl Genet 116:577–587

Li Q, Yang X, Bai G, Warburton ML, Mahuku G, Gore M, Dai J, Li J, Yan J (2010) Cloning and characterization of a putative GS3 ortholog involved in maize kernel development. Theor Appl Genet 120:753–763

Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J, Wanga Y (2009) *DWARF27*, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice Tiller bud outgrowth. Plant Cell 21:1512–1525

Mangombe N, Mabasa S, Obilana AT (2000) Breeding sorghum for *Striga* resistance in Zimbabwe. In: Haussmann BIG, Hess DE, Koyama ML, Grivet L, Rattunde HFW, Geiger HH (eds) Breeding for *Striga* resistance in cereals. Margraf, Ibadan, p 335

Matusova R, Rani K, Verstappen FWA, Franssen MCR, Beale MH, Bouwmeester HJ (2005) The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiol 139:920–934

Mbwaga AM, Riches C, Ejeta G (2007) Integrated *Striga* management to meet sorghum demand in Tanzania. In: Ejeta G, Gressel J (eds) Integrating new technologies for *Striga* control: towards ending the witch-hunt. World Scientific Publishing Company PTE LTD, Singapore, pp 253–264

Michelmore RW, Paran I, Kessel RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mimmo T, Marzadori C, Gessa C (2009) Does the degree of pectin esterification influence aluminium sorption by the root apoplast? Plant and Soil. Springer, Netherlands, pp 159–168

Mohamed KI, Musselman LJ, Riches CR (2001) The genus *Striga* (Scrophulariaceae) in Africa. Ann Missouri Bot Garden 88:60–103

Mutengwa CS, Tongoona PB, Sithole-Niang I (2005) Genetic studies and a search for molecular markers that are linked to *Striga asiatica* resistance in sorghum. Afr J Biotech 4:1355–1361

Netzly DH, Riopel JL, Ejeta G, Butler LG (1988) Germination stimulants of witchweed (*Striga asiatica*) from hydrophobic root exudate of sorghum (*Sorghum bicolor*). Weed Sci 36:441–446

Nimbal CI, Pedersen JF, Yerkes CN, Weston LA, Weller SC (1996) Phytotoxicity and distribuition of sorgoleone in grain sorghum. J Agric Food Chem 44:1343–1347

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A et al (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Price AH (2006) Believe it or not, QTLs are accurate. Trends Plant Sci 11:213–216

Ramaiah KV (1987) Breeding cereal grains for resistance to witchweed. In: Musselman LJ (ed) Parasitic weeds in agriculture, vol 1. CRC Press, Boca Raton, pp 227–242

Ramaiah KV, Chidley VL, House LR (1990) Inheritance of *Striga* seed germination stimulant in sorghum. Euphytica 45:33–38

Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ (2008) Biosynthetic considerations could assist the structure

elucidation of host plant produced rhizosphere signalling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. Plant Physiol Biochem 46:617–626

Rich PJ, Grenier C, Ejeta G (2004) *Striga* resistance in wild relatives of sorghum. Crop Sci 44:2221–2229

Rodenburg J, Bastiaans L, Kropff MJ, Van Ast A (2006) Effects of host plant genotype and seedbank density on *Striga* reproduction. Weed Res 46:251–263

Saghai-Maroof MA, Soliman KM, Jorgenen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Satish K, Srinivas G, Madhusudhana R, Padmaja PG, Nagaraja Reddy R, Murali Mohan S, Seetharama N (2009) Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 119(8):1425–1439

Sauerborn J (1991) The economic importance of the phytoparasites *Orobanche* and *Striga*. In: 5th international symposium on parasitic weeds. Nairobi, Kenya, pp 137–143

Saxton AM (1998) A macro for converting mean separation output to letter groupings in Proc Mixed. In: Proceedings of the 23rd SAS users group international. SAS Institute, Cary, pp 1243–1246

Scholes JD, Press MC (2008) *Striga* infestation of cereal crops—an unsolved problem in resource limited agriculture. Curr Opin Plant Biol 11:180–186

Siame BA, Weerasuriya Y, Wood K, Ejeta G, Buttler LG (1993) Isolation of strigol, a germination stimulant for *Striga asiatica*, from host plants. J Agric Food Chem 41:1486–1491

Swarbrick PJ, Huang K, Liu G, Slate J, Press MC, Scholes JD (2008) Global patterns of gene expression in rice cultivars undergoing a susceptible or resistant interaction with the parasitic plant *Striga hermonthica*. New Phytol 179(2):515–529

Swarbrick PJ, Scholes JD, Press MC, Slate J (2009) A major QTL for resistance of rice to the parasitic plant *Striga* hermonthica is not dependent on genetic background. Pest Manag Sci 65:528–532

Tesso T, Deressa A, Gutema Z, Ejeta G (2007) An integrated *Striga* management (ISM) option offers effective control of *Striga* in Ethiopia. In: Ejeta G, Gressel J (eds) Integrating new technologies for *Striga* control: Towards ending the witch-hunt. World Scientific Publishing Company PTE LTD, Singapore, pp 199–212

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455:U129–U195

Van Ooijen JW (2005) Map-QTL® 5: software for the mapping quantitative trait loci in mapping populations. Kyazma BV, Wageningen

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0® software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vasudeva Rao MJ (1985) Techniques for screening sorghums for resistance to *Striga*. In: Musselman LJ (ed) Parasitic weeds in agriculture. CRC Press, Inc., Boca Raton, pp 281–304

Vogler RK, Ejeta G, Butler LG (1996) Inheritance of low production of *Striga* germination stimulant in sorghum. Crop Sci 36:1185–1191

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTL. J Hered 93:77–78

Vu GTH, Wicker T, Buchmann JP, Chandler PM, Matsumoto T, Graner A, Stein N (2010) Fine mapping and syntenic integration of the semi-dwarfing gene sdw3 of barley. Funct Integr Genomics 10:509–521

Weber WE, Wrickle G (1994) Genetic markers in plants breeding. Adv Plant Breed, Parey Scientific Publ., Berlin No. 16

Williams CN (1959) Resistance of *Sorghum* to witchweed. Nature 184:1511–1512

Xie X, Yoneyama K, Kusumoto D, Yamada Y, Takeuchi Y, Sugimoto Y, Yoneyama K (2008) Sorgomol, germination stimulant for root parasitic plants, produced by *Sorghum bicolor*. Tetrahedron Lett 49:2066–2068

Xie X, Yoneyama K, Yoneyama K (2010) The strigolactone story. Annu Rev Phytopathol 48:93–117

Xin Z, Velten JP, Oliver MJ, Burke JJ (2003) High-throughput DNA extraction method suitable for PCR. BioTechniques 34:820–826

Yoder JI, Scholes JD (2010) Host plant resistance to parasitic weeds; recent progress and bottlenecks. Curr Opin Plant Biol 13:478–484

Yonemaru J, Ando T, Mizubayashi T, Kasuga S, Matsumoto T, Yano M (2009) Development of genome-wide simple sequence repeat markers using whole-genome shotgun sequences of sorghum (*Sorghum bicolor* (L.) Moench). DNA Res 16:187–193

Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y, Yoneyama K (2007) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227:125–132

Yoneyama K, Awad AA, Xie X, Yoneyama K, Takeuchi Y (2010) Strigolactones as germination stimulants for root parasitic plants. Plant Cell Physiol 51:1095–1103