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ORIGINAL PAPER

Identification and validation of genomic regions that affect shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract Shoot fly is one of the most important pests affecting the sorghum production. The identification of quantitative trait loci (QTL) affecting shoot fly resistance enables to understand the underlying genetic mechanisms and genetic basis of complex interactions among the component traits. The aim of the present study was to detect QTL for shoot fly resistance and the associated traits using a population of 210 RILs of the cross 27B (susceptible) × IS2122 (resistant). RIL population was phenotyped in eight environments for shoot fly resistance (deadheart percentage), and in three environments for the component traits, such as glossiness, seedling vigor and trichome density. Linkage map was constructed with 149 marker loci comprising 127 genomic-microsatellite, 21 genic-microsatellite and one morphological marker. QTL analysis was performed by using MQM approach. 25 QTL (five each for leaf glossiness and seedling vigor, 10 for deadhearts, two for adaxial trichome density and three for abaxial trichome density) were detected in individual and across environments. The LOD and R^2 (%) values of QTL ranged from 2.44 to 24.1 and 4.3 to 44.1%, respectively. For most of the QTLs, the resistant parent, IS2122 contributed alleles for resistance; while at two QTL regions, the susceptible parent 27B also contributed for resistance traits. Three genomic regions affected multiple traits, suggesting the phenomenon of pleiotrophy or tight linkage. Stable QTL were identified for the traits across different environments, and genetic backgrounds by comparing the QTL in the study with previously reported QTL in sorghum. For majority of the QTLs, possible candidate genes were identified. The QTLs identified will enable marker assisted breeding for shoot fly resistance in sorghum.

Keywords Deadheart percentage · Quantitative trait loci · Candidate genes · Co-localization · Gene pyramiding

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops globally, after wheat, maize, rice, barley because of its good adaptation to a wide range of ecological conditions and low input cultivation, and diverse uses. It has a predominat role in the food and fodder security for millions of rural families in arid and semi-arid regions of the world. During the past three decades, sorghum production has increased, especially of rainy season, by exploiting hybrid vigor through the use of cytoplasmic male sterility. But the yield potential of sorghum cultivars could not be realized in the farmers' fields due to many biotic stresses, of which shoot fly [*Atherigona soccata* (Rondani)] is the most important one. Shoot fly is one of the most destructive pests in Asia, Africa and the Mediterranean Europe limiting sorghum production. Grain yield losses up to 50% have been reported in India (Jotwani 1982), but the loss at times may be over 90% (Rao and Gowda 1967). In view of the seriousness of the shoot fly problem in sorghum and owing to the limitations (high

costs and toxicity hazards) in chemical control, it is necessary to develop new varieties or hybrids with resistance to this pest. Genetic manipulation to impart resistance against this important biotic stress is essential for sustained improvement of sorghum to meet the future demand of both grain and nutritious fodder. Plant resistance to insects can be an effective component of the integrated pest management program. Utilization of sorghum cultivars with high levels of plant resistance would reduce the need for application of chemical insecticides, resulting in positive environmental, health and economic benefits.

Shoot fly attacks the sorghum seedlings during 5–25 days after emergence. The female fly lays white, elongated, cigar-shaped eggs singly on the abaxial (lower) surface of the leaves, parallel to the midrib. The shoot fly larvae cut the growing tip, resulting in deadheart formation (Deeming 1971). Infestation causes deadhearts in seedlings as well as in tillers of older plants, resulting in considerable damage to the crop. Its incidence is higher in crops sown late during rainy season, and in the early-sown crops during post-rainy season (Jotwani et al. 1970).

In the conventional pest resistance breeding, although there have been some notable successes for improved resistance to insects, the breeding process is often slow and laborious, and sufficient levels of resistance have not been achieved due to the fact that resistance to various insects is most often a quantitatively inherited trait with a strong effect of environment (Tao et al. 2003). Classic genetic analysis using phenotypic data demonstrated that inheritance of sorghum shoot fly resistance is complex, polygenically inherited with high influence of environment (Rana et al. 1975; Agarwal and Abraham 1985; Singh et al. 2004; Aruna and Padmaja 2009). Studies on shoot fly resistance mechanisms suggested that deadhearts, oviposition, leaf glossiness, trichomes on the abaxial surface of the leaf can be used as marker traits to select for resistance to shoot fly in sorghum (Dhillon et al. 2005; Anandan et al. 2009).

Identification of genomic regions/ quantitative trait loci (QTL) governing traits of biotic stress can create a base for rapid, detailed, and direct genetic manipulation through marker-assisted selection (MAS). The identification of QTLs controlling the important traits would improve our understanding of inheritance of these traits, enable us to analyze association between these traits, clarify the relationships of QTLs to candidate genes and finally provide the basis for MAS of these traits. Since the advent of molecular marker techniques and statistical models for detection and mapping of QTL, many QTLs for different traits have been identified in sorghum for different pests, including shoot fly (Satish et al. 2009), 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). Once the QTLs for important traits are identified, it is equally important to validate these putative QTLs across various genetic backgrounds before embarking upon marker-assisted introgression of selection. One approach to validate genetic markers across populations is to develop multiple mapping populations with different genetic backgrounds and identify QTLs, which are common to two or more populations (Haussmann et al. 2002).

Recently, Satish et al. (2009) identified QTLs for shoot fly resistance in a recombinant inbred line (RIL) population of the cross, 296B × IS18551 wherein, IS 18551 originated from Ethiopia was the resistant parent. In the present study, we developed another RIL population of the cross, 27B × IS2122, where a different resistant source, IS2122 (originated from USA) was used. The main objectives of the present study were to identify new QTL for shoot fly resistance and associated traits, and to validate the QTLs and linked markers identified for shoot fly resistance earlier.

Materials and methods

Development of a recombinant inbred population

A RIL population consisting of 210 F_6 RILs derived by single-seed descent method from a cross between two parents, 27B and IS2122 was used to identify QTLs controlling shoot fly resistance traits. The parents used for the mapping population are contrasting for shoot fly resistance and associated traits (Table 1). The susceptible parent, 27B is the female parent of the popular rainy season hybrid, CSH16. It has non-glossy leaves with low density of trichomes on both abaxial and adaxial surfaces of leaves. The resistant parent, IS2122 is a germplasm line (land race), and shows contrasting characters with those of the susceptible parent. It has glossy leaves with high trichome density. From F_2 generation of the cross between 27B ×

Table 1 Mean phenotypic values of parents, RILs, standard error of difference and heritability for component traits of shoot fly resistance across environments

Trait	27 B	IS 2122	Recombinant Inbred lines				h^2 (%)
	Mean	Mean	Mean	SED±	Min	Max	
GS	2.12	4.86	3.02	0.23	1.17	4.67	59
SV	3.09	4.19	3.03	0.24	1.36	4.61	46
TDU	4.01	52.2	19.68	1.63	4.01	53.0	90
TDL	3.26	25.1	13.1	1.15	2.11	38.7	89
DH %	79.1	41.5	55.4	2.62	41.0	80.1	31

GS Glossiness, *SV* Seedling vigor, *TDU* Trichome density on upper surface of leaf, *TDL* Trichome density on lower surface, *DH %* Deadheart percentage, *SED* Standard error of difference

IS2122, 385 single plant progenies were selected at random and were advanced by single seed descent method to F_6 during 2003–2005. Out of these, phenotyping and genotyping was done for 210 RILs.

Field trials and evaluation for shoot fly resistance

The 210 RILs along with the two parents (27B and IS2122) were used for phenotypic characterization during 2006, 2007 and 2008 at three locations. The experiment was conducted in a total of eight environments. It was sown during the rainy seasons of three consecutive years (2006–2008) at Directorate of Sorghum Research (DSR), Hyderabad and Maharana Prathap University of Agriculture and Technology (MPUAT), Udaipur; and for 2 years (2006 and 2008) at Dr Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola. The test environments were abbreviated as follows: DSR06, DSR07, DSR08, UD06, UD07, UD08, AK06 and AK08 representing DSR 2006, DSR 2007, DSR 2008, Udaipur 2006, Udaipur 2007, Udaipur 2008, Akola 2006 and Akola 2008, respectively. The experiment was carried out in a randomized complete block design (RCBD) with three replications. Each RIL entry was planted in a single row plot (4 m long) and the spacing between the rows was 45 cm. At 10 days after seedling emergence (DAE), the seedlings were thinned to a plant to plant spacing of 15 cm within a row. Field evaluations were conducted under high shoot fly stress condition using the standard interlard fish meal technique (Soto 1974). In this method, moistened fish meal in plastic bags is kept uniformly at regular intervals across the field one week after seedling emergence, to attract shoot flies from the surrounding areas. All the recommended agronomic practices, except plant protection measures for shoot fly, were followed to raise a good crop.

Phenotyping and data analysis for shoot fly resistance and associated traits

Observations were recorded on five component traits of shoot fly resistance, viz., leaf surface glossiness (abbreviated as GS), seedling vigor (SV), deadheart percentage (DH%) and leaf surface trichome density on adaxial (upper; TDU) and abaxial (lower; TDL) leaf surfaces. Observations on shoot fly resistance in terms of deadheart percentage were recorded in all eight environments, while GS, SV, TDU and TDL were recorded at DSR, Hyderabad location for 3 years (2006–2008). Observations were recorded in each replication during all the 3 years. The intensity of GS was recorded at 10 DAE on a scale of 1–5, where 1 = non-glossy (dark green, dull, broad, and drooping leaves), and 5 = higher glossy (light green, shining, narrow and erect leaves). Leaf glossiness was observed during the morning hours when there was maximum reflection of light. SV was scored at 10 DAE on a scale of 1–5, where 1 = low vigor (plants showing minimum growth, low leaf area and weak growth) and 5 = high vigor (plants showing maximum height, leaf area and robustness).

To record leaf trichome density, the central portion of the fifth leaf from the base was taken from three randomly selected seedlings in each entry at 12–15 DAE. Leaf samples (5 mm^2) were placed in small vials with 20 ml of acetic acid: alcohol (2:1) overnight. The cleared samples were transferred into 90% lactic acid and stored for observation. The leaf samples were mounted in a drop of lactic acid on a microscopic slide and observed under the microscope at a magnification of ×20 (Maiti and Bidinger 1979). The number of trichomes was counted in three microscopic fields selected at random on both abaxial (lower) and adaxial (upper) leaf surfaces and expressed as trichome density (no/mm^2). In the present study, the two parents differ in trichome morphology (the susceptible parent 27B possesses bicellular and blunted trichomes, whereas the resistant parent IS2122 possesses unicellular and pointed trichomes). Hence, trichome morphology i.e. bicellular blunted or unicellular pointed were scored in the RILs to map its position on the linkage map. Overall resistance was recorded as the percentage of deadhearts (DH%) caused by shoot fly infestation. 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.

The statistical software Windostat (http://www.windostat.org/) was used for the statistical analysis of the phenotypic data. The data from individual environments for all the traits were subjected to analysis of variance (ANOVA). The data from across environments were also used to determine the effect of genotype (RILs), environment, genotype x environment (G × E) and error variance. Pearson correlation analysis was performed to study the relationship between shoot fly resistance (DH%) and the component traits such as GS, SV, TDU and TDL using the statistical software, 'Statistix' (version 8.1). Broad sense heritability (h^2b, defined as the ratio of genetic variance to the sum of genetic variance and environmental variance) was estimated for all the traits according to Falconer (1989).

DNA extraction, PCR amplification, electrophoresis

The procedure for DNA extraction, PCR amplification and electrophoresis was followed as detailed earlier (Srinivas et al. 2009a). In brief, the genomic DNA was extracted using the CTAB method (Saghai-Maroof et al. 1984). Touchdown PCR was used to reduce non-specific

amplification. Temperature cycling was carried out for both EST-SSRs and genomic-SSR markers using the Bio-Rad iCycler version 3.3032. Briefly, a hot start of 94°C for 10 s, 61°C for 20 s, and 72°C for 30 s, then 9 cycles during which the annealing temperature dropped by 1°C per cycle, then 30 cycles of 94°C for 10 s, 54°C for 20 s, and 72°C for 30 s, and a 20 min final extension at 72°C (Smith et al. 2000). Reactions were run in 5 µl reaction in 96-well PCR plates (Axygen, PCR-96-HS-C) with each PCR containing 2–4 pmol of primer, 1.0–4.0 mM $MgCl_2$, 0.1–0.2 mM dNTP, 0.2 U *Taq* DNA polymerase and 1x PCR buffer (Invitrogen S. Giuliana Milanese, Italy) and 50 ng of template genomic DNA. After amplification, the presence of amplification products was tested by agarose gel electrophoresis. The successful PCR products were assayed on a Bio-Rad Sequi-Gen^{TM} sequencing electrophoresis apparatus in 5% polyacrylamide gels containing 8 M urea and 1X TBE buffer at 80 W of constant power. The DNA fragments were visualized by silver staining (Fritz et al. 1999) and scored as either parental (A or B), heterozygous (H), or missing data (–).

Linkage map construction and QTL analysis

In all, more than 500 microsatellites markers were considered for screening polymorphism between the mapping parents 27B and IS2122. A total of 149 polymorphic markers (consisting of 127 genomic-microsatellite markers, 21 genic-microsatellite markers and one morphological marker) were used for genotyping the RILs. 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). The construction of the linkage map was done as described by Srinivas et al. (2009a, b).

The QTL analysis was performed both with trait mean values in each environment, and the averaged mean values of each trait across the three environments using the software MAPQTL version 5 (Van Ooijen 2005). First, interval mapping (IM) analyses was performed (Lander and Botstein 1989) to locate preliminary QTL positions on the map, and to select markers significantly associated with the trait to constitute an initial set of cofactors. A backward elimination procedure was applied to the initial set of cofactors. Only significant markers at $P < 0.02$ were used as cofactors in the multiple QTL method (MQM) (Jansen 1993; Jansen and Stam 1994) analysis for QTL detection. After the selection of cofactors, MQM analyses were performed. Putative QTLs were identified at LOD 2.5. A 1-LOD support interval was calculated for each QTL to obtain a 95% confidence interval. Adjacent QTLs on the same chromosome were considered different when the curve had a minimum between peaks that were at least one-LOD unit below either peak or when the support intervals were not overlapping. The phenotypic variance explained by a single QTL was calculated as the square of the partial correlation coefficient with the observed variable, adjusted for cofactors. The additive effect of a putative QTL was estimated by half the difference between two homozygous classes.

Designation of the traits and QTLs was followed as per Satish et al. (2009), where glossiness, seedling vigor, deadhearts and trichome density on upper and lower leaf surfaces were designated as Gs, Sv, 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. In cases where more than one QTL controlling a trait were detected in the same chromosome, they were sub numbered. In the present QTL analysis, a positive additive value implied that the 27B allele increased the phenotypic value, whereas a negative value implied that the 27B 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).

Results

Phenotypic trait variation, distribution and correlations

The mean phenotypic values of the component traits of shoot fly resistance for the parents and their RILs over environments are presented in Table 1. Highly significant differences were observed between the parents for all the component traits. A wide range in trait expression was observed among the RILs. The phenotypic distributions for all the component traits showed normal distributions. The wide range of variation for the studied traits in the RIL population (Table 1) and normal distributions (data not shown) suggested a polygenic inheritance of the traits studied. The component traits showed low (31%) to high (90%) heritability. ANOVA indicated highly significant differences ($P < 0.001$) among the 210 RILs, and also highly significant environmental effects on the traits (Table 2).

Table 2 ANOVA for genotype, environment, genotype x environment interactions of shoot fly resistance and associated traits in the RIL population derived from the cross 27B × IS 2122

Source of variation	DF	MSS	DF	Mean sum of squares (MSS)			
		DH %		GS	SV	TDU	TDL
Genotype	209	263.4**	209	0.71**	0.59**	239.4**	89.9**
Environments	7	84031**	2	1.26**	2.40**	192.7**	150.3**
G x E	1463	132.7	418	0.12	0.14**	63.5**	27.39**

GS Glossiness, *SV* Seedling vigor, *TDU* Trichome density on upper surface of leaf, *TDL* Trichome density on lower surface, *DH %* Deadheart percentage

* $P < 0.05$; ** $P < 0.01$

Table 3 Pearson correlation coefficients between the traits associated with shoot fly resistance

	DH %	GS	SV	TDU
GS	−0.38**			
SV	−0.35**	0.73**		
TDU	−0.39**	0.35**	0.28**	
TDL	−0.26**	0.34**	0.28**	0.81**

* $P < 0.05$; ** $P < 0.01$, *GS* Glossiness, *SV* Seedling vigor, *TDU* Trichome density on upper surface of leaf, *TDL* Trichome density on lower surface, *DH %* Deadheart percentage

The correlation co-efficients between resistance component traits were estimated based on RIL means over the environments (Table 3). Correlations among the traits for shoot fly resistance were found to be significant. DH% was negatively associated with the component traits GS, SV, TDU and TDL. The highest correlations were found between TDU and TDL ($r = 0.81$**), followed by the correlation between GS and SV ($r = 0.73$**). DH% was observed to have significant negative associations with GS ($r = -0.38$**), SV ($r = -0.35$**), TDU ($r = -0.39$**) and TDL ($r = -0.26$**).

Genetic linkage map construction

Out of more than 500 SSR markers used, 149 revealed polymorphism between parents. These 149 polymorphic SSR markers were evenly distributed on the 10 chromosomes, and therefore were used for construction of the genetic linkage map with the RIL population. The genetic map spanned 700 cM in length, with an average distance of 4.7 cM between adjacent markers. To dissect and map the underlying QTL for resistance, multiple QTL mapping (MQM) analysis was conducted on component traits using genotypic data from 149 marker loci. The QTL results were based on average trait values of the component traits over eight environments for DH%, and over three environments for GS, SV, TDU and TDL. 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 25 QTL for shoot fly resistance, 5 each for leaf GS and SV, 10 for DH%, 2 for TDU and 3 for TDL. The LOD values of QTL ranged from 2.44 to 24.1, and R^2 (%) from 4.3 to 44.1%. The QTL detected for the component traits are listed in Table 4 and illustrated in Fig. 1.

Mapping QTLs underlying component traits

Glossiness (GS)

Five QTL, which were distributed on four chromosomes (SBI-01, 02, 04 and 10), were identified for GS. The phenotypic variation explained by individual QTL ranged from 5.7 to 14.7%. At all the QTL regions, alleles contributed by 27B resulted in decreasing the trait. Among the 5 QTL, 3 (*QGs.dsr-1*, *QGs.dsr-4.1* and *QGs.dsr-10*) were detected in combined analysis and 2 QTL (*QGs.dsr-2* and *QGs.dsr-4.2*) were specific to the environment K07. MQM identified one major QTL on chromosome SBI-10 (near the marker Xtxp320), explaining 14.7% of the phenotypic variation.

Seedling vigor (SV)

Five QTL located on four chromosomes (2 on SBI-01, one each on SBI-02, SBI-09 and SBI-10) were identified for this trait. Individual QTL for this trait accounted for 5.1–7.9% of the phenotypic variation. For all the QTL, alleles from 27B decreased the trait. Among all the QTL, four were detected in combined analysis and one (*QSv.dsr-10*) was detected specifically in the environment K08.

Trichome density (upper and lower leaf surfaces)

Two QTL distributed on 2 chromosomes (one each on SBI-07 and SBI-10) were identified for TDU, explaining 4.3–44.1% of the phenotypic variation. At the QTL position, *QTdu.dsr-7*, alleles from 27B increased the trait. The QTL on

Table 4 Quantitative trait loci (QTL) associated with component traits of shoot fly resistance in sorghum RILs from the cross 27 B × IS 2122

Trait	S no.	QTL name	Environment	Chromosome	Flanking markers	QTL position	LOD	R^2 [a]	Additive effect[b]	Comments
GS	1	*QGs.dsr-1*	Av, K06	SBI-01	Xtxp357*-Xtxp32	9.48	3.08	5.9	−0.13	New
	2	*QGs.dsr-2*	K07	SBI-02	Xisp10336*-Xtxp1	32.0	4.09	7.6	−0.19	New
	3	*QGs.dsr-4.1*	Av	SBI-04	Xtxp24*-Xtxp41	4.9	3.05	5.7	−0.12	New
	4	*QGs.dsr-4.2*	K07	SBI-04	Xcup20-Xtxp343*	9.89	4.27	7.5	−0.18	New
	5	*QGs.dsr-10*	Av, K06, K07	SBI-10	Xtxp320*-Xcup16	38.26	6.95	14.7	−0.19	Satish et al. (2009)
SV	1	*QSv.dsr-1.1*	Av, K08	SBI-01	gpsb3-Xtxp357*	8.48	4.35	7.9	−0.13	Knoll et al. (2008)
	2	*QSv.dsr-1.2*	Av, K08	SBI-01	Xcup6-Xtxp302*	8.89	2.78	4.9	−0.10	Knoll et al. (2008)
	3	*QSv.dsr-2*	Av	SBI-02	Xisep1145*-Xtxp197	21.82	2.9	5.1	−0.10	New
	4	*QSv.dsr-9*	Av, K08	SBI-09	Xisp102271*-Xtxp101	39.58	3.33	7.1	−0.12	New
	5	*QSv.dsr-10*	K08	SBI-10	Xisep0625-Xgap1*	22.32	2.92	5.90	−0.12	Satish et al. (2009)
TDU	1	*QTdu.dsr-7*	Av, K08	SBI-07	Xcup57*-Xtxp99	0	2.8	4.3	1.85	New
	2	*QTdu.dsr-10*	Av, K06, K07, K08	SBI-10	Xtxp320*-Xcup16	18.94	24.1	44.1	−6.27	Satish et al. (2009)
TDL	1	*QTdl.dsr-3*	K06	SBI-03	Xtxp70*-Xtxp48	34.05	2.44	5.00	1.76	New
	2	*QTdl.dsr-10.1*	Av, K06, K07	SBI-10	Xgap1*-Xtxp320	29.29	12.31	24.1	−2.69	Satish et al. (2009)
	3	*QTdl.dsr-10.2*	K08	SBI-10	Xtxp320-Trit*	44.01	5.92	12.7	−2.68	Satish et al. (2009)
DH%	1	*QDh.dsr-1.1*	DSR06	SBI-01	Xisp10322*-Xtxp316	74.06	3.62	8.00	2.95	New
	2	*QDh.dsr-1.2*	UDAv	SBI-01	Xisp10314-Xtxp208*	1.00	2.53	5.00	1.56	New
	3	*QDh.dsr-2*	UD08	SBI-02	Xisp10336*-Xtxp1	31.00	2.71	6.40	2.34	New
	4	*QDh.dsr-6.1*	Av	SBI-06	Xisep0432*-Xtxp45	11.00	2.5	5.4	1.43	New
	5	*QDh.dsr-6.2*	UDAv, UD07	SBI-06	Xisp10347-gpsb18*	53.81	5.66	7.50	2.50	New
	6	*QDh.dsr-7.1*	Av	SBI-07	Xcup70*-Xgap342	42.74	3.36	7.1	1.57	New
	7	*QDh.dsr-7.2*	UDAv, AKAv DSR08,	SBI-07	Xtxp278*-Xisp10233	59.74	4.08	9.80	3.93	New
	8	*QDh.dsr-9*	AKAv	SBI-09	Xtxp101*-Xcup21	52.00	2.79	6.40	3.10	Satish et al. (2009)
	9	*QDh.dsr-10.1*	Av, UDAv, UD07, DSR06	SBI-10	Xtxp320*-Trit	39.01	6.01	12.8	2.14	Satish et al. (2009)
	10	*QDh.dsr-10.2*	DSRAv, DSR07	SBI-10	Xisep0625-Xgap1*	26.31	4.38	9.40	2.17	Satish et al. (2009)

GS Glossiness, *SV* Seedling vigor, *TDU* Trichome density on upper surface of leaf, *TDL* Trichome density on lower surface, *DH %* Deadheart percentage

* Flanking marker closest to the QTL position. Environments K06, K07, K08 and Av (average) indicate QTL detected in the environments Kharif (2006), Kharif (2007), Kharif (2008)and across all environments, respectively. DSR, UD and AK are the locations Directorate of Sorghum Research, Udaipur and Akola

[a] Percentage of phenotypic variation explained by the QTL

[b] Additive effect of the susceptible parent, 27B. A positive value implies that the 27B allele increased phenotypic value, whereas a negative value implies that 27B allele decreased phenotypic value

SBI-10 is a major QTL contributing for 44.1% of phenotypic variation, and was identified in all the environments.

Three QTL were detected for TDL which were distributed on two chromosomes, one on SBI-03 and two on SBI-10. The phenotypic variation explained by individual QTL ranged from 5.0 to 24.1%. At one QTL position, *QTdl.dsr-3*, 27B contributed positive alleles, and at other QTL positions it contributed negative alleles. Among all QTL, one (*QTdl.dsr-10.1*) was detected in combined analysis, and *QTdl.dsr-3* was specific to the environment K06 and *QTdl.dsr-10.2* was specific to K08.

Deadheart percentage (DH%)

Ten QTL were identified for this trait, which is a direct measure of resistance. These were distributed on six chromosomes with one each on SBI-02 and SBI-09, two each on SBI-01, SBI-06, SBI-07 and SBI-10. The phenotypic variation explained by individual QTL ranged from 4.5 to 12.8%. Three out of 10 QTL (*QDh.dsr-6.1*, *QDh.dsr-7.1* and *QDh.dsr-10.1*) were detected in combined analysis of all the environments, 5 were detected in location averages (*QDh.dsr-1.2* and *QDh.dsr-6.2* at Udaipur, *QDh.dsr-9* at Akola, *QDh.dsr-7.2* at Udaipur and Akola, and *QDh.dsr-10.2* at DSR), and 2 were detected in specific environments (*QDh.dsr-1.1* at DSR06 and *QDh.dsr-2* at UD08). Three major QTL, *QDh.dsr-10.1* (explaining 12.8% of phenotypic variation), *QDh.dsr-10.2* (explaining 9.4% variation) on SBI-10 and *QDh.dsr-7.2* (explaining 9.8% of phenotypic variation) on SBI-07 were detected near the markers Xtxp320, Xgap1 and Xtxp278 respectively. For all the QTL, alleles from the susceptible

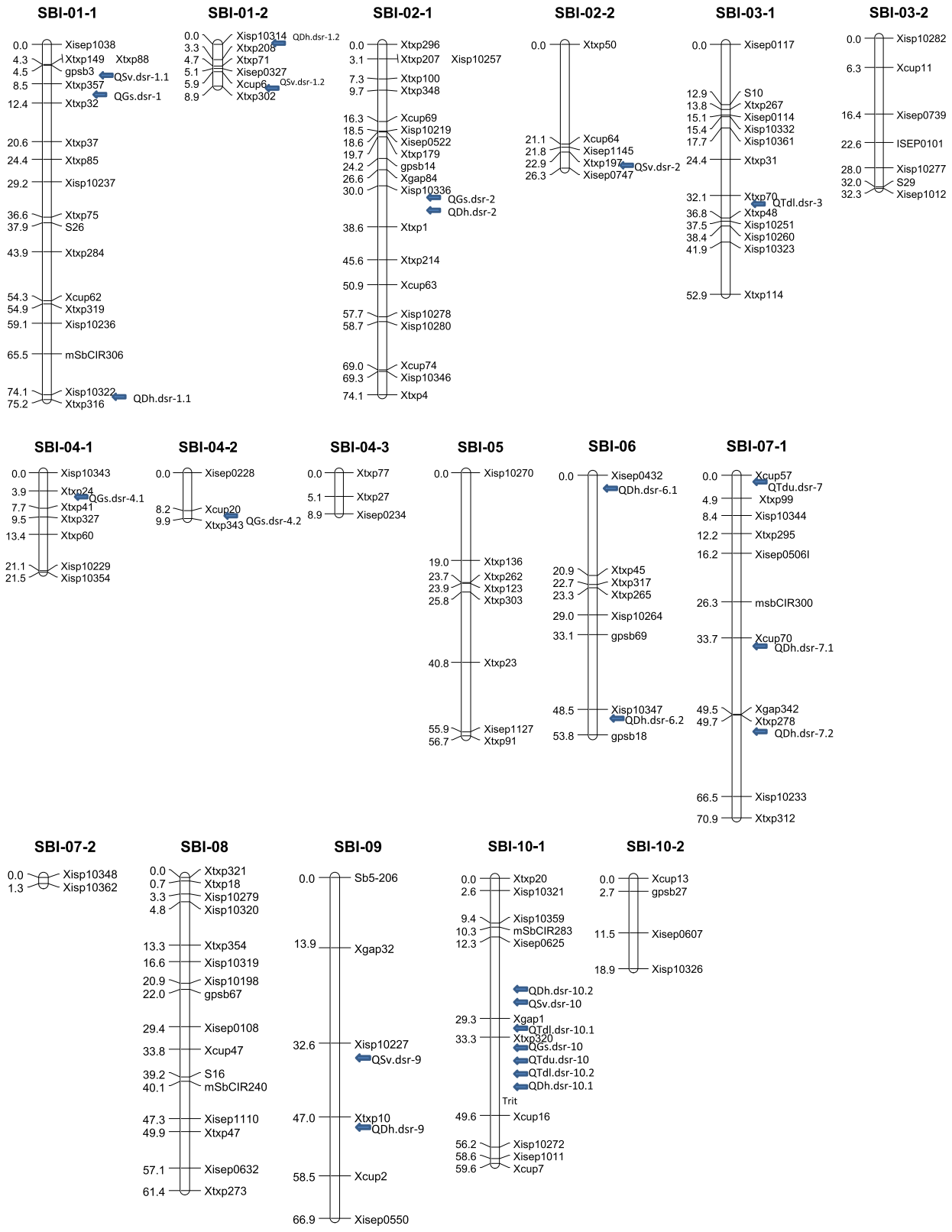

Fig. 1 Genetic linkage map of sorghum showing 25 quantitative trait loci (QTL) identified for shoot fly resistance and associated traits in the 27B × IS2122 RIL population

parent 27B increased deadhearts, thus contributing for susceptibility.

Co-localization of QTL

In the mapping population, co-localization of QTL was observed for component traits. The co-localization was mainly observed on chromosomes SBI-02 (2 QTL) and SBI-10 (7 QTL) (Table 4; Fig. 1). The co-localizing QTL for traits GS and DH% were identified on chromosome SBI-02 between the markers Xisp10336-Xtxp1. Similarly, on chromosome SBI-10, two co-localizing clusters, one with 2 and one with 4 QTL, were detected. They were identified between the markers Xisep0625-Xgap1 (cluster I; one SV QTL and one DH QTL) and Xtxp320-Xcup16 (cluster II; one GS QTL, two trichome QTL and one DH% QTL).

Discussion

Pursuing resistance to shoot fly is one of the most important objectives in sorghum improvement programs. The progress in improving resistance levels of sorghum cultivars for the last 30 years by sorghum scientists, using the identified resistance sources through conventional selection methods has been slow, because of the involvement of many mechanisms of resistance, polygenic nature of inheritance, and high influence of environment. To study the inheritance of such traits, whose expression is highly influenced by environment, recombinant inbred lines (RILs) developed by the single seed descent method are highly useful (Singh et al. 1999). Phenotyping the RIL population for shoot fly resistance and traits associated with resistance, in different environments is a very important step for detection of molecular markers linked to QTLs associated with quantitative resistance genes. In the present study, phenotyping for shoot fly resistance was carried out in 8 environments. A total of 25 QTLs distributed on eight chromosomes (SBI-01, 02, 03, 04, 06, 07, 09 and 10) were identified for the component traits of shoot fly resistance.

Quantitative trait loci for component traits of shoot fly resistance

The leaf GS at seedling stage probably has a strong influence on the orientation of shoot fly females due to reflection of light in sorghum, thereby reducing the oviposition (Agarwal and Abraham 1985; Sharma 1993; Kamatar and Salimath 2003). The glossy trait is associated with resistance to biotic stress such as shoot fly (Agarwal and House 1982), and also to abiotic stresses (Maiti et al. 1984). The intensity of GS of the leaves at the seedling stage is positively associated with resistance to shoot fly. The results of the present study indicate significant negative correlation between shoot fly deadheart percentage and GS as reported earlier (Nwanze et al. 1990; Kamatar and Salimath 2003).

The major QTL for GS was identified on SBI-10 between the markers Xtxp320-Xcup16 (*QGs.dsr-10*, explaining 14.7% of the phenotypic variation), corresponds with the QTL reported earlier by Satish et al. (2009), indicating that it is a stable QTL which can be used for the improvement of this trait by MAS. Besides this, four new minor QTLs for GS were identified in this study. One of the new QTLs for GS, *QGs.dsr-1* on SBI-01 although explained less phenotypic variation (5.9%) when taken on average, explained 10.4% of phenotypic variation in kharif 2006. Such QTLs needs to be validated further to see their suitability for MAS.

SV is a very important component trait of shoot fly resistance, and early season cold tolerance (Knoll et al. 2008) in sorghum. High SV inhibits the establishment of the shoot fly larva in causing damage to the seedling (Jayanthi et al. 2002). Faster growing plants remain in the favorable height (susceptible stage) for a relatively shorter period than the slower growing susceptible plants (Khurana and Verma, 1985). Shoot fly resistant lines have rapid initial plant growth, greater seedling height, longer stems and longer internodes (Mote et al. 1986). Significant negative correlation of SV with shoot fly damage was reported earlier (Taneja and Leuschner 1985), supporting the results in the present study. Of the five QTLs identified for this trait, *QSv.dsr-10* on SBI-10 corresponds with the one identified by Satish et al. (2009). Knoll et al. (2008) identified three QTL for SV, of which two were on SBI-01 and one on SBI-04. Two minor QTLs in the present study, *QSv.dsr-1.1* and *QSv.dsr-1.2* correspond with those reported by Knoll et al. (2008). These QTLs on SBI-01 were validated in various genetic backgrounds, and the utility of MAS for a quantitative trait was demonstrated (Knoll and Ejeta 2008).

Trichomes are non-glandular hairs that are microscopic in size and protrude above the epidermis (Gibson and Maiti, 1983). The presence of trichomes provides a mechanical barrier against insect pests, for egg laying or larval movement, and thereby reduce plant damage. The present study showed that trichome density has a positive correlation with resistance to shoot fly in sorghum supporting the earlier reports (Jadhav et al. 1986; Dhillon et al. 2005, 2006). Density of trichomes is genetically controlled (Maiti and Gibson 1983). Besides trichome density, trichome morphology has been reported to play an important role in imparting shoot fly resistance (Padmaja et al. 2010a). In the present study, the susceptible parent (27B) had bicellular blunt trichomes in contrast to the resistant

parent (IS2122), which had unicellular pointed trichomes as reported earlier (Padmaja et al. 2010b). The RILs derived from these parents were also found to have differences in trichome morphology. Each of the RILs showed either bicellular blunt or unicellular pointed trichomes, indicating the monogenic inheritance of the trait. The gene for trichome morphology (*Trit*) in the RILs was mapped as a morphological marker on SBI-10, between the markers Xtxp320-Xcup16, where four important QTL for shoot fly resistance traits (one QTL each for GS, TDU, TDL and DH%) were identified. Satish et al. (2009) also mapped the trichome morphology gene on SBI-10.

Five QTLs were identified for leaf surface trichome density (two for TDU and three for TDL). Out of five, only one QTL on chromosome SBI-10 (Xtxp320-Xcup16) was associated with trichome density on both surfaces. The QTL position was different at this genomic region for both the surfaces, suggesting the involvement of different genes. At the QTL positions, *QTdu.dsr-7* and *QTdl.dsr-3*, the susceptible parent, 27B had contributed positive alleles for trichome density. Positive additive effect of 27B at *QTdl.dsr-3* and transgressive segregation of RIL population for TDL indicates that 27B had contributed for increased TDL, and ultimately towards resistance to shoot fly. This suggests that even the susceptible cultivars possess some favorable alleles for shoot fly resistance, and are helpful in improving the level of resistance through gene pyramiding. Contribution of favorable alleles from susceptible parents was reported earlier in sorghum with respect to shoot fly resistance (Satish et al. 2009), grain mould resistance (Audilakshmi et al. 2000) and head bug resistance (Deu et al. 2005).

Deadhearts are the resultant of shoot fly damage and serve as a direct measure of shoot fly resistance in sorghum. Ten QTL were identified for this trait, of which three are major QTL. Two major QTL identified for this trait were on SBI-10 between the markers Xtxp320-Xcup16 explaining 12.8%, and Xisep0625-Xgap1 explaining 9.4% of the phenotypic variance. Another major QTL (*QDh.dsr-7.2*) for DH% was observed on chromosome 7, explaining 9.8% of phenotypic variance. Out of ten QTLs identified, 3 (2 on SBI-10 and 1 on SBI-09) were common with those identified by Satish et al. (2009). The rest of seven were new QTLs identified in the present study. The major QTL on SBI-07 (*QDh.dsr-7.2*) is a new QTL for DH% which needs to be validated further. Out of 10 QTLs identified, seven were found to be specific either to the location or to the environment suggesting significant influence of environment on shoot fly resistance (Aruna et al. 2011).

During the last few years, emphasis has shifted towards the development of molecular markers from the transcribed region of the genome to associate the molecular polymorphisms of genes (sequence variation) with phenotypic variability of the traits. Construction of genetic linkage map by mapping functionally defined genes permits evaluation of co-location between genic-markers and QTLs of any trait (Aubert et al. 2006). It may also increase our understanding of the biochemical pathways and mechanisms affecting the important traits (Mathews et al. 2001; Zhang et al. 2004). Sorghum gene sequences in the major QTL regions were scanned based on the sequence information provided by the phytozome project to identify the putative candidate genes for shoot fly resistance. Some of the important putative candidate genes in the major QTL intervals based on literature are presented in Table 5. Scanning of the sorghum genome sequence at one of the important DH% QTL regions (*QDh.dsr-10.1*) on SBI-10 (Xtxp320-Xcup16; which was found to be co-localized with the QTL for GS, TDU and TDL, and explained 12.8, 14.7, 44.1 and 12.7% of the phenotypic variation for traits DH%, GS, TDU and TDL, respectively) revealed the presence of several important candidate genes. Some of the important candidate genes identified for plant resistance in this major QTL region include Cysteine protease Mir1 (deter insect feeding and prevent additional herbivory; Pechan et al. 2002) near the marker Xtxp320, NAC1 protein (involved in the developmental process like meristem development and plant defense in arabidopsis; Duval et al. 2002), Nramp1 (associated with stress resistance in arabidopsis; Alonso et al. 1999), Class III peroxidase 89 precursor (protects plants from oxidative damage due to biotic and abiotic stresses; Cosio and Dunand 2009) and HcrVf1 protein (conferring resistance to biotic stress; Dilworth et al. 2005). These would be the possible candidate genes for shoot fly resistance in this major QTL interval. Similarly, the sequence scan at another major DH% QTL interval on SBI-10 between the markers Xisep0625-Xgap1 (co-localized with the QTL for SV) also revealed some putative candidate genes, such as glossy 15 (responsible for wax biosynthesis and resistance to fall armyworm and southwestern corn borer in maize; Brooks et al. 2004), S receptor kinase (gene responsible for biotic stress resistance in rice; Chen et al. 2006); NBS-LRR disease resistance gene (Belkhadir et al. 2004). The gene for auxin-responsive protein IAA23 (responsible for faster plant growth and developmental responses; Kulaeva and Prokoptseva 2004) would be the candidate gene for seedling vigor in this region. The gene for WRKY1 protein (responsible for trichome development and resistance to biotic stress in Arabidopsis; Eulgem et al. 2000) is the candidate gene for trichomes on the leaf surface.

Recent sorghum QTL mapping studies revealed that multiple genomic regions of sorghum were involved in the resistance to shoot fly, and eight QTLs identified in the present study (*QGs.dsr-10*, *QSv.dsr-10*, *QTdu.dsr-10*,

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

Chromosome	QTL interval	Sorghum gene ID	Description	Functional role	Reference
SBI-07	Xtxp278-Xisp10233 (DH%; 4 Mb)	Sb07g020090	DRE binding factor 1	Activates plant's defense genes/ abiotic stress resistance	Chen et al. (2006)
		Sb07g020420	Cytochrome P450	Biosynthesis of defensive compounds	Munro et al. (2007)
		Sb07g020500	NB-ARC domain	Disease resistance and immune response	Ooijen et al. (2008)
		Sb07g021360	Zinc finger A20 and AN1 domain-containing stress-associated protein 12	Stress associated proteins involved in immune response	Vij and Tyagi (2006)
		Sb07g025620	Resistance protein T10rga 2-1A	Pest and disease resistance	Vander Biezen and Jones (1998)
		Sb07g025650	Leucine rich repeats Pi-b protein	Disease resistance and immune response	Rotheberg et al. (1990)
SBI-10	Xtxp320-Xcup16 (GS, TDU, TDL and DH%; 2.4 Mb)	Sb10g027980	Cysteine protease Mir 1	Insect resistance	Pechan et al. (2002)
		Sb10g026300	Auxin efflux carrier	Plant growth	Blakeslee et al. (2005)
		Sb10g027100	NAC domain protein NAC1	Developmental process like meristem development and plant defense	Duval et al. (2002)
		Sb10g027130	Integral membrane protein Nramp1	Resistance associated	Alonso et al. (1999)
		Sb10g027490	Class III peroxidase 89 precursor	Defensive response to biotic and abiotic stresses	Cosio and Dunand (2009)
		Sb10g027695	HcrVf1 protein	Biotic stress resistance and immune response	Dilworth et al. (2005)
SBI-10	Xisep0625-Xgap1 (SV, DH%; 7 Mb)	Sb10g022000	Thaumatin-like protein 1	Systematically acquired resistance and stress response in plants	Datta et al. (1998)
		Sb10g022690	S-receptor kinase	Universal stress protein-Response to stress	Chen et al. (2006)
		Sb10g022710	Serine/threonine-protein phosphatase PP2A-5 catalytic subunit	Regulation of cell functions	Xu et al. (2007)
		Sb10g023210	Auxin-responsive protein IAA23	Plant growth and developmental responses	Kulaeva and Prokoptseva (2004)
		Sb10g025053	Glossy15	Insect resistance and wax synthesis	Brooks et al. (2004)
		Sb10g025283	NBS-LRR disease resistance protein	Disease resistance and immune response	Belkhadir et al. (2004)
		Sb10g025300	NBS-LRR disease resistance	Disease and pest resistance	Hulbert et al. (2001)
		Sb10g025590	WRKY1 protein	Plant defense and trichome development	Eulgem et al. (2000)

QTdl.dsr-10.1, *QTdl.dsr-10.2*, *QDh.dsr-9*, *QDh.dsr-10.1* and *QDh.dsr-10.2*) correspond to the QTLs identified for shoot fly resistance in the early study by Satish et al. (2009) indicating that they are the stable QTLs across different genetic backgrounds, and reliable selection can be made based on these QTLs. In the present study, some new QTLs were identified for shoot fly resistance and associated traits on different chromosomes. The QTLs, *QDh.dsr-7.2* and *QDh.dsr-1.1* for DH%, and *QGs.dsr-1* for GS, identified in our mapping population are new QTLs for shoot fly resistance which needs further validation.

Among the QTLs identified in this study, the QTLs on SBI-10 conferred a major portion of the phenotypic resistance to shoot fly and are the valuable source for improving shoot fly resistance of commercial sorghum hybrids. We suggest the simultaneous use of the two closest markers Xtxp320 and Xgap1, or the marker intervals, *i.e.* four markers Xtxp320-Xcup16 and Xisep0625-Xgap1 of the two QTLs will precisely assist the selection by clarifying the inheritance of shoot fly resistance in breeding materials. The SSR markers close to the QTLs would be a valuable tool for breeding resistant parents for developing commercial sorghum hybrids.

Clustering of QTLs affecting the shoot fly resistance

The phenomenon of clustering of QTLs affecting different traits was observed in the present study. A major QTL cluster was located on the chromosome SBI-10, near the morphological marker (*Trit*), is significantly associated with four of the five traits measured. It is more likely that such clustering of QTLs for different traits may result from either tight linkage of several genes controlling these traits (Faris et al. 2000; Sandhu et al. 2001), otherwise their inheritance is functionally linked through a single gene affecting multiple characters (pleiotropic effect) (Veldboom et al. 1994; Xiao et al. 1996). However, the mapping resolution achieved in this study is not sufficient to determine whether pleiotrophy or tight linkage is the genetic cause of the association of the multiple QTLs in this region. Further investigations using more closely linked markers are needed to address these issues.

Implications in shoot fly resistance breeding

Conventional breeding methods supplemented by MAS would facilitate to improve the level of resistance. These newly identified SSR markers and the information on chromosomal location of the shoot fly resistance QTLs will facilitate our continued research towards isolation of shoot fly resistance gene through a map based cloning approach. The identified QTLs can be used in the breeding program for developing shoot fly resistant varieties and hybrids. Eventhough there are some common QTLs identified for shoot fly resistance and associated traits in the two populations studied (296B × IS18551 by Satish et al. 2009 and 27B x IS2122 in the present study), there are some more regions in each of the populations conferring resistance to shoot fly. These genomic regions are helpful for enhancing the efficiency of selection and shortening the course of breeding cycle to screen the target genotypes directly for related traits. Based on the finding that host immunity is absent and inheritance of shoot fly resistance is quantitative (polygenic) in nature, the resistance can gradually be built up through accumulation of desirable alleles. Pyramiding of the alleles from both the populations will improve the level of resistance and help in developing lines with higher level of resistance. If the objective of the breeding program is to develop insect resistant hybrids, the genes conferring resistance to insect pest need to be transferred into both male sterile and restorer lines. Hybrids based on resistant × resistant parents exhibit greater resistance than the hybrids based on other cross combinations (Sharma et al. 2006). It is always better to use different resistant sources on male and female sides, so that enough diversity is maintained between them and maximum heterosis is harvested. In that case, different resistant sources can be used for incorporation of shoot fly resistance in to the male and female parents, which ultimately may give raise to a shoot fly resistant hybrid.

Conclusions

The present study identified 25 QTLs for shoot fly resistance and the component traits using the RIL population of the cross 27B × IS2122. The important QTLs identified between the markers Xtxp320-Xcup16 (for GS, TDU, TDL and DH%), Xisep0625-Xgap1 (for DH% and SV), Xtxp278-Xisp10233 (for DH%), Xgap1-Xtxp320 (TDL), gbsb3E-Xtxp357 and Xisp102271-Xtxp101 for SV, should be highly useful for improving shoot fly resistance in sorghum through marker-assisted breeding (MAB). Majority of the QTLs influencing the traits in the study were consistent among the environments studied indicating that they are highly reliable for using in MAS. Furthermore, stable QTLs were identified for the traits across diverse genetic backgrounds by comparing the QTLs of the study with previous QTL studies for shoot fly resistance in sorghum. In addition, the alleles for resistance were also contributed by the susceptible parent (27B) at two QTL regions, which may be utilized for gene pyramiding.

The resistance QTLs identified would help breeders to bring in the beneficial allelic combinations and accelerate breeding programs for the development of resistant cultivars, and allow design of appropriate marker-assisted breeding strategies for improving shoot fly resistance in sorghum. Application of molecular marker technology, functional genomics, marker-assisted selection coupled with conventional breeding are the promising approaches for crop improvement for complex traits, such as shoot fly resistance.

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References

Agarwal BL, Abraham CV (1985) Breeding sorghum for resistance to shoot fly and midge. In: Proceedings of the international sorghum entomology workshop, Texas A&M University, College Station, Texas, USA, 15–21 July 1984. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India, pp 371–383

Agarwal BL, House LR (1982) Breeding for pest resistance in sorghum. In: Sorghum in eighties, Proceedings of the International symposium on sorghum, 2–7 November 1981. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India, pp 435–446

Agrama HA, Widle GE, Reeses JC, Campbell LR, Tuinstra MR (2002) Genetic mapping of QTL associated with green bug resistance and tolerance in *Sorghum bicolor*. Theor Appl Genet 104:1373–1378

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN 2, a bifunctional transducer of Ethylene and stress responases in Arabidopsis. Science 284:2148–2152

Anandan A, Huliraj H, Veerabadhiran P (2009) Analysis of resistance mechanism to *Atherigona soccata* in crosses of sorghum. Plant Breed 128:443–450

Aruna C, Bhagwat VR, Sharma V, Hussain T, Ghorade RB, Khandalkar HG, Audilakshmi S, Seetharama N (2011) Genotype × environment interactions for shoot fly resistance in sorghum (*Sorghum bicolor* (L.) Moench): response of recombinant inbred lines. Crop prot (Accepted)

Aruna C, Padmaja PG (2009) Evaluation of genetic potential of shoot fly resistant sources in sorghum (*Sorghum bicolor* (L.)). J Agri Sci 147:71–80

Audilakshmi S, Stenhouse JW, Reddy TP (2000) Genetic analysis of grain mould resistance in coloured sorghum genotypes. Euphytica 116:95–103

Aubert G, Morin J, Jacquin F, Loridon K, Quillet MC, Petit A, Rameau C, Lejeune-He'naut I, Huguet T, Burstin J (2006) Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. Theor Appl Genet 112:1024–1041

Belkhadir Y, Subramanian R, Dangi J (2004) Plant disease resistance protein signaling: NBS-LRR protein and their parameters. Curr Opin Plant Boil 7:391–399

Blakeslee JJ, Peer WA, Murphy AS (2005) Auxin transport. Curr Opin Plant Biol 8:494–500

Brooks TD, Willcox MC, Williams WP, Luthe DS (2004) Quantitative trait loci conferring resistance to fall Army worm and southern corn borer leaf feeding damage. Crop Sci 45:2430–2434

Chen M, Wang QY, Cheng XG, Xu ZS, Li LC, Ye X, Xia L, Ma Y (2006a) *Gm DREB2*, a soybean DRE binding transcription factor, conferred drought and high salt tolerance in transgenic plants. Biochem Biophys Res Comm 353:299–305

Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G, Ma B, Wang Y, Zhao X, Li S, Zhu L (2006b) AB-lectin receptor kinase gene conferring rice blast resistance. Plant J 46:794–804

Cosio C, Dunand C (2009) Specific functions of individual class III peroxidase genes. J Expt Bot 60:391–408

Datta K, Velazhahan R, Oliva N, Ona I, Mew T, Khush GS, MuthuKrishnan S, Datta SK (1998) Over expression of the cloned rice thaumatin like protein (PR-5) gene in transgenic rice plants enhances environmental friendly resistance to *Rhizoctonia solani* causing sheath blight disease. Theo Appl Genet 98:1138–1145

Deeming JC (1971) Some species of *Atherigona rondani* (Diptera: Muscidae) from northern Nigeria, with special reference to those injurious to cereal crops. Bull Entomol Res 61:133–190

Deu M, Ratnadass A, Hamada MA, Noyer JL, Diabate M, Chantereau J (2005) Quantitative trait loci for head-bug resistance in sorghum. African J Biotech 4:247–250

Dhillon MK, Sharma HC, Reddy BVS, Ram S, Naresh JS, Kaizhu (2005) Relative susceptibility of different male-sterile cytoplasms in sorghum to shoot fly, *Atherigona soccata*. Euphytica 144:275–283

Dhillon MK, Sharma HC, Reddy BVS, Ram-Singh, Naresh JS (2006) Inheritance of resistance to sorghum shoot fly, *Atherigona soccata*. Crop Sci 46:1377–1383

Dilworth ES, Beese S, Paris R, Belfanti E, Tartarini S, Sansavini S, Patocchi A, Gessler C (2005) Identification of functional apple scab resistance gene promoters. Theor Appl Genet 110:1119–1126

Duval M, Hsieh TF, Kim SY, Thomas TL (2002) Molecular characterization of AtNAM: a member of the Arabidopsis NAC domain superfamily. Plant Mol Biol 50:237–248

Eulgem T, Rushton PJ, Robartzek S, Somssich IE (2000) The WRKY super family of plant transcription factors. Trends Plant Sci 5:199–206

Falconer DS (1989) Introduction to quantitative genetics. Wiley, New York

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154:823–835

Fritz AK, Caldwell S, Worral WD (1999) Molecular mapping of Russsian wheat aphid resistance from triticale accession, PI 386156. Crop Sci 39:1707–1710

Gibson PT, Maiti RK (1983) Trichomes in segregating generations of sorghum matings. I. Inheritance of presence and density. Crop Sci 23:73–75

Haussmann BIG, Mahalakshmi V, Reddy BVS, Seetharama N, Hash CT, Geiger HH (2002) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:133–142

Hulbert SH, Webb CA, Smith SM, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol 39:285–312

Jadhav SS, Mote UN, Bapat DR (1986) Biophysical plant characters contributing to shoot fly resistance. Sorghum Newsletter 29:70

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Jayanthi PDK, Reddy BVS, Gour TB, Reddy DDR (2002) Early seedling vigour in sorghum and its relationship with resistance to shoot fly *Atherigona soccata* (Rond). J Entomological Res 26:93–100

Jotwani MG (1982) Factors reducing sorghum yields-insect pests. In: Sorghum in Eighties. Proceedings International Symposium on sorghum. 2–7 Nov. 1981. ICRISAT, Patancheru, pp 251–255

Jotwani MG, Marawaha KK, Srivastava KM, Young WR (1970) Seasonal incidence of shoot fly (*Atherigona soccata* Rond.) in jowar hybrids at Delhi. Indian J Ento 32:7–15

Kamatar MY, Salimath PM (2003) Morphological traits of sorghum associated with resistance to shoot fly, *Atherigona soccata* Rondani. Indian J Plant Prot 31:73–77

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, Ejeta G (2008) Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. Theor Appl Genet 116:541–553

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

Khurana AD, Verma AN (1985) Some physical plant characters in relation to stemborer and shoot fly resistance in sorghum. Indian J Entom 47:14–19

Kulaeva ON, Prokoptseva OS (2004) Recent advances in the study of mechanism of action of phytohormones. Biochem Mosc 69:233–247

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits by using RFLP linkage maps. Genetics 121:1447–1455

Maiti RK, Bidinger FR (1979) A simple approach to identification of shoot fly tolerance in sorghum. Indian J Plant Prot 7:135–140

Maiti RK, Gibson PT (1983) Trichomes in segregating generations of sorghum matings. II. Association with shoot fly resistance. Crop Sci 23:76–79

Maiti RK, Prasada Rao KE, Raju PS, House LR (1984) The glossy trait in sorghum: its characteristics and significance in crop improvement. Field Crops Res 9:279–289

Mathews BF, Devine TE, Weisemann JM, Beard HS, Lewers KS, McDonald MH, Park Y-B, Maiti R, Lin J-J, Kuo J, Pedroni MJ, Cregan PB, Saunders JA (2001) Incorporation of sequenced cDNA and genomic markers into the soybean genetic map. Crop Sci 41:516–521

Munro AW, Girvan HM, McLean KJ (2007) Cytochrome P450-redox partner fusion enzymes. Biochim Biophys Acta 1770:345–359

Mote UN, Kadam JR, Bapat DR (1986) Antibiosis mechanisms of resistance to sorghum shoot fly. J Maharashtra Agric Univ 11:43–46

Nagaraj N, Reese JC, Tuinstra MR, Smith CM, Amand PS, Kirkham MB, Kofoid KD, Campbell LR, Wild GE (2005) Molecular mapping of sorghum genes expressing tolerance to damage by green bug (Homoptera: Aphididae). J Econ Entomol 98:595–602

Nwanze KF, Reddy YVR, Soman P (1990) The role of leaf surface wetness in larval behavior of the sorghum shoot fly *Atherigona soccata*. Entomol Exp Appl 56:187–195

Padmaja PG, Madhusudhana R, Seetharama N (2010a) Epicuticular wax and morphological traits associated with resistance to shoot fly, *Atherigona soccata* Rondani in sorghum, *Sorghum bicolor*. Entomon 34:137–146

Padmaja PG, Madhusudhana R, Seetharama N (2010b) Sorghum shoot fly. Directorate of Sorghum Research, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India. ISBN 81-89335-29-4. Pp 82

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

Pechan T, Cohen A, Williams QP, Luther DS (2002) Insect feeding mobilizes a unique plant defense protease that disrupts the pleiotrophic matrix of caterpillar. Proc Natl Acad Sci USA 99:13319–13323

Rana BS, Tripathi DP, Balakotaiah K, Damodar R, Rao NGP (1975) Genetic analysis of some exotic x Indian crosses in sorghum. XI. Selection of shoot fly resistance. Indian J Genet 35:350–354

Rao M, Gowda S (1967) A short note on the bionomics and control of jowar fly. Sorghum Newsletter 10:55–57

Rotheberg JM, Jacobs JR, Goodman CS, Artavanis-Tsakonas S (1990) Slit: an extra cellular protein necessary for development of midline glia and commissural axon pathways contains both EGF and LRR domains. Genes Dev 4:2169–2187

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

Sandhu D, Champoux JA, Bondareva SN, Gill KS (2001) Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group IA chromosomes. Genetics 157:1735–1747

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]. Theo Appl Genet 119:1425–1439

Sharma HC (1993) Host plant resistance to insects in sorghum and its role in integrated pest management. Crop Prot 12:11–34

Sharma HC, Dhillon MK, Reddy BVS (2006) Expression of resistance to *Atherigona soccata* in F1 hybrids involving shoot fly resistant and susceptible cytoplasmic male-sterile and restorer lines of sorghum. Plant Breed 125:473–477

Singh BU, Padmaja PG, Seetharama N (2004) Stability of biochemical constituents and their relationship with resistance to shoot fly *Atherigona soccata* (Rond.) in seedling sorghum. Euphytica 136:279–289

Singh H, Grewal TS, Pannu PPS, Dhaliwal HS (1999) Genetics of resistance to Karnal bunt disease of wheat. Euphytica 105:125–131

Smith JSC, Kresovich S, Hopkins MS, Mitchell SE, Dean RE, Woodman WL, Lee M, Porter K (2000) Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. Crop Sci 40:226–232

Soto PE (1974) Ovipositional preference and antibiosis in relation to resistance to sorghum shoot fly. J Econ Entomol 67:165–167

Srinivas G, Satish K, Madhusudhana R, Seetharama N (2009a) Exploration and mapping of microsatellite markers from subtracted drought stress ESTs in *Sorghum bicolor* (L.) Moench. Theor Appl Genet 118:703–717

Srinivas G, Satish K, Madhusudhana R, Nagaraja Reddy R, Murali Mohan S, Seetharama N (2009b) Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor Appl Genet 118:1439–1454

Taneja SL, Leuschner K (1985) Resistance screening and mechanisms of resistance in sorghum to shoot fly. In: Proceedings of the international sorghum entomology workshop, 15–21 July, 1984, Texas A&M University, College Station, Texas, USA, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India, pp 115–129

Tao YZ, Hardy A, Drenth J, Henzell RG, Franzmann BA, Jordan DR, Butler DG, McIntyre CL (2003) Identification of two different mechanisms for sorghum midge resistance through QTL mapping. Theor Appl Genet 107:116–122

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 Res Int, Wageningen

Vander Biezen EA, Jones D (1998) The NB-ARC domain: a novel signaling motif shared by plant resistance gene products and regulators of cell death in animals. Curr Biol 8:226–227

Veldboom LR, Lee M, Woodman WL (1994) Molecular facilitated studies of morphological traits in an elite maize population. II. Determination of QTL for grain yield and yield components. Theor Appl Genet 89:451–458

Vij S, Tyagi AK (2006) Genome-wide analysis of the stress associated protein (SAP) gene family containing A20/AN1 Zinc-finger(s) in rice and their phylogenetic relationship with Arabidopsis. Mol Genet Genom 276:565–575

Wu Y, Huang Y (2008) Molecular mapping of QTL for resistance to the green bug *Schizaphis graminum* (Rondani) in *Sorghum bicolor* (Moench). Theor Appl Genet 117:117–124

Xiao J, Li J, Tanksley SD (1996) Identification of QTL affecting traits of agronomic importance in a recombinant inbred population derived from a sub specific rice cross. Theor Appl Genet 92:230–244

Xu C, Jing R, Mao X, Jia X, Chang X. (2007) A wheat (*Triticum aestivum*) protein phosphatase 2A catalytic subunit gene provides enhanced drought tolerance in Tobacco. Ann Bot. doi: 10.1093/aob/mc1285

Zhang WK, Wang YJ, Luo GZ, Zhang JS, He CY, Wu XL, Gai JY, Chen SY (2004) QTL mapping of ten agronomic traits on the soybean (*Glycine max* L. Merr.) genetic map and their association with EST markers. Theor Appl Genet 108:1131–1139