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ORIGINAL PAPER

Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract The shoot fly is one of the most destructive insect pests of sorghum at the seedling stage. Deployment of cultivars with improved shoot fly resistance would be facilitated by the use of molecular markers linked to QTL. The objective of this study was to dissect the genetic basis of resistance into QTL, using replicated phenotypic data sets obtained from four test environments, and a 162 microsatellite marker-based linkage map constructed using 168 RILs of the cross 296B (susceptible) × IS18551 (resistant). Considering five component traits and four environments, a total of 29 QTL were detected by multiple QTL mapping (MQM) viz., four each for leaf glossiness and seedling vigor, seven for oviposition, six for deadhearts, two for adaxial trichome density and six for abaxial trichome density. The LOD and R^2 (%) values of QTL ranged from 2.6 to 15.0 and 5.0 to 33%, respectively. For most of the QTL, IS18551 contributed resistance alleles; however, at six QTL, alleles from 296B also contributed to resistance. QTL of the related component traits were co-localized, suggesting pleiotropy or tight linkage of genes. The new morphological marker *Trit* for trichome type was associated with the major QTL for component traits of resistance. Interestingly, QTL identified in this study correspond to QTL/genes for insect resistance at the syntenic maize genomic regions, suggesting the conservation of insect resistance loci between these crops. For majority of the QTL, possible candidate genes lie within or very near the ascribed confidence intervals in sorghum. Finally, the QTL identified in the study should provide a foundation for marker-assisted selection (MAS) programs for improving shoot fly resistance in sorghum.

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Introduction

Sorghum bicolor (L.) Moench is one of the most important crops in the world because of its adaptation to a wide range of ecological conditions, suitability for low input cultivation and diverse uses (Doggett 1988). It is grown on about 10.4 Mha in India, with an annual grain production of 8 Mt (FAO 2002). During its cultivation, the crop is exposed to several stresses, starting from the seedling stage to harvest, and biotic stresses have the maximum impact on crop growth (Dhillon et al. 2005). More than 150 species of insect pests damage sorghum, of which sorghum shoot fly, *Atherigona soccata* (Rondani), is the most important pest in Africa, Asia and Mediterranean Europe (Sharma 1993) during early stage of crop growth and establishment. Shoot flies of the genus *Atherigona* are also known to cause 'deadhearts' in a number of tropical grass species (Pont 1972) and wheat (Pont and Deeming 2001). In India, the losses due to shoot fly damage have been estimated to reach as high as 90% of grain, and 45% of fodder yield (Sukhani and Jotwani 1980; Jotwani 1982). In India, the annual economic losses in sorghum due to this pest have been estimated at US$200 million (ICRISAT 1992).

The shoot fly attacks sorghum seedlings between 7 and 28 days after the emergence of the seedling (Nwanze et al. 1990). The female fly lays white, elongated, cigar-shaped

eggs singly on the abaxial (lower) surface of the leaves, parallel to the midrib. The eggs hatch in 1–2 days of incubation and larvae enter the seedling's whorl of the central leaf, where it cuts the growing point, and feed on the decaying leaf tissue, resulting in a typical wilting and drying of the central whorl leaf, a condition called "deadheart" (Pont 1972). As a result of deadheart formation, the young seedlings may be killed outright or they may produce axial tillers, which are rarely productive. The axial tillers serve as a mechanism of recovery resistance if they remain undamaged, but if shoot fly infestation continues, the seedling may die or present a rosette appearance and fail to produce any grain (Dhillon et al. 2005). The pest is especially serious in late-sown crops, but sometimes appears with early sowing also, when the preceding dry season is interrupted by frequent showers of rain (Nimbalkar and Bapat 1987). The levels of infestation may go up to 90–100% under delayed sowing (Hiremath and Renukarya 1966).

Earlier studies on the genetics of shoot fly resistance suggested that the component traits of resistance are complex and quantitatively inherited (Goud et al. 1983; Hallali et al. 1983; Agrawal and Abraham 1985), with predominantly additive gene effects (Nimbalkar and Bapat 1992). Shoot fly resistance in sorghum was classified into three components, viz., non-preference for oviposition, antibiosis and tolerance (Soto 1974). Under field conditions, resistance to shoot fly is primarily due to non-preference for oviposition (also called antixenosis, observed as reduction in the number of eggs laid on the seedling) (Jotwani et al. 1971). Many other important component traits (reviewed by Sukhani 1987) such as leaf glossiness, leaf trichomes, seedling vigor, epicuticular wax (Nwanze et al. 1992) and biochemical factors (Singh et al. 2004) are also associated with shoot fly resistance in sorghum.

The severity of shoot fly infestation can be reduced by good management practices, of which the use of resistant cultivars is the most effective, economical and eco-friendly approach to control the pest. Although many notable successes have been achieved through conventional breeding in the improvement of plant resistance to insects, the breeding process is often slow and laborious, and sufficient levels of resistance have not been achieved due to the quantitative nature of resistance (Tao et al. 2003). However, concerted efforts toward breeding for shoot fly resistance have resulted in some progress, and a number of genotypes with resistance to shoot fly have been identified (Singh and Rana 1996; Kumar et al. 2000; Sharma et al. 2003). Unfortunately, all high-yielding sorghum cultivars presently under cultivation in India are highly susceptible to shoot fly, prompting the national program to fix a threshold level of resistance before any cultivar can be officially released for cultivation. In addition to the quantitative nature of resistance, the progress in improving resistance levels of sorghum cultivars using the identified resistance sources and other wild resistant genotypes through conventional breeding methods has been slow, in part due to difficulty in crossing cultivated sorghum with wild genotypes.

Given the economic impact of shoot fly, the improvement of genetic resistance to this pest is one of the major goals in sorghum breeding programs in India. In this context, a better understanding of the inheritance of resistance and the identification of genomic regions/QTL that influence resistance can help the breeders to develop more efficient and effective breeding and selection schemes through marker-assisted selection (MAS). Use of molecular markers and identification of quantitative trait loci (QTL) have been demonstrated in sorghum for other insect pests such as green bug (Agrama et al. 2002; Nagaraj et al. 2005; Wu and Huang 2008), head bug (Deu et al. 2005) and midge (Tao et al. 2003).

The objectives of the present study were to genetically map QTL for different component traits of shoot fly resistance and to identify QTL-associated molecular markers for use in marker-assisted breeding. In addition, we report the development and mapping of some new genomic-microsatellite markers on to the previously constructed microsatellite linkage map (Srinivas et al. 2009b).

Materials and methods

Plant material

Mapping was performed using a set of 168 F_7 recombinant inbred lines (RILs) developed from a sorghum cross between highly susceptible Indian seed parent 296B and the stable resistant germplasm line IS18551 through single-seed descent method. The shoot fly-susceptible parent, 296B, is a well-adapted, rainy season genotype, characterized by semi-compact ear head, creamy grains, tan plant color and dwarf plant height. The resistant parent IS18551 is a line of sorghum *durra* race from Ethiopia having highly contrasting characters in comparison to the susceptible parent. The distinctive features of this parent are its narrow, pointed and shiny leaves, ear head with straw-colored grain, larger glumes and tall stature. Of the several thousand lines screened for resistance, IS18551 was identified as one of the stable resistant lines for both shoot fly and stem borer (Nwanze et al. 1991; Bantilan et al. 2004).

Field evaluation of shoot fly resistance

The 168 F_7 RILs along with the two parents (296B and IS18551) were evaluated in four test environments [two rabi

(post-rainy) and two kharif (rainy) environments] during 2003–2005 at the research farm of the Directorate of Sorghum Research (DSR), Hyderabad, India. The test environments were abbreviated as follows: R03, R04, K04 and K05, representing the environments rabi 2003, rabi 2004, kharif 2004 and kharif 2005, respectively. The experiment was carried out in a randomized complete block design (RCBD) with three replications. Each entry was planted in a single row plot (4 m long) and the spacing between the rows was 45 cm. The seedlings were thinned at 10 days after seedling emergence (DAE) to a plant-to-plant spacing of 15 cm with a total plant stand of 26 plants per plot. Field evaluations were conducted under high stress condition using the interland fish-meal technique (Soto 1974). All the recommended agronomic practices, except plant protection measures, were followed to raise a good crop.

Phenotyping and data analysis

Phenotypic data on shoot fly resistance were recorded on five component traits, viz., leaf surface glossiness (abbreviated as GS), seedling vigor (SV), oviposition [number of eggs laid on seedling at 21 and 28 days after seedling emergence (DAE), abbreviated as EG21 and EG28, respectively], deadhearts (DH%) and leaf surface trichome density on adaxial (upper; TDU) and abaxial (lower; TDL) leaf surfaces. Leaf glossiness was visually scored on a scale of 1–5 scores at 12 DAE [1 = non-glossy (dark green, dull, broad, and drooping leaves, and 5 = high glossy (light green, shiny, narrow and erect leaves)]. Seedling vigor (height, leaf growth, robustness) was visually scored at 14 DAE on a 1–5 scale, where 1 = low vigor (plants showing minimum growth, less leaf expansion) and 5 = high vigor (plants showing maximum height, leaf expansion and robustness). Ovipositional non-preference was expressed in terms of eggs per plant and it was recorded by counting the total number of eggs laid on five seedlings at random from each plot. The mean number of eggs per seedling were calculated on 21 and 28 DAE. Overall resistance was recorded as the percentage of deadhearts (DH%) caused by shoot fly infestation. To record data on DH%, the total number of plants was initially recorded, and the number of plants with deadhearts were subsequently recorded on 21 and 28 DAE. The mean values of DH% (ratio of the number of deadhearts/total number of plants × 100) recorded on 28 DAE were used for QTL identification. Trichome density on the adaxial leaf surface (TDU) and abaxial surface (TDL) was recorded at 14 DAE on the central portion of the fifth leaf from the base, in three randomly selected seedlings in each plot. For measuring trichome density, leaf segments (approximately 2 cm^2) were cleared in acetic acid:alcohol (2:1) and transferred to 90% lactic acid in small vials (Maiti et al. 1980). The leaf segments were then mounted on a slide in a drop of water and observed under stereomicroscope at a magnification of 20×. The number of trichomes on both abaxial and adaxial leaf surface was counted in three microscopic fields at random and expressed as trichome density (no./mm^2). The two parents of the present study also differ in the trichome morphology (the susceptible parent 296B possesses bicellular and blunted trichomes, whereas the resistant parent IS18551 possesses unicellular and pointed trichomes). Hence, the trichome morphology (bicellular blunted vs. unicellular pointed) was also scored in the RILs segregating for either type to map the trait as a morphological marker (*Trit*) on the linkage map.

Phenotypic data were analyzed using the statistical software, Minitab (http://www.minitab.com). The data from individual environments for all traits were subjected to analysis of variance (ANOVA). However, the data from across environments were also used to determine the effect of genotype (RIL), environment, genotype × environment (G × E) and error variance. While performing ANOVA for environments, the replications were nested within environments treating replications, environments and all other effect involving environment as random, and genotypes (RILs) as fixed factors. Estimates of the genotypic variances $\left(\sigma_g^2\right)$ and genotype by environment interaction variances $\left(\sigma_{ge}^2\right)$ were estimated by equating the computed mean squares to their respective expectations and solved as $\sigma_g^2 = (\mathrm{MSG} - \mathrm{MSGE})/\mathrm{RE}$ and $\sigma_{ge}^2 = (\mathrm{MSGE} - \mathrm{MSE})/\mathrm{R}$; and the phenotypic variances were estimated as $\sigma_{pv}^2 = (\mathrm{MSG}/\mathrm{RE})$; i.e., $\sigma_{pv}^2 = \left(\sigma_g^2 + \sigma_{ge}^2/\mathrm{E}\right) + \left(\sigma_e^2/\mathrm{RE}\right)$. Here, MSG, MSGE and MSE are the mean squares of the RILs, of the genotype by environment interaction, and of the error; and R and E are the number of replications and environments, respectively. Heritability estimates across environments were calculated on entry mean basis as described by Hallauer and Miranda (1981), using the formula given below, and exact confidence intervals for heritability were computed following the procedure described by Knapp et al. (1985). Genotypic correlations among traits were estimated using the method of moments (Mode and Robinson 1959).

$$h^2 = \frac{\sigma_g^2}{\frac{\sigma_e^2}{re} + \frac{\sigma_{ge}^2}{e} + \sigma_g^2}.$$

Linkage map

The genetic linkage map of 296B × IS18551 RIL population previously reported by our group (Srinivas et al. 2009b) was further updated in the present study by adding seven new polymorphic genomic-microsatellite markers

(named as Xnhsbm series), two genomic-microsatellite markers (unmapped in our previous map) and one new morphological marker (*Trit*; for trichome morphology). Collectively, the genetic linkage map was generated with 162 marker loci consisting of 100 genomic-microsatellite markers, 49 genic-microsatellite markers and four morphological markers (including the new morphological marker *Trit* mapped in this study). To increase marker density in a large chromosomal gap in our previously reported map on SBI-10 [between the markers pepC–Xtxp141 (according to Bhattramakki et al. 2000)], we developed new genomic-microsatellite markers by specifically targeting the gap, using the sorghum whole shotgun genome sequences (Paterson et al. 2009; http://www.phytozome.org). The parameters set for marker development and PCR amplification conditions were followed as described in our previous studies (Srinivas et al. 2008, 2009a). Genotyping of RILs, linkage map construction and naming of chromosomes were also described in our previous reports (Srinivas et al. 2008, 2009a, b). Briefly, touchdown PCR amplification profile (Smith et al. 2000) was used for all microsatellite markers. The amplicons were separated on 6% denaturing polyacrylamide gel electrophoresis (PAGE) and visualized by silver staining (Fritz et al. 1995). The alleles were manually scored in RILs as A (parent 1 allele), B (parent 2 allele) and H (heterozygote-carrying alleles from both parents). The linkage map was constructed using the software JOINMAP 3.0 (Van Ooijen and Voorrips 2001) and the linkage groups were named according to Kim et al. (2005).

QTL mapping

The QTL mapping was performed for mean values of traits in each environment and mean values of each trait averaged across the four environments using the software MapQTL 5® (Van Ooijen 2005). The interval mapping (Lander and Botstein 1989) was performed initially for the presence of a QTL for each position on the map. The threshold value for a genome-wide significance level of 5% ('suggestive linkage' as proposed by Lander and Kruglyak 1995) was chosen following Van Ooijen (1999). In the region of the putative QTL, the nearest flanking marker loci were chosen as co-factors for multiple QTL mapping (MQM) following the backward elimination technique (Jansen and Stam 1994). When LOD (logarithm of odds) values in other regions reached a significant level, the MQM was repeated by adding new markers as co-factors until a stable LOD profile was reached. The position of the QTL was estimated as the point of maximum LOD value in the region under consideration. Adjacent QTL on the same chromosome were considered as different when the curve had a minimum between peaks that were at least 1-LOD unit below either peak or when the support intervals were non-overlapping (with at least 20 cM). A thousand-permutation test was applied to each data set to decide the LOD thresholds ($P = 0.05$) for considering significant level of identified QTL (Churchill and Doerge 1994). The phenotypic variance explained by a single QTL was calculated as the square of the partial correlation coefficient (R^2) with the observed variable, adjusted for co-factors. The additive effect of a putative QTL was estimated by half the difference between two homozygous classes.

Glossiness, seedling vigor, EG21, EG28, deadhearts and trichome density on upper and lower leaf surfaces were designated as *Gs, Sv, Eg21, Eg28, Dh, Tdu* and *Tdl*, respectively. The identified QTL were designated with italicized symbol composed of *Q* along with a trait name, a code for the institute (for example, *dsr* in the QTL name denotes Directorate of Sorghum Research) and the chromosome number in which the QTL is located. For instance, the QTL name *QDh.dsr-5* refers to the DH% QTL detected on sorghum chromosome SBI-05. In cases where more than one QTL controlling a trait were detected in the same chromosome, they were subnumbered (for example four QTL on chromosome SBI-10 for a trait DH% were designated as *QDh.dsr-10.1*, *QDh.dsr-10.2*, *QDh.dsr-10.3* and *QDh.dsr-10.4*, respectively). In the present QTL analysis, a positive additive value implied that the 296B allele increased the phenotypic value, whereas a negative value implied that the 296B allele decreased the phenotypic value.

The recent availability of complete genome sequence of *Sorghum bicolor* (Paterson et al. 2009) enabled the search for possible candidate genes near the mapped QTL. This study used the sorghum physical map, based on sequence information provided by the Phytozome project (http://www.phytozome.net/sorghum), as a framework to locate putative candidate genes at the QTL intervals for shoot fly resistance (QTL regions were located on the sorghum genome sequence using BLAST analysis with sequence-based markers). Among cereals, maize (*Zea mays*) is more closely related to sorghum, and extensive studies have been conducted for insect resistance. Hence, we attempted to study the synteny at the identified QTL regions with maize to locate conserved insect resistance QTL/genes for further validation [*In-silico* comparative analysis was conducted using the gramene cMap tool (http://www.gramene.org/cmap)].

Results

Phenotypic data analysis

The phenotypic trait means of the parents (296B and IS18551) and their RIL population for five component traits over environments are given in Table 1. Differences

Table 1 Mean phenotypic values of parental lines, RILs (min, max and mean) and standard error of difference for component traits of shoot fly resistance across environments

Trait	Parental lines		RIL population			SED[a]
	296B	IS18551	Min	Max	Mean	
GS	2.7	4.5	2.6	4.5	3.4	0.2
SV	2.8	3.8	2.8	4.2	3.5	0.2
EG21	1.5	0.6	0.5	1.8	1.0	0.1
EG28	1.8	0.7	0.6	2.0	1.2	0.2
DH%	74.6	28.6	28.0	74.6	48.8	5.5
TDU (No/mm^2)	17.5	69.8	5.7	83.9	37.2	4.4
TDL (No/mm^2)	16.8	25.8	7.5	49.8	22.1	2.3

GS glossiness, *SV* seedling vigor, *EG21* mean eggs at 21 days after seedling emergence (DAE), *EG28* mean eggs on 28 DAE, *DH%* mean deadheart percentage on 28 DAE, *TDU* trichome density on upper leaf surface, *TDL* trichome density on lower leaf surface

[a] Standard error of difference; parental means were significantly different at 0.01 probability level for all traits

Table 2 Genotypic correlations among component traits of shoot fly resistance based on average mean value over four environments

Traits	SV	EG21	EG28	DH%	TDU	TDL
GS	0.33	−0.62	−0.64	−0.60	0.25	0.15
SV		−0.28	−0.34	−0.27	0.06	0.04
E21			0.81	0.94	−0.64	−0.21
E28				0.99	−0.44	−0.14
DH%					−0.54	−0.25
TDU						0.36

GS glossiness, *SV* seedling vigor, *EG21* mean eggs at 21 days after seedling emergence (DAE), *EG28* mean eggs on 28 DAE, *DH%* mean deadheart percentage on 28 DAE, *TDU* trichome density on upper leaf surface, *TDL* trichome density on lower leaf surface

between the parental lines were highly significant for all component traits. A wide range in trait expression among the RILs was observed. The phenotypic distributions for all the component traits showed normal distributions. ANOVA indicated highly significant differences ($P < 0.001$) among the 168 RILs and also highly significant environmental effects on traits and significant genotype × environment interactions (provided in supplementary Table). The calculated heritabilities for component traits were moderate to high and ranged from 42 to 79%.

Genotypic correlations between the component traits were estimated based on RIL means over four environments (Table 2). DH% was negatively associated with the component traits GS, SV, TDU and TDL, and positively associated with oviposition (EG21 and EG28). The association of leaf glossiness with the component traits SV ($r = 0.33$), TDU ($r = 0.25$) and TDL ($r = 0.15$) was positive, whereas the correlation with E21 ($r = -0.62$), E28 ($r = -0.64$) and DH% ($r = -0.60$) was negative, indicating that leaf glossiness is one of the most important component traits for shoot fly resistance. Seedling vigor was positively correlated with GS and negatively correlated with EG21 ($r = -0.28$), EG28 ($r = -0.34$) and DH% ($r = -0.27$). TDU showed a negative association with EG21 ($r = -0.64$), EG28 ($r = -0.44$) and DH% ($r = -0.54$). The correlation between TDL and EG21 ($r = -0.21$), EG28 ($r = -0.14$) and DH% ($r = -0.25$) was also negative.

QTL mapping

The genetic linkage map previously reported by our group (Srinivas et al. 2009b) was updated in this study with the addition of ten new markers (seven newly developed genomic-microsatellite markers, one new morphological marker *Trit* and two genomic-microsatellite markers Xcup16 and Xgap1, which were unmapped in our previous map). The primer sequence information of seven new genomic microsatellites is given in Table 3. The information of the genic microsatellites and morphological markers mapped on the map other than this study can be found in our earlier reports (Srinivas et al. 2008, 2009a, b). Mapping of new markers in this study on SBI-10, resulted in two linkage groups (SBI-10a and 10b), and increased the total number of linkage groups in the previous study from 15 to 16 and the total map length from 1,098.7 to 1,143 cM.

To dissect and map the underlying QTL for resistance, multiple QTL mapping (MQM) analysis was conducted on component traits using genotypic data from 162 marker loci. The QTL results were based on average trait values of component traits over four environments. In the case where QTL were not identified from average data of a trait, but identified from mean data of other environments studied, those QTL were also presented corresponding to the environment. MQM analysis identified a total of 29 QTL for shoot fly resistance, four each for leaf glossiness and seedling vigor, seven for oviposition, six for deadhearts, two for adaxial trichome density and six for abaxial trichome density. The LOD and R^2 (%) values of QTL ranged from 2.6 to 15.0 and 5.0 to 33%, respectively. The newly mapped genomic-microsatellite markers on chromosome SBI-10 were associated with the major QTL component traits of resistance, indicating their importance in resistance breeding. The QTL detected for the component traits are listed in Table 4 and illustrated in Fig. 1. For majority of the QTL mapped in this study, possible candidate genes lie within or very near to the ascribed confidence intervals. Some of the important putative candidate genes in the three major QTL intervals based on literature are presented in Table 5.

Table 3 Primer sequence information of new genomic microsatellites developed in this study

Marker name	Repeat motif	Sequence of forward primer	Sequence of reverse primer	Ann temp (°C)	Expected size (bp)
Xnhsbm1008	$(TCTA)_{18}$	TGAATGGCAATGTGTTTGGT	ACGTGTTCCCGTAGGTTGTC	54	204
Xnhsbm1011	$(TTC)_{17}$	TGGGATGCCATATTCTTTTTG	GTTCCTGGTGTTCGTTTGCT	54	150
Xnhsbm1013	$(GT)_{13}$	GCAACTCGTGACACCAGAGA	TGCCGATTCATCTTCCAAAT	54	150
Xnhsbm1033	$(GA)_{19}$	GGCCTTTTGGTTATGATTGC	GGGTCTATTGTGCCTTGACG	54	212
Xnhsbm1043	$(AGAT)_{13}$	TTTCTCATCGCGACTCACAC	TGGATGAGACATCGACCTTG	54	190
Xnhsbm1044	$(TATG)_{16}$	GCGCACCAGAGTCATATTGTT	GCCCTTTTGCAACGTCTAAA	54	180
Xnhsbm1048	$(ACTCT)_5$	CGAACCCCCTACTCCACTCT	CGCGATTTTCTTTCACACAA	54	161

In the population, co-localization of QTL was observed for component traits. The co-localization was mainly observed on chromosomes SBI-03 (2 QTL), SBI-05 (3 QTL), SBI-07 (2 QTL), SBI-09 (2 QTL) and SBI-10 (10 QTL) (Table 4 and Fig. 1). The co-localizing QTL for traits GS and SV were identified on chromosomes SBI-03 between the markers Xtxp59–Xtxp336. Similarly, on chromosome SBI-10, two co-localizing clusters, each with five QTL, were detected. They were identified between the markers Xnhsbm1044–Xnhsbm1013 (cluster I; two oviposition QTL, two trichome QTL and one DH% QTL) and Xnhsbm1043–Xgap1 (cluster II; one QTL each for GS, SV and DH%, and two trichome QTL) for QTL clusters I and II, respectively. Likewise, GS QTL on SBI-05 (between Xtxp65–Xtxp30) was co-localized with the QTL for oviposition and DH%. Finally, a co-localizing QTL for oviposition and DH% was detected on SBI-09 between the markers Xcup02–Xtxp355.

QTL for component traits

Glossiness

Four QTL, which were distributed on four chromosomes (SBI-03, 05, 06 and 10), were identified for this trait. The phenotypic variation explained by individual QTL ranged from 7.6 to 14.0%. For all the QTL regions, alleles were contributed by 296B resulting in decreasing the trait. Among the four QTL, three (*QGs.dsr-3*, *QGs.dsr-5* and *QGs.dsr-10*) were detected in combined analysis and one QTL (*QGs.dsr-6*) was specific to the environment K05. MQM identified two major QTL, one each on chromosomes SBI-03 (near the marker Xtxp336) and SBI-05 (near Xtxp65), explaining 10.1 and 14.0% of the phenotypic variation, respectively.

Seedling vigor

Four QTL located on three chromosomes (one each on SBI-03 and SBI-10, two on SBI-06) were identified for this trait. Individual QTL for this trait accounted for 7.4–11.8% of the phenotypic variation. For all the QTL, except *QSv.dsr-3*, alleles from 296B decreased the trait. A major QTL, *QSv.dsr-3*, was identified on SBI-03 near the marker Xtxp336, which explained 11.8% of the phenotypic variation. Among all the QTL, three were detected in combined analysis and one (*QSv.dsr-10*) was detected specifically in the environment K05.

Oviposition (EG21 and EG28)

Seven QTL were identified for oviposition and were distributed on five chromosomes (two each on SBI-07 and SBI-10, one each on SBI-01, SBI-05 and SBI-09). The phenotypic variation explained by individual QTL ranged from 5.0 to 19.0%. For the majority of the QTL regions, alleles contributed by 296B resulted in increasing oviposition. Interestingly, for the two QTL detected on SBI-01 (*QEg21.dsr-1*) and SBI-09 (*QEg21.dsr-9*), 296B contributed alleles for decreasing oviposition trait values. A major QTL for this trait was detected on chromosome SBI-10 near the marker Xnhsbm1044, explaining 19.0 and 16.1% of the phenotypic variation for EG21 and EG28, respectively.

Deadhearts

Six QTL were detected for this trait, which is a direct measure of resistance. These were distributed on three chromosomes with one each on SBI-05, SBI-09 and four on SBI-10. The phenotypic variation explained by individual QTL ranged from 5.5 to 15.0%. Five out of six QTL (*QDh.dsr-5, QDh.dsr-9*, *QDh.dsr-10.2, QDh.dsr-10.3* and *QDh.dsr-10.4*) were detected in combined analysis and the remaining one (*QDh.dsr-10.1*) was specifically detected in R03. Two major QTL, *QDh.dsr-10.2* (explaining 11.4% of the phenotypic variation) and *QDh.dsr-10.3* (explaining 15.0% of the phenotypic variation) were detected on SBI-10, near the markers Xcup16 and Xnhsbm1013, respectively. For all the QTL, alleles from the susceptible parent

Table 4 Quantitative trait loci (QTL) associated with component traits of shoot fly resistance in sorghum F_7 RILs from the cross 296B × IS18551

Trait	S no	QTL name	Environment	Chromosome	Flanking markers	QTL Position	LOD	Additive effect[a]	R^2 [b]
GS	1	*QGs.dsr-3*	AV, R03, R04, K04	SBI-03	Xtxp59-Xtxp336*	2.9	3.6	−0.111	10.1
	2	*QGs.dsr-5*	AV, R03, R04, K04	SBI-05	*Xtxp65-Xtxp30	1.5	5.5	−0.198	14.0
	3	*QGs.dsr-6*	K05	SBI-06	GlumeT-Mrco*	4.0	3.0	−0.180	8.4
	4	*QGs.dsr-10*	AV, R04, K05	SBI-10	*Xnhsbm1043-Xgap1	4.0	2.7	−0.166	7.6
SV	1	*QSv.dsr-3*	AV, R03, R04, K04	SBI-03	Xtxp59-Xtxp336*	3.0	4.6	0.128	11.8
	2	*QSv.dsr-6.1*	AV, R03, R04	SBI-06	Dsenhsbm2-Xtxp6*	2.5	3.1	−0.156	8.6
	3	*QSv.dsr-6.2*	AV, K05	SBI-06	*Xtxp145-Xtxp274	4.0	3.0	−0.123	7.4
	4	*QSv.dsr-10*	K05	SBI-10	Xnhsbm1011-Xgap1*	1.3	3.0	−0.127	7.7
EG21	1	*QEg21.dsr-1*	AV	SBI-01	*Xcup24-Dsenhsbm64	4.0	3.0	−0.070	8.3
	2	*QEg21.dsr-7*	AV	SBI-07	Xtxp36-Xtxp312*	11.0	2.7	0.063	6.7
	3	*QEg21.dsr-9*	AV	SBI-09	*Xcup02-Xtxp355	3.2	2.6	−0.046	5.0
	4	*QEg21.dsr-10*	AV,R03	SBI-10	*Xnhsbm1044-Xnhsbm1013	3.7	8.0	0.102	19.0
EG28	1	*QEg28.dsr-5*	AV, R03, R04	SBI-05	*Xtxp65-Xtxp30	1.6	2.8	0.080	7.2
	2	*QEg28.dsr-7*	AV	SBI-07	Xtxp36-Xtxp312*	11.0	2.7	0.072	6.7
	3	*QEg28.dsr-10*	AV, R04	SBI-10	*Xnhsbm1044-Xnhsbm1013	3.4	7.5	0.140	16.1
DH%	1	*QDh.dsr-5*	AV	SBI-05	Xtxp65-Xtxp30*	1.8	2.7	1.732	6.0
	2	*QDh.dsr-9*	AV, R04	SBI-09	*Xcup02-Xtxp355	5.3	3.4	−3.236	8.0
	3	*QDh.dsr-10.1*	R03	SBI-10	*Xtxp217-SvPEPcA	3.1	3.9	3.392	8.8
	4	*QDh.dsr-10.2*	AV	SBI-10	Xnhsbm1033-Xcup16*	5.0	3.9	2.840	11.4
	5	*QDh.dsr-10.3*	AV, R03, R04	SBI-10	Xnhsbm1044-Xnhsbm1013*	3.5	5.4	4.823	15.0
	6	*QDh.dsr-10.4*	AV	SBI-10	*Xnhsbm1043-Xgap1	4.0	2.6	1.744	5.5
TDU	1	*QTdu.dsr-10.1*	AV, K04, K05	SBI-10	Xnhsbm1044-Xnhsbm1013*	0.7	5.8	−9.571	15.7
	2	*QTdu.dsr-10.2*	AV, R03, R04	SBI-10	*Xnhsbm1043-Xgap1	2.3	15.0	−6.325	33.0
TDL	1	*QTdl.dsr-1.1*	K04	SBI-01	Xtxp32-Xtxp88*	1.5	4.2	−3.937	8.8
	2	*QTdl.dsr-1.2*	AV, R03	SBI-01	*Dsenhsbm80-xtxp302	1.6	2.8	−2.700	5.3
	3	*QTdl.dsr-4*	K04	SBI-04	Xcup48-Ungnhsbm32*	0.7	4.1	−3.636	8.5
	4	*QTdl.dsr-6*	K04	SBI-06	*Xtxp317-Xtxp274	0.0	2.6	2.792	5.2
	5	*QTdl.dsr-10.1*	AV, R03, R04, K04, K05	SBI-10	*Xnhsbm1048-Xnhsbm1013	2.0	8.1	3.290	15.1
	6	*QTdl.dsr-10.2*	AV, R03, R04, K04, K05	SBI-10	*Xnhsbm1043-Xgap1	2.0	10.0	−4.276	22.7

The flanking marker indicated by an *asterisk* is the closest to the QTL position, QTL position 0 indicates that the QTL position is exactly the same as the corresponding flanking marker. Environments R03, R04, K04, K05 and AV (average) indicate QTL detected in the environments Rabi 2003, Rabi 2004, Kharif 2004, Kharif 2005, and across all environments, respectively

GS glossiness, *SV* seedling vigor, *EG21* mean eggs on 21 days after seedling emergence (DAE), *EG28* mean eggs on 28 DAE, *DH%*: mean deadheart percentage on 28 DAE, *TDU*: trichome density on upper leaf surface, *TDL*: trichome density on lower leaf surface

[a] Additive effect of 296B (susceptible parent). A positive value implies that the 296B allele increased phenotypic value, whereas a negative value implies that the 296B allele decreased phenotypic value

[b] R^2 (%) is percentage of phenotypic variation explained by individual QTL

296B increased deadhearts, except for the QTL on chromosome SBI-09 (*QDh.dsr-9*), where the allele from 296B decreased deadhearts, similar to the EG21 QTL (*QEg21.dsr-9*).

Trichome density (upper and lower leaf surfaces)

Two major QTL, *QTdu.dsr-10.1* and *QTdu.dsr-10.2*, were detected for TDU on chromosome SBI-10, explaining 15.7 and 33.0% of the phenotypic variation, respectively. At both the QTL regions, alleles from 296B decreased the trait. Six QTL were detected for TDL and were distributed on four chromosomes with two on SBI-01, one each on SBI-04 and SBI-06, and two on SBI-10. The phenotypic variation explained by individual QTL ranged from 5.2 to 22.7%. At four of the six QTL, 296B contributed negative alleles, and for the remaining two QTL regions contributed positive alleles. Among all QTL, three were detected in combined analysis and three were specific to the environment K04.

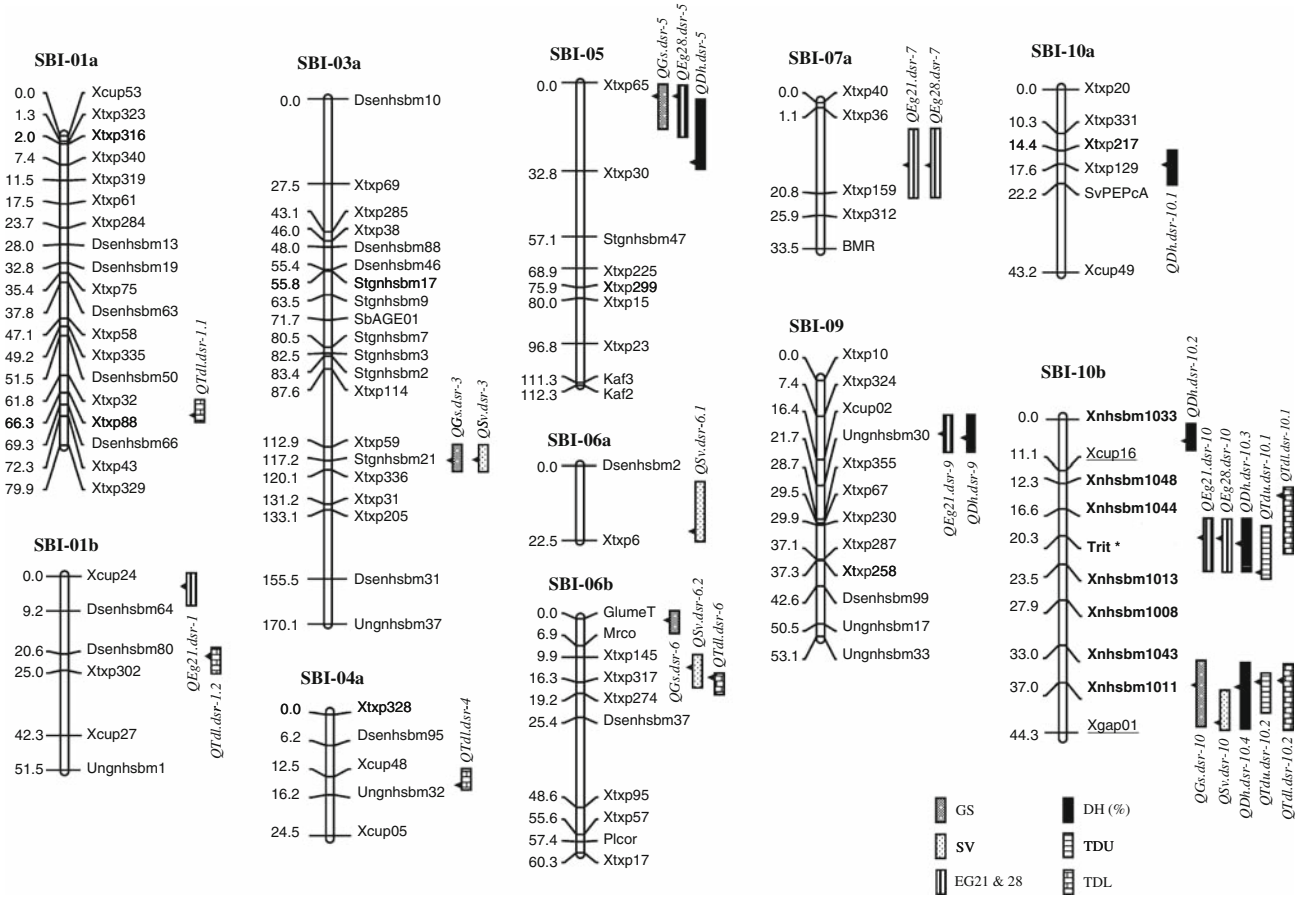

Fig. 1 Genetic linkage map of sorghum showing 29 quantitative trait loci (QTL) identified for the five component traits of shoot fly resistance studied in the 296B × IS18551 RIL population. The linkage map shows only the linkage groups harboring the QTL of this study. Separate graphical designs were used to indicate QTL bars for each trait. The length of *vertical bars* indicates 1-LOD support intervals for each QTL. LOD maximum is indicated as a sharp point on the *bar*, which shows QTL position on chromosomes. The *underlined markers* were previously unmapped in our map, but now mapped. The markers in *bold* are new genomic microsatellites mapped in this study. A new morphological marker (*Trit*) for trichome morphology mapped in this study is tagged with an *asterisk*

Discussion

Development of cultivars resistant to shoot fly is one of the important goals of sorghum improvement programs in India. Despite the breeding efforts made over the past three decades by utilizing the available sources on shoot fly resistance, the level of resistance achieved in the cultivars so far is limited. In this context, marker-assisted selection (MAS) using the markers linked to genomic regions/QTL for resistance may increase the efficiency of breeding to shoot fly resistance. In the present study, a more precise evaluation of the RILs for resistance to shoot fly under field conditions was done by performing 3 years of phenotypic experimentation (in four environments), and a total of 29 QTL distributed on eight chromosomes (SBI-01, 03, 04, 05, 06, 07, 09, and 10) were identified for five component traits of shoot fly resistance. These results will enable further characterization of consistent QTL regions and the development of the genetic material for breeding programs, which forms the foundation for MAS in sorghum for improving shoot fly resistance. To our knowledge, this is the first report on QTL mapping for shoot fly resistance in sorghum.

An interesting finding of this study is that both the resistant and susceptible parents contributed alleles for resistance. For most of the QTL, parental alleles had the expected effect on phenotypic traits. Alleles from the resistant parent (IS18551) contributed to resistance, while alleles from the susceptible parent (296B) had an opposite effect. However, at six QTL regions (*QSv.dsr-3*, *QEg21.dsr-1*, *QEg21.dsr-9, QDh.dsr-9*, *QTdl.dsr-6* and *QTdl.dsr-10.1*), the susceptible parent (296B) contributed the alleles for resistance. This suggests that the level of resistance available in IS18551 can be further improved by pyramiding alleles from the susceptible parent 296B. Thus, even cultivars susceptible to shoot fly, such as 296B, may harbor some favorable alleles, which can be helpful in gene pyramiding. Similar cases of contribution of favorable

Table 5 A list of the putative candidate genes identified in the three major QTL intervals for shoot fly resistance in sorghum

Chromosome	QTL interval	Sorghum gene ID	Description	Functional role	Reference
SBI-05	Xtxp65-Xtxp30 (GS, EG28 and DH%; 2.6 Mb)	Sb05g001740	Long chain acyl-CoA synthetase	Cuticular wax biosynthesis	Schnurr et al. (2004)
		Sb05g001770	GDSL-like Lipase/ Acylhydrolase	Cuticular wax biosynthesis	Cominelli et al. (2008)
		Sb05g002270	Lipase-like	Wax metabolism	Mintz-Oron et al. (2008)
		Sb05g003700	Epoxide hydrolase	Wax metabolism and biotic stress	Gomi et al. (2003)
		Sb05g003730	Digalctosyldiacylglycerol synthase 1	Lipid metabolism	Kelly et al. (2003)
		Sb05g002520	Strictosidine synthase	Insect resistance	Sarosh and Meijer (2007)
		Sb05g003920	NBS-LRR type R protein, Nbs1-Pi2	Disease and pest resistance	Hulbert et al. (2001)
		Sb05g003830	NB-ARC domain containing protein	Disease resistance	Takken et al. (2006)
		Sb05g003400	NO apical meristem (NAM) protein	Biotic and abiotic stress	Olsen et al. (2005)
		Sb05g002410	EF hand family protein	Biotic and abiotic stress	Day et al. (2002)
SBI-10	Xnhsbm1044-Xnhsbm1013 (EG21, EG28, DH%, TDU, and TDL; 2 Mb)	Sb10g027980	Cysteine protease Mir1	Insect resistance	Pechan et al. (2000)
		Sb10g028060	Glycosyl hydrolase family 1 protein	Biotic, abiotic stresses and cell wall remodeling	Opassiri et al. (2006)
		Sb10g029150	Cycloartenol synthase	Sterol biosynthesis, biotic and abiotic stresses	Tholl (2006)
		Sb10g029175	Beta-amyrin synthase	Triterpene synthesis and biotic stress	Ormaetxe et al. (2003)
		Sb10g026190	Homogentisate phytyl transferase VTE2-1	Tocopherol biosynthesis and defense	Sattler et al. (2006)
		Sb10g027490	Class III peroxidase 89 precursor	Defensive responses to wounding	Espelie et al. (1986)
SBI-10	Xnhsbm1043-Xgap1 (GS, SV, DH%, TDU, and TDL; 2.3 Mb)	Sb10g025053	Glossy15	Insect resistance and wax synthesis	Williams et al. (2000)
		Sb10g026548	Hydroxyproline-rich glycoprotein-like	Biotic stress	Showwalter et al. (1985)
		Sb10g025300	NBS-LRR disease resistance protein	Disease and pest resistance	Hulbert et al. (2001)
		Sb10g027100	NAC domain protein NAC1	Biotic and abiotic stress	Olsen et al. (2005)
		Sb10g026340	Leucine-rich repeat transmembrane protein kinase	Meristem growth and defense	Dievart and Clark (2004)
		Sb10g025850	GDSL-lipase-like	Cuticular wax biosynthesis	Mintz-Oron et al. (2008)
		Sb10g026720	Speckle-type POZ protein-like	Expressed in *Arabidopsis* trichomes	Jakoby et al. (2008)

alleles from susceptible parents have been reported with resistance to head bug in sorghum (Deu et al. 2005) and European corn borer in maize (Bohn et al. 2000).

QTL for component traits of shoot fly resistance

Expression of leaf glossiness (GS) in sorghum is an important trait for multiple insect pest resistance, including shoot fly (Agrawal and House 1982), and tolerance to abiotic stresses, such as drought, salinity, high temperature and low nutrient availability (Maiti et al. 1984). Glossiness affects the quality of light reflected from leaves and influences the orientation of shoot flies toward their host plants, thereby reducing the oviposition (Sharma 1993). Scanning election microscopy (SEM) studies of glossy and non-glossy sorghum plants revealed that the glossy leaf

surface is characterized by reduction in the number of wax crystals, whereas the non-glossy leaf surface is characterized by a high density of wax crystals (Tarumoto et al. 1981). Similar to sorghum, glossiness was also reported in recessive mutants of *Zea mays* (named as *glossy* or *gl* mutants), where it was used as a morphological marker for bacterial resistance (Marcell and Beattie 2002). In maize, at least 18 glossy genes have been found to affect the quantity and/or the composition of cuticular waxes on the leaf surface of seedlings (Neuffer et al. 1997). Most of these mutants have been genetically mapped and some (*gl1*, *gl2*, *gl8*, and *gl*15) have been cloned and characterized (Sturaro et al. 2005).

A negative and highly significant correlation between percentage of deadhearts caused by shoot fly and glossiness of leaves was reported in sorghum (Nwanze et al. 1990; Kamatar and Salimath 2003). The results of those studies are in conformity with the correlations observed in the present study. The major glossiness (GS) QTL identified on SBI-05 between the markers Xtxp65–Xtxp30 (*QGs.dsr-5*, explaining 14% of the phenotypic variation) was syntenic to maize chromosome 4 (bins 4.08/4.09), where a mutant *glossy3* gene (Hayes and Chang 1939) was reported. To know whether any gene involved in wax synthesis was present at this QTL region, the sorghum genome sequence was scanned for genes related to wax synthesis. Interestingly, a gene coding for *Long chain Acyl-CoA synthetase* was present close to Xtxp65 marker (about 9.5 kb away). Since, this gene was reported to be involved in cuticular wax biosynthesis in *Arabidopsis* (Schnurr et al. 2004), it could be one of the probable candidate genes for glossiness at this QTL region. Some of the other important putative candidate genes involved in wax synthesis in this QTL interval are presented in Table 5. The second important GS QTL region on SBI-03, between the markers Xtxp59–Xtxp336 (*QGs.dsr-3*, explaining 10.1% of phenotypic variation), was syntenic to a segment of maize chromosome 3 (bin 3.09). This maize chromosomal segment contains some important genes related to biotic and abiotic stresses such as *glossy9* [one of the glossy mutant gene (Emerson et al. 1935)], phospholipid transfer protein homolog2 [*plt2*; involved in plant resistance for bacterial and fungal pathogens in barley and maize (Molina et al. 1993)], thiamine biosynthesis2 [*thi2*; involved in leaf senescence (Griffiths et al. 1995)] and major QTL for resistance to important insect pests such as corn earworm and southwestern corn borer (Byrne et al. 1996; CIMMYT 1994). Sequence analysis of the sorghum genome in this QTL interval revealed the presence of some important genes such as wax synthase (involved in wax synthesis), and genes for drought tolerance such as *Sec13* and *LEA* (Guterman et al. 2003; QiaoYing et al. 2008). The presence of genes related to abiotic stress resistance at this GS QTL in sorghum supports earlier reports on the association of leaf glossiness with drought and other abiotic stress tolerance (Maiti et al. 1984). Hence, this GS QTL may be utilized to improve sorghum for resistance to shoot fly besides other biotic and abiotic stresses. The preliminary synteny analysis at major sorghum glossiness QTL regions with maize indicated the presence of some conserved glossy genes, which are also associated with disease and insect resistance QTL, in these two crops.

Seedling vigor (SV) is one of the most important component traits of shoot fly resistance in sorghum, which inhibits the establishment of the shoot fly larva in causing damage to the seedling (Jayanthi et al. 2002). Rapid seedling growth delays the first instar larvae from reaching the growing tip, thereby reducing the chance of deadheart formation. In contrast, slow growth due to poor seedling vigor, low fertility or environmental stress increases the shoot fly damage (Taneja and Leuschner 1985). Shoot fly resistant lines have rapid initial plant growth, greater seedling height, longer stems and longer internodes (Mote et al. 1986). In the present study, seedling vigor was negatively associated with shoot fly damage (negatively correlated with EG21, EG28 and DH%), supporting an earlier study (Taneja and Leuschner 1985). Sorghum sequence analysis at a major SV QTL (*QSv.dsr-3*) on SBI-03 (Xtxp59–Xtxp336) region revealed an important plant growth-related gene *Indole 3 acetic acid-amino synthase GH3.5* near the marker Xtxp336. Since this gene was known to be associated with plant growth, light and stress adaptation responses in *Arabidopsis* (Eun Park et al. 2007), it could be one of the probable candidate genes for seedling vigor in this QTL interval.

Non-preference to oviposition (also called antixenosis) is reported to be a primary mechanism of resistance to shoot fly in sorghum (Jotwani et al. 1971; Taneja and Leuschner 1985). A major QTL for this trait was identified on SBI-05 (Xtxp65–Xtxp30), which was found to be co-localized with the GS and DH% QTL. If we look into the QTL position at this region, the GS and EG28 QTL were closer to the marker Xtxp65, whereas the QTL for DH% was closer to Xtxp30 for DH%, suggesting the involvement of different genes. Interestingly, analysis of the sorghum genome sequence in this major QTL interval identified some probable candidate genes involved in wax synthesis (associated with glossiness), as well as genes involved in insect and disease resistance (Table 5). Some of the important genes identified include *Srictosidine synthase* [which is reported to be differentially expressed upon diamondback moth larvae feeding in *Brassica* (Sarosh and Meijer 2007)], *No apical meristem protein* gene (involved in biotic and abiotic stresses in plants Olsen et al. 2005), *NBS-LRR protein Nbs-pi2* (involved in resistance against the full taxonomic range of plant pests Hulbert et al. 2001)

and *NB-ARC domain protein* coding gene (involved in disease resistance Takken et al. 2006).

Deadhearts are the direct measure of shoot fly resistance in sorghum. Two major QTL were identified for this trait on SBI-10 between the markers Xnhsbm1044–Xnhsbm1013 and Xnhsbm1033–Xcup16, explaining 15.0 and 11.4% of the phenotypic variance, respectively. Furthermore, all the QTL on SBI-10 (*QDh.dsr-10.1* to *10.4*) showed synteny to the maize chromosome 9 (bins 03/04). This syntenic maize region contains major insect resistance QTL for corn earworm and sugarcane cornborer resistance (Byrne et al. 1996; Brooks et al. 2005). Scanning of the sorghum genome sequence at one of the important DH% QTL region (*QDh.dsr-10.2*) on SBI-10 (Xnhsbm1033–Xcup16; explaining 11.4% of the phenotypic variation) revealed the presence of an important insect resistance gene *Cysteine protease Mir1* near the marker Xcup16, which could be a possible candidate gene for shoot fly resistance in this interval. It was reported that maize lines resistant to lepidopteran insect pests rapidly mobilize a unique 33 KDa cysteine protease (a novel insect defense mechanism) in response to caterpillar feeding in midwhorl, which is highly correlated with a significant reduction in caterpillar growth that resulted from impaired nutrient utilization due to disruption of insect peritrophic matrix (Pechan et al. 2000). Furthermore, Mexican sweetcorn callus transformed with *Mir1* gene coding for *Cysteine protease* expressed the protease, and the growth of caterpillars reared on the transgenic callus was reduced by 60–80% (Pechan et al. 2000). Similarly, the sequence scan at the major DH% QTL interval on SBI-10 between the markers Xnhsbm1044–Xnbsbm1013 (which was found to be co-localized with the QTL for EG21 and EG28, and explained 15.0, 16.1 and 19.0% of the phenotypic variation for traits DH%, EG28 and EG21, respectively) also revealed some putative candidate genes, such as *Homogentisate phytyl transferase vte2* (involved in *Arabidopsis* biotic stress response Sattler et al. 2006), a gene coding for *Hydroxyproline rich glycoprotein* (reported in bean fungal response Showwalter et al. 1985) and *NAC1* gene (NAC-domain genes have been implicated in plant responses to biotic and abiotic stresses Olsen et al. 2005).

Trichomes are cellular appendages that protrude above the epidermis and their presence provides a physical barrier against insect pests, for egg laying or larval movement, and thereby reduce plant damage. Earlier studies in sorghum reported that the presence of trichomes on the leaf surface hinders egg laying by shoot flies (Biradar et al. 1986), resulting in the indirect reduction of deadhearts. Density of trichomes is genetically controlled (Maiti and Gibson 1983) and is reported to be negatively correlated with oviposition and deadhearts in sorghum (Sandhu et al. 1988; Dhillon et al. 2005). It is possible that besides trichome density, trichome morphology (pointed unicellular vs. blunt bicellular trichome) may also play an important role in restricting the oviposition preference or larval movement leading to reduced deadheart percentage. We observed that in a set of sorghum genotypes analyzed, the resistant sorghum genotypes usually had unicellular pointed trichomes, while the susceptible genotypes had bicellular blunt trichomes (data not shown). The susceptible parent (296B) in this study had bicellular blunt trichomes in contrast to the resistant parent (IS18551), which had unicellular pointed trichomes. The differences observed in trichome morphology between the parents were also reflected in the RILs, as each of the RIL showed either bicellular blunt or unicellular pointed trichomes, indicating the monogenic inheritance/control of the trait. The gene segregating for trichome morphology (*Trit*) in the RILs were mapped as a morphological marker on SBI-10, between the markers Xnhsbm1044–Xnhsbm1013, where five important QTL for shoot fly component resistance traits (two QTL each for oviposition and trichome density, and one for DH%) were identified.

A total of eight QTL were identified for leaf surface trichome density (two for upper and six for lower surface). Out of eight, only two QTL on chromosome SBI-10 (Xnhsbm1044–Xnhsbm1013 and Xnhsbm1043–Xgap1) were associated with trichome density on both surfaces with one QTL (Xnhsbm1044–Xnhsbm1013) showing an opposite additive effect of 296B (additive effects of −9.571 and 3.290 for upper and lower surfaces, respectively). Moreover, the QTL position was also different at this genomic region for both the surfaces, suggesting the involvement of different genes. Lauter et al. (2004) identified four genomic regions for leaf macro hair (trichome) density in maize subspecies Teosinte (*Zea mays* ssp. *parviglumis*). They reported a major QTL region for macrohair density on the long arm of chromosome 9.03/04 between the flanking markers glossy15-umc95 (chromosome bin 9.03/04), which is syntenic to the two major trichome density QTL identified in the present study on chromosome SBI-10, between the markers Xnhsbm1044–Xnhsbm1013 and Xnhsbm1043–Xgap1.

In this maize syntenic region, the *gl15* (glossy 15) gene was reported as a candidate insect resistance gene for fall armyworm, southwestern corn borer and European corn borer (Brooks et al. 2005; Williams et al. 2000). The loss-of-function mutations at the maize *gl15* locus alter the normal transition from juvenile-to-adult plant growth by conditioning the abbreviated expression of juvenile epidermal cell traits (epicuticular waxes, leaf hairs and cell wall composition) and the coordinate precocious expression of adult epidermal cell features (Moose and Sisco 1994), which is also known as an early phase transition. The early phase transition from juvenile-to-adult vegetative

stage is a primary mechanism affecting resistance to fall armyworm and southwestern corn borer in maize (Williams et al. 2000). Interestingly, the sorghum genome sequence analysis at the major trichome density QTL region on SBI-10 (Xnhsbm1043–Xgap1), which was also found to be co-localized with the QTL for GS, SV and DH%, identified a gene orthologous to *gl15*. This could be one of the possible candidate genes associated with shoot fly resistance in sorghum. The presence of *gl15* orthologous gene in sorghum at the major shoot fly resistance QTL interval (identified for multiple component resistance traits) also suggests the possibility of a similar resistance mechanism operating for multiple insect pest resistance in sorghum.

Another important gene for macrohair initiation and density (*mhl1*) was reported in the syntenous maize chromosomal region (bin 9.04) (Moose et al. 2004), which corresponds to the newly mapped morphological marker for trichome morphology (*Trit*) on sorghum chromosome SBI-10 (between the markers Xnhsbm1044–Xnhsbm1013) in the present study. Interestingly, this sorghum region also harbors two major QTL for trichome density in this study. Thus, the preliminary analysis clearly suggests the presence of conserved genomic regions/genes influencing leaf morphological traits (leaf surface wax and trichomes) and insect resistance between the two cereal crops sorghum and maize.

Co-localization of QTL

Co-localization of QTL for different traits may result from either tight linkage of several genes (Sandhu et al. 2001), or the pleiotropic effect of a gene (Veldboom et al. 1994; Xiao et al. 1996). In the present study too, co-localization of QTL for component traits of shoot fly resistance could be explained by the tight linkage of genes or pleiotropy of a single gene. Unfortunately, the resolution between QTL-linked markers in the present map does not allow to distinguish between tight linkage of genes and pleiotropic gene effects. Further investigations using more closely linked markers and near-isogenic lines (NILs) are needed to address these questions.

Conclusion and outlook

In the present study, a total of 29 QTL were identified for five component traits of shoot fly resistance using the RIL population of the cross 296B × IS18551. The important QTL between the markers Xnhsbm1043–Xgap1 (for GS, SV, DH%, and trichome density), Xnhsbm1044–Xnhsbm1013 (for oviposition, DH% and trichome density), Xnhsbm1033–Xcup16 (DH%), Xtxp65–Xtxp30 (for GS, oviposition, and DH%), Xtxp59–Xtxp336 (for GS and SV), Xtxp36-Xtxp312 (for oviposition), Xtxp217–SvPEPcA (for DH%) and Xcup02–Xtxp355 (for oviposition and DH%) should be valuable for improving shoot fly resistance in sorghum through marker-assisted breeding (MAB). Additionally, there are other important QTL regions, where resistance alleles were contributed by the susceptible parent (296B) that may be utilized for MAS.

The information on shoot fly resistance QTL, their syntenic relationships with maize, and identified putative candidate genes at the important QTL regions will facilitate our continued efforts toward mapping of candidate genes for functional association with the QTL, allele mining, isolation of resistance genes through map-based cloning, comparative genomic studies with other cereal crops and characterization of sorghum genotypes for novel resistance mechanisms.

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