RESEARCH ARTICLE

Mapping of shoot fly tolerance loci in sorghum using SSR markers

D. B. APOTIKAR[1†], D. VENKATESWARLU[1†], R. B. GHORADE[2], R. M. WADASKAR[3], J. V. PATIL[4] and P. L. KULWAL[1,4*]

[1]*Department of Agricultural Botany, Dr. Panjabrao Deshmukh Agricultural University, Akola 444 104, India*
[2]*Sorghum Research Unit, Dr. Panjabrao Deshmukh Agricultural University, Akola 444 104, India*
[3]*Pulses Research Unit, Dr. Panjabrao Deshmukh Agricultural University, Akola 444 104, India*
[4]*State Level Biotechnology Centre, Mahatma Phule Agricultural University, Rahuri 413 722, India*

Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important crops in the semiarid regions of the world. One of the important biotic constraints to sorghum production in India is the shoot fly which attacks sorghum at the seedling stage. Identification of the genomic regions containing quantitative trait loci (QTLs) for resistance to shoot fly and the linked markers can facilitate sorghum improvement programmes through marker-assisted selection. A simple sequence repeat (SSR) marker-based skeleton linkage map of two linkage groups of sorghum was constructed in a population of 135 recombinant inbred lines (RIL) derived from a cross between IS18551 (resistant to shoot fly) and 296B (susceptible to shoot fly). A total of 14 SSR markers, seven each on linkage groups A and C were mapped. Using data of different shoot fly resistance component traits, one QTL which is common for glossiness, oviposition and dead hearts was detected following composite interval mapping (CIM) on linkage group A. The phenotypic variation explained by this QTL ranged from 3.8%–6.3%. Besides the QTL detected by CIM, two more QTLs were detected following multi-trait composite interval mapping (MCIM), one each on linkage groups A and C for the combinations of traits which were correlated with each other. Results of the present study are novel as we could find out the QTLs governing more than one trait (pleiotropic QTLs). The identification of pleiotropic QTLs will help in improvement of more than one trait at a time with the help of the same linked markers. For all the QTLs, the resistant parent IS18551 contributed resistant alleles.

[Apotikar D. B., Venkateswarlu D., Ghorade R. B., Wadaskar R. M., Patil J. V. and Kulwal P. L. 2011 Mapping of shoot fly tolerance loci in sorghum using SSR markers. *J. Genet.* **90**, 59–66]

Introduction

Sorghum is one of the most important cereal crops in the semiarid tropics (SAT) and are the fifth most important cereal crop worldwide after wheat, rice, maize and barley (Bantilan *et al.* 2004). Insect pests are the major biotic constraints for the production and productivity of sorghum causing economic losses over US $ 1 billion annually in the SAT. Among 100 insect pests that attack sorghum, one of the most important biotic constraints to sorghum production in India is the shoot fly (*Atherigona soccata* Rond.), which causes damage when sowings are delayed. The late sown crops tend to be affected by shoot fly damage as compared to the early sown crop. The infestation of shoot fly is high when sorghum sowings are staggered due to erratic rainfall distribution which is common in the SAT (Kumar *et al.* 2008). Shoot flies of the genus *Atherigona* are known to cause 'dead hearts' in a number of tropical grass species (Deeming 1971). They attack sorghum 5–25 days after emergence. The shoot fly larvae cut the growing tip which results in dead heart formation. Infestation causes dead hearts in seedlings as well as in tillers of old plants, resulting in considerable damage to the crop (Aruna and Padmaja 2009). Many approaches have been employed to minimize the loss caused by shoot fly. These include agronomic practices, natural enemies, synthetic insecticides and host plant resistance (Kumar *et al.* 2008). However, it is not always feasible to implement all these approaches in practice. For example, early sowing is not always feasible due to short sowing window, whereas chemical control is a limiting factor for majority of farmers. The seriousness of the shoot fly problem in sorghum, combined with the high costs and toxicity hazards of using chemical control render it necessary to develop new varieties or hybrids that are resistant to this pest.

*For correspondence. E-mail: pawankulwal@gmail.com.
†These authors contributed equally to this work.

Keywords. sorghum; shoot fly; SSR; linkage mapping; QTL mapping; pleiotropic QTL.

DNA marker-assisted breeding for a range of traits (particularly to overcome diseases and pests) has become one of the most important applications of biotechnology in recent times. Molecular markers are being used worldwide to tag specific chromosome segments containing the desired gene(s) to be introgressed into the breeding lines. In this way, indirect selection with co-dominant molecular markers (like SSR) tightly linked to gene(s) controlling characters of interest improves response to selection. DNA based marker-assisted selection (MAS) can supplement conventional breeding and therefore will become an integral part of the plant breeding practices in the coming years (for details about MAS and successful examples see Gupta *et al.* 2010).

Considering the economic importance of the pest, improving the genetic makeup of the plant is an important objective in the sorghum breeding programmes. Therefore, in order to better understand the inheritance of resistance, identification of QTLs and the linked markers are important. This will eventually help in successful introgression of the QTLs through marker-assisted breeding. Studies carried out in the past on shoot fly resistance in sorghum suggest quantitative nature of the trait (Sajjanar 2002; Folkertsma *et al.* 2003). For instance, in a recent study, Satish *et al.* (2009) identified a total of 29 QTLs for five component traits of shoot fly resistance with a varying degree of phenotypic variation in a 168 RIL mapping population derived from the cross 296B × IS18551. In the present study, a RIL mapping population derived from the reciprocal cross of the above i.e. IS18551 × 296B was used, first to prepare the skeleton map of two linkage groups and then QTL interval mapping following CIM and MCIM for identification of main effect as well as pleiotropic QTL for shoot fly component traits.

Material and methods

Plant material

A mapping population in the form of F_8 254 recombinant inbred lines (RILs), which was developed from a cross between genotypes IS18551 (shoot fly resistant) and 296B (shoot fly susceptible) was kindly provided by Dr B. V. S. Reddy, International Crops Research Institute for the Semi Arid Tropics (ICRISAT), India. The two parental genotypes are contrasting in terms of different shoot fly resistance characters. The distinguishing features of these parental genotypes are available in Satish *et al.* (2009).

The field experiment was conducted in late kharif (rainy) season at Sorghum Research Unit, Dr Panjabrao Deshmukh Agricultural University, Akola, India, during 2008–09. The experiment was carried out in a simple lattice design with two replications. Each entry was planted in a single row and the spacing between rows was 45 cm. To attain uniform shoot fly pressure under field conditions, the fishmeal technique (Nwaze 1997) was adopted to ensure shoot fly infestation in the experimental material. Plant protection measures were avoided.

Phenotyping and data analysis

Phenotypic data on shoot fly resistance was scored on 254 F_8 RILs and the two parental genotypes on different component traits viz., leaf glossiness (T1), pigmentation (T2), oviposition (number of eggs laid on seedling at 14 and 21 days after seedling emergence (DAE)) (T3 and T5), dead hearts (DH% recorded on 14th and 21st DAE) (T4 and T6), tiller dead heart (T7) and trichome density on abaxial (lower) leaf surface (T8). Leaf glossiness was visually scored on a scale of 1–5 scores at 10 DAE (1, highly glossy (light green, shinning, narrow and erect leaves) and 5, nonglossy (dark green, dull broad and dropping leaves)), pigmentation was assessed at 5 DAE (where 1, plumule or leaf sheath with dark pigment and 5, plumule or leaf sheath with green colour) following Sharma (1997). Oviposition was recorded by taking an average of the total number of eggs laid on 10 seedlings in a row. The number of eggs per seedling was calculated at 14 and 21 DAE. To record data on DH%, the total numbers of plants were initially recorded and the numbers of plants with dead hearts were subsequently recorded on 14th and 21st DAE. The mean values of DH% (number of dead hearts / total number of plants × 100) were recorded on 14th and 21st DAE. Tillers with dead hearts symptoms following shoot fly damage at 28 DAE were computed as per cent of the total number of tillers with DH. Trichome density was recorded at 14 DAE on the abaxial leaf surface on the central portion of the fifth leaf from the base, in three randomly selected seedlings in each row in each replication as per the procedure outlined by Sharma (1997). Briefly, the leaf segments (~ 2 cm^2) were cleared in acetic acid : alcohol (2 : 1) and transferred to 90% lactic acid in small vials. The leaf segments were then mounted on a slide in a drop of water and observed under stereomicroscope at a magnification of 40X. The number of trichomes on abaxial leaf surface was counted in three microscopic fields at random and expressed as trichome density (no./mm^2). The phenotypic correlations among different shoot fly component traits were estimated using MS Excel program (office.microsoft.com/).

Bulk segregant analysis

Based on the phenotypic observations, two bulks; resistant (B_1) comprising six resistant RILs (lines having high glossiness, more number of trichomes and less or without oviposition incidence and dead heart symptoms) and susceptible (B_2) comprising of six susceptible RILs (lines having no glossiness, less number / without trichomes and high oviposition incidence and more dead hearts) were made by pooling DNA samples in equal quantities. Bulk segregant analysis (Michelmore *et al.* 1991) was carried out for two bulks along with the parental genotypes using a set of 38 SSR primers (see table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/) to identify the linked polymorphic markers for resistance to shoot fly component traits.

Table 1. Mean phenotypic data of shoot fly resistance component traits in the parents IS18551 (resistant), 296B (susceptible) and in the RIL population.

Character	IS18551	296B	RILs
Leaf glossiness (1–5 scale)	1.00	4.50	2.79
Pigmentation (1–5 scale)	1.50	4.50	3.91
Oviposition per cent at 14, 21 DAE	0.90, 1.30	4.90, 6.00	2.00, 2.80
Dead hearts per cent at 14, 21 DAE	10.00, 15.00	55.00, 67.50	22.70, 31.74
Tillers dead hearts per cent at 28 DAE	0	33.30	46.41
Trichome density (no./mm^2)	31.00	2.00	9.17

Preparation of linkage map

The polymorphic SSR markers identified to be associated with shoot fly resistance component traits by bulk segregant analysis were utilized further for genotyping of the randomly selected 135 RILs. These markers corresponded to the linkage groups A and C of sorghum as per the published literature.

The genetic linkage map was constructed using MAPMAKER/EXP 3.0b (Lander *et al.* 1987). The criteria used were the minimum LOD of 2.0 and maximum recombination frequency of 49%. The recombination frequencies were converted into genetic distances using Kosambi mapping function (Kosambi 1944). Based on the genetic distances obtained from MAPMAKER, the map figures were drawn using QTL Cartographer 2.5 (Wang *et al.* 2007).

QTL interval mapping

The molecular and phenotypic data obtained were analysed first by simple linear regression method to know the association between the markers and the shoot fly resistant component traits. Linear regression was carried out following single marker analysis using QTL Cartographer 2.5. QTL interval mapping was performed for mean values of traits. Based on the data of various shoot fly component traits and the genotypic data of the SSR markers and the genetic distances, QTL interval mapping was carried out following CIM using QTL Cartographer 2.5. A LOD score of 2.0 was taken as criteria to define a putative QTL. Cartographer's Zmap QTL, model 6 with a window size of 10 cM was used for CIM analyses. The number of markers for the background control was set to 5. With the objective of improving precision of QTL mapping and also for detecting QTL which affected more than one trait (pleiotropic QTLs), MCIM was performed for the correlated traits using pair-wise combination of traits. The module JZmapqtl available in QTL Cartographer was used for conducting MCIM.

Results

Phenotypic data analysis

The phenotypic trait means of the parents (IS28551 and 296B) and their RIL population for different shoot fly component traits are given in table 1. Differences between the parental lines were significant for all component traits. The wide variation observed among the RILs for shoot fly resistance component traits also provided a scope for grouping them into resistant and susceptible one.

Phenotypic correlations between the component traits were estimated based on RIL means (table 2). Glossiness exhibited significant positive correlation with pigmentation and trichome density and significant negative correlation with oviposition at 14 and 21 DAE and dead hearts at 14 and 21 DAE. Pigmentation was negatively associated with traits oviposition at 14 and 21 DAE, dead hearts at 14 and 21 DAE, tillers dead hearts, while positively correlated with trichome density. Oviposition exhibited positive correlation with dead hearts at 14 and 21 DAE, tillers dead hearts and negative with trichome density. Dead hearts exhibited negative correlation with trichome density. Tiller dead hearts were negatively correlated with trichome density. The correlations between these traits were used to find out the pleiotropic QTL for the correlated traits following MCIM.

Table 2. Phenotypic correlations among components of resistance to shoot fly in sorghum RIL mapping population.

Trait	Glossiness	Pigmentation	Oviposition 14 DAE	Oviposition 21 DAE	Dead heart 14 DAE	Dead heart 21 DAE	Tillers dead heart
Pigmentation	0.40**						
Oviposition 14 DAE	−0.44**	−0.40**					
Oviposition 21 DAE	−0.51**	−0.52**	0.72**				
Dead heart 14 DAE	−0.44**	−0.40**	0.99**	0.72**			
Dead heart 21 DAE	−0.52**	−0.54**	0.72**	0.99**	0.72**		
Tillers dead hearts	−0.10	−0.17**	0.19**	0.31**	0.19**	0.30**	
Trichome density	0.28**	0.27**	−0.17**	−0.24**	−0.18**	−0.26**	−0.14*

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

Bulk segregant analysis

Of the total 234 RILs, randomly selected 135 RILs were used for further analyses. The banding pattern obtained in bulk segregant analysis (BSA) using SSR markers suggested possible association of two markers *Xtxp88* and *Xtxp228* with shoot fly component traits (figure 1). The single marker analysis performed using the genotypic data of these two markers suggested significant association of *Xtxp88* with seedling vigour and *Xtxp228* with leaf glossiness (results not shown). From the earlier published literature, it was found that SSR marker *Xtxp88* was genetically mapped on linkage group A, while marker *Xtxp228* was mapped on linkage group C. Based on this preliminary information, additional SSR markers specific to these linkage groups and showing polymorphism in the parental genotypes were used to genotype the mapping population and used for preparation of skeleton map and for QTL interval mapping. Thus, a total of 14 SSR markers specific to the linkage groups A and C (seven each) were used for genotyping the population.

Framework linkage map

Based on the genotypic data of 14 SSR markers, framework linkage map of groups A and C was prepared. Each linkage group had seven SSR markers mapped on it. The details on linkage group to which 14 SSR markers were assigned and genetic distances among the marker loci are given in figure 2. The total length of linkage group A was 247.6 cM while, that of linkage group C was 294.5 cM. The intermarker distances obtained were relatively more in the present study as compared to earlier published maps. However, the observed order of markers in these two sorghum linkage groups in the study was more or less same as compared to the earlier published genetic maps of sorghum. Therefore, the skeleton map prepared in the present study could be used for QTL mapping.

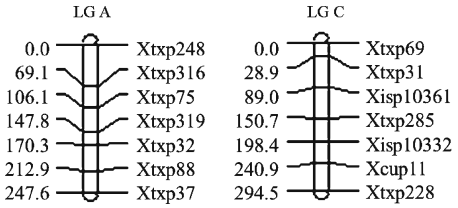

Figure 2. Framework linkage map of two linkage groups (LG) A and C of the RIL population derived from the cross IS18551 × 296B. The names of the markers are on the right while the cumulative genetic distances (cM) are on the left of the map.

QTL analysis

Phenotypic and genotypic data for 135 RILs were subjected to QTL analysis. The results of CIM analysis are given in table 3 and figure 3. CIM revealed presence of a single QTL in the marker interval *Xtxp248–Xtxp316*. This QTL was coincident for three traits viz., glossiness, oviposition at 21 DAE and dead hearts at 21 DAE. The LOD score values ranged from 2.4 to 4.3 with phenotypic variation explained ranging

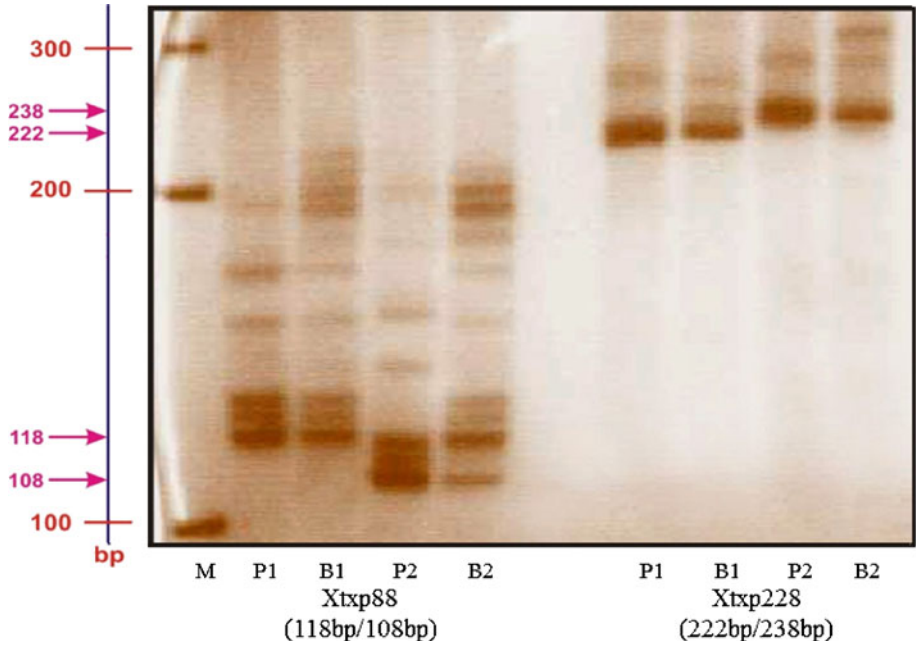

Figure 1. Results of bulk segregant analysis as obtained using the resistant parent IS18551 (P1), susceptible parent 296B (P2) and their respective bulks (B1 and B2) with two SSR markers; M, molecular weight marker; product size of the markers are given below marker names in parentheses.

Table 3. QTL associated with components of resistance to shoot fly based on composite interval mapping in a RIL population derived from the cross IS18551 × 296B.

Trait	Marker interval*	Distance	LOD	Additive effect	R^2 (%)
Glossiness	*Xtxp248–**Xtxp316***	56.01 cM	2.4	0.31	3.8
Oviposition at 21 DAE	*Xtxp248–**Xtxp316***	32.00 cM	3.4	0.94	6.0
DH at 21 DAE	*Xtxp248–**Xtxp316***	32.10 cM	4.3	10.60	6.3

*Marker nearest to the QTL is highlighted in bold. R^2, phenotypic variation explained.

from 3.8% to 6.3%. Positive additive effects obtained suggested that resistant parent IS18551 contributed favourable alleles for the QTL identified for all these three traits (table 3).

The multitrait-composite interval mapping was done using pair-wise combination of traits to find out presence of pleiotropic QTL. The eight traits (six traits with data for two traits recorded at two intervals) generated a total of 84 trait combinations (total number of combinations equal to $3n(n-1)/2$, where n is the number of traits). Analysis was performed so that QTL Cartographer analysed trait one first, followed by trait two, then traits one and two together. Likewise, there were a total of 84 trait combinations. The analyses confirmed the results obtained using CIM. In addition, it suggested presence of two more QTLs, one in the distal region of linkage group A and another on linkage group C (table 4; figure 4). In all these cases, positive additive effects were observed.

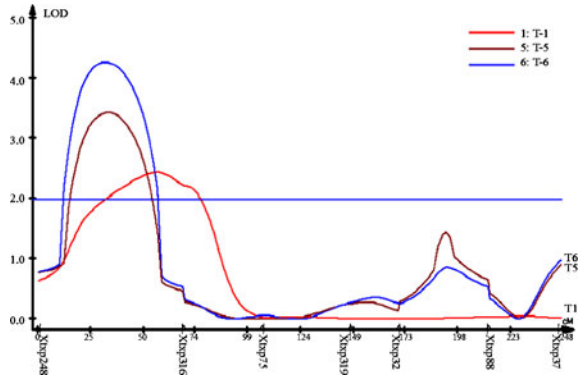

Figure 3. QTL Cartographer plot showing QTL peaks as obtained using composite interval mapping on linkage group A for leaf glossiness (T1), oviposition at 21DAE (T5) and dead heart per cent at 21DAE (T6).

Discussion

Development of cultivars resistant to shoot fly is one of the major goals in sorghum breeding programmes. The identification of genomic regions/QTLs that influence resistance can help breeders to introgress them into the breeding lines using the linked molecular markers. Besides, it also helps to increase the efficiency of selection in the breeding programmes. In the present study, the two parental genotypes differed considerably for all the observed shoot fly resistance component traits. The wide variation also observed among the RILs for shoot fly resistance component traits provided a scope for grouping them into resistant and susceptible one facilitating BSA and QTL interval mapping.

For all the shoot fly component traits, significant correlations were observed in the present study except between glossiness and tiller dead hearts. A negative but significant

Table 4. QTLs identified following multi-trait composite interval mapping.

Particular	Number	LOD score (range)
Total number of trait combinations	84	–
Combinations in which QTL is detected only between markers *Xtxp248–Xtxp316* on linkage group A	14	2.16–2.97
Combinations in which QTL is detected only between markers *Xtxp32–Xtxp88* on linkage group A	04	2.34–2.64
Combinations in which QTL is detected between markers *Xtxp248–Xtxp316* and *Xtxp32–Xtxp88* on linkage group A	09	2.16–2.97
Combinations in which QTL is detected only between markers *Xtxp11–Xtxp228* on linkage group C	06	2.01–3.94

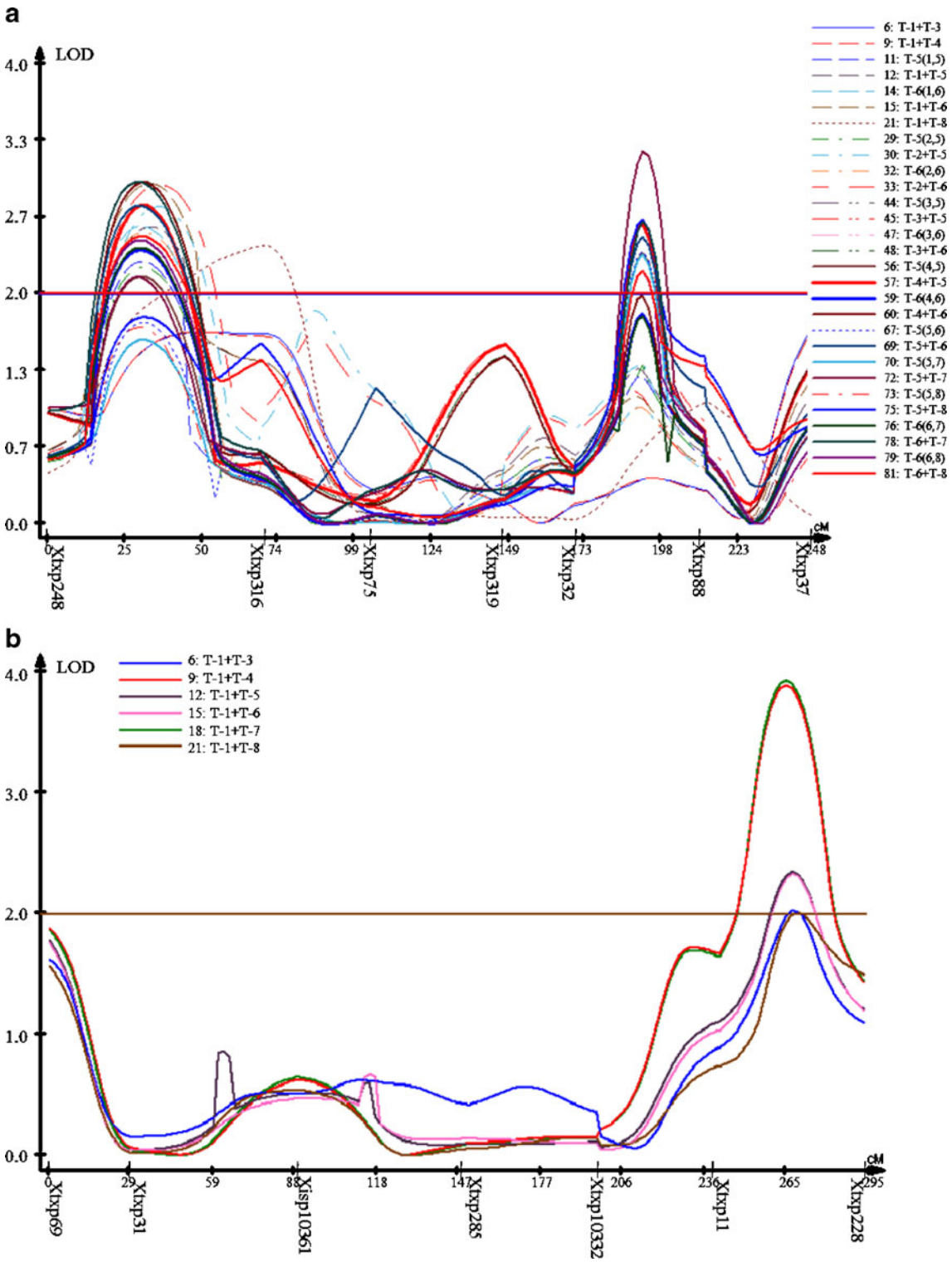

Figure 4. QTL Cartographer plot showing QTL peaks as obtained using multi-trait composite interval mapping on (a) linkage group A and (b) linkage group C for different traits and their combinations.

correlation of glossiness of leaves with that of oviposition and dead heart percentage was reported in sorghum (Nwaze *et al.* 1990; Kamatar and Salimath 2003; Satish *et al.* 2009). The results observed in the present study are in conformity with the above studies. Similarly, negative correlation between oviposition and glossiness was reported by Sajjanar (2002), while a negative correlation between oviposition and trichome density was reported by Hallali *et al.* (1982),

Karanjkar *et al.* (1992) and Sajjanar (2002). A highly positive and significant correlation was recorded between oviposition and dead heart percentage as was also reported by Hallali *et al.* (1982), Patel and Sukhani (1990), Sajjanar (2002) and Satish *et al.* (2009). Trait correlations are common, but important phenomenon in biology which helps plant breeders in deciding the strategy for improvement of a particular trait (Chen and Lubberstedt 2010). The significant correlations between these different component traits enabled the MCIM for identification of pleiotropic QTLs.

Sorghum (diploid with chromosome numbers $2n = 2x = 20$), has 10 linkage groups. Based on earlier published reports, two to four SSR markers per linkage group were selected for bulk segregant analysis using this mapping population. The BSA suggested association of two SSR markers viz., *Xtxp88* and *Xtxp228* with seedling vigour and glossiness, respectively (Venkateswarlu 2009). Data on seedling vigour could not be used for QTL mapping in the present study as it could not be recorded at appropriate time. These markers were reported to be mapped on the linkage groups A and C as per Bhattramakki *et al.* (2000) and Haussmann *et al.* (2002). Therefore, it was decided initially to prepare the skeleton map of these two linkage groups for QTL interval mapping. The skeleton map was generated using additional SSR markers. Thus, a total of seven SSR markers each was mapped on both the linkage groups. The distance between the adjacent markers is more in the present map as compared to the earlier published maps. This may be due to the segregation pattern of the genotypic data and also because of relatively less number of markers mapped on the individual linkage map in the present study.

Composite interval mapping detected one QTL between the markers *Xtxp248–Xtxp316* on linkage group A. The QTL is important because it was detected for three traits: glossiness, oviposition at 21 DAE and DH at 21 DAE. The detection of common QTL for oviposition at 21 DAE and DH at 21 DAE at same genetic location is not surprising because there was a strong significantly positive correlation between these traits ($r = 0.99$). The QTL explained up to 6.3% of the variation for the trait, which explains complex genetic nature of these different traits and possibility of effect of environment influencing these traits. The positive additive effect suggested that the resistant parent (IS18551) contributed alleles for resistance.

A number of reports on linkage and QTL mapping for various traits have been published in sorghum. However, only one comprehensive report of QTL interval mapping for shoot fly resistance in sorghum has been published so far (Satish *et al.* 2009). In this study, the mapping population used was derived from the cross 296B × IS18551. In the present study, the RIL population derived from a reciprocal cross of the above i.e., IS18551 × 296B was used. The QTL identified in the present study on linkage group A was not detected by Satish *et al.* (2009). Use of reciprocal mapping population can be one of the reasons for this. Moreover, it is expected that new recombinations will help in identifying new QTLs. Therefore, the genomic region identified in the present study can be important besides the QTLs identified by Satish *et al.* (2009). This also suggests that more than one mapping population should be used for detailed genetic dissection of the trait in terms of quantitative trait loci; otherwise there are possible chances of missing important genomic regions containing QTLs.

Besides CIM, MCIM was also performed for pair-wise combination of traits to find out the pleiotropic QTL. Out of a total 84 combinations, in 23 cases the QTL as identified by CIM was detected. Besides this, two more QTLs, one each on both the linkage groups were detected. It is interesting to note that only one QTL was detected on linkage group A using CIM. However, using MCIM, an additional QTL was detected on linkage group A in the distal region between the markers *Xtxp32* and *Xtxp88* for different trait combinations including that for trichome density. A QTL for trichome density on lower leaf surface was also detected by Satish *et al.* (2009) on linkage group SBI-01 between the same two markers. Thus, the QTL identified between the markers *Xtxp32* and *Xtxp88* in the present study seems to be the same as detected by Satish *et al.* (2009). The QTL exhibited positive additive effect, explaining that resistant parent IS18551 contributed alleles for resistance. Another pleiotropic QTL identified following MCIM was on linkage group C between the markers *Xtxp11* and *Xtxp228*. This QTL is also a new one which was also not detected following CIM. It should be noted that following BSA, the two SSR markers viz., *Xtxp88* and *Xtxp228* were found to have possible association with the shoot fly resistance component traits. However, no QTL was detected in the vicinity of any of these markers following CIM. MCIM could resolve these two QTLs in the vicinity of these markers. Every method of QTL mapping has certain advantages accompanied with some drawbacks. It is therefore important that the analysis is performed following different approaches in order not to miss the QTLs, particularly those which explain relatively less phenotypic variation.

Shoot fly resistance component traits viz., leaf glossiness, pigmentation, oviposition (%), dead heart (%), tillers dead heart and trichome density could be utilized as simple criteria for phenotypic selection for resistance to shoot fly in sorghum. However, phenotypic selection based on these traits is ineffective due to the complex, quantitative inheritance of these traits and may sometimes lead to type I and type II errors. Therefore, identification of QTLs and the linked markers for these component traits is very important from the marker-assisted selection point of view. Glossiness has been considered as an important trait conferring resistance against variety of insets including shoot fly in sorghum. Therefore, the new QTL identified for glossiness in the present study which is also a coincident QTL with two more traits i.e., oviposition at 21 DAE and DH at 21 DAE is an important one.

In the present study, a RIL mapping population was used which was derived from a reciprocal cross between the genotypes which were earlier used by Satish *et al.* (2009). The

present study helped us to identify a common QTL as was detected in the above study, besides two additional QTLs. It is therefore important to use more than one mapping population as well as more than one approaches of QTL mapping to find out as many QTLs as possible for their effective use in the marker-assisted breeding programmes.

The QTLs identified in the present study explained relatively less phenotypic variation. Therefore, in order to introgress them effectively into the elite sorghum genotypes via marker-assisted backcrossing, the region of the QTL needs to be saturated with an additional set of markers. This will further help in increasing the precision about the variation explained by the QTL. In addition, the identification of pleiotropic QTL can help in improvement of more than one trait at a time using the same linked markers.

Acknowledgements

Financial support to PLK in the form of research grants from Indian Council of Agricultural Research (ICAR) under Indo–US Agricultural Knowledge Initiative is acknowledged. Thanks are also due to Dr B. V. S. Reddy, ICRISAT for the supply of seed material of the mapping population. Authors are also thankful to the suggestions of the two anonymous reviewers.

References

Aruna C. and Padmaja P. G. 2009 Evaluation of genetic potential of shoot fly resistant sources in sorghum *Sorghum bicolor* (L.) Moench. *J. Agric. Sci.* **147**, 71–80.

Bantilan M. C. S., Deb U. K., Gowda C. L. L., Reddy B. V. S., Obilana A. B. and Evenson R. E. (ed.) 2004 *Sorghum genetic enhancement: research process, dissemination and impacts*, pp. 320. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.

Bhattramakki D., Dong J., Chhabra A. K. and Hart G. E. 2000 An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. *Genome* **43**, 988–1002.

Chen Y. and Lubberstedt T. 2010 Molecular basis of trait correlations. *Trends Plant Sci.* **15**, 454–461.

Deeming J. C. 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.

Folkertsma R. T., Sajjanar G. M., Reddy B. V. S., Sharma H. C. and Hash C. T. 2003 Genetic mapping of QTL associated with sorghum shoot fly (*Atherigona soccata*) resistance in sorghum (*Sorghum bicolor*). In *Final abstracts guide, plant and animal genome XI*, p. 462. Town and Country Hotel, San Diego, USA (http://www.intlpag. org/11/abstracts/P5d P462 XI.html).

Gupta P. K., Kumar J., Mir R. R. and Kumar A. 2010 Marker-assisted selection as a component of conventional plant breeding. *Plant Breed. Rev.* **33**, 145–217.

Hallali M. S., Gowda B. T. S., Kulkarni K. A. and Goud J. V. 1982 Inheritance of resistance to shootfly (*Atherigona soccata* Rond.) in sorghum. *SABRAO J.* **14**, 165–170.

Haussmann B. I. G., Hess D. E., Seetharama N., Welz H. G. and Geiger H. H. 2002 Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps. *Theor. Appl. Genet.* **105**, 629–637.

Kamatar M. Y. and Salimath P. M. 2003 Morphological traits of sorghum associated with resistance to shoot fly, *Atherigona soccata* Rondani. *Ind. J. Plant Prot.* **31**, 73–77.

Karanjkar R. R., Chundurwar R. D. and Borikar S. T. 1992 Correlation and path analysis of shoot fly resistance in sorghum. *J. Maharashtra Agric. Univ.* **17**, 389–391.

Kosambi D. D. 1944 The estimation of map distances from recombination values. *Ann. Eugen.* **12**, 172–175.

Kumar A. A., Reddy B. V. S., Sharma H. C. and Ramaiah B. 2008 Shoot fly (*Atherigona soccata*) resistance in improved grain sorghum hybrids. *E-Journal of SAT Agric. Res.* **6**, 1–4. doi:10.3914/lCRISAT.0039.

Lander E. S., Green P., Abrahamson J., Barlow A. and Daly M. J. 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174–181.

Michelmore W. R., Paran I. and Kesseli R. V. 1991 Identification of marker linked to diseases resistance genes by bulked segregant analysis, a rapid method to detect the markers in specific genetic region by using the segregating population. *Proc. Natl. Acad. Sci. USA* **88**, 9828–9832.

Nwaze K. F. 1997 Screening for resistance to sorghum shoot fly. In *Plant resistance to insect in sorghum.* (ed. H. C. Sharma, F. Singh and K. F. Nwaze), pp. 35–37. International Crops Research Institute for the Semi- Arid Tropics, Patancheru, India.

Nwaze K. F., Reddy Y. V. R. and Soman P. 1990 The role of leaf surface wetness in larval behaviour of the sorghum shoot fly *Atherigona soccata*. *Entomol. Exp. Appl.* **56**, 187–195.

Patel G. M. and Sukhani T. R. 1990 Biophysical plant characters associated with shoot fly resistance. *Indian J. Ent.* **52**, 14–17.

Sajjanar G. M. 2002 *Genetic analysis and molecular mapping of components of resistance to shoot fly* (*Atherigona soccata*) *in sorghum* (*Sorghum bicolor* (L.) Moench). Ph.D. thesis, University of Agricultural Sciences, Dharwad, India.

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

Sharma H. C. 1997 Plant resistance to insects: Basic principles. In *Plant resistance to insects in sorghum* (ed. H. C. Sharma, F. Singh and K. F. Nwanze) pp. 24–31. ICRISAT, Patancheru, India.

Venkateswarlu D. 2009 *Marker-trait association study for shoot fly tolerance in sorghum using SSR markers*. M.Sc. thesis, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, India.

Wang S., Basten C. J. and Zeng Z. B. 2007 Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, USA (http://statger.ncsu.edu/qtlcart/WQTL Cart.htm).

Received 9 July 2010, in revised form 20 August 2010; accepted 25 August 2010
Published on the Web: 19 May 2011