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Molecular mapping of QTLs for resistance to the greenbug *Schizaphis graminum* (Rondani) in *Sorghum bicolor* (Moench)

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Abstract Sorghum is a worldwide important cereal crop and widely cultivated for grain and forage production. Greenbug, *Schizaphis graminum* (Rondani) is one of the major insect pests of sorghum and can cause serious damage to sorghum plants, particularly in the US Great Plains. Identification of chromosomal regions responsible for greenbug resistance will facilitate both map-based cloning and marker-assisted breeding. Thus, a mapping experiment was conducted to dissect sorghum genetic resistance to greenbug biotype I into genomic regions. Two hundred and seventy-seven (277) F_2 progeny and their $F_{2:3}$ families from a cross between Westland A line (susceptible parent) and PI550610 (resistant parent) combined with 118 polymorphic simple sequence repeat (SSR) markers were used to map the greenbug resistance QTLs. Composite interval mapping (CIM) and multiple interval mapping (MIM) revealed two QTLs on sorghum chromosome nine (SBI-09) consistently conditioned the resistance of host plant to the greenbug. The two QTLs were designated as *QSsgr-09-01* (major QTL) and *QSsgr-09-02* (minor QTL), accounting for approximately 55–80%, and 1–6% of the phenotypic variation for the resistance to greenbug feeding, respectively. These resistance QTLs appeared to have additive and partially dominant effects. The markers Xtxp358, Xtxp289, Xtxp67 and Xtxp230 closely flanked the respective QTLs, and can be used in high-throughput marker-assisted selections (MAS) for breeding new resistant parents and producing commercial hybrids.

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Introduction

Sorghum bicolor (L.) Moench is among the most important crops in the world because of its good adaptation to a wide range of ecological conditions and low input cultivation, and diverse uses (Doggett 1988). It is widely cultivated in the Great Plains, USA, where its major insect pest is greenbug, *Schizaphis graminum* Rondani, which sucks juice from and injects toxins into sorghum plant tissues, and consequently causes damage to the plants (Teetes and Pendleton 2000). Since 1968, greenbug biotypes C, E, I, and K have been identified as inflicting serious damage to grain sorghum production in the area (Harvey and Hackerott 1969; Porter et al. 1982; Harvey et al. 1991, 1997). Developing resistant cultivars and hybrids as a component of the integrated pest management systems for sorghum production has been effective in controlling greenbug damage and remains the focus of many sorghum-breeding programs (Rooney 2004). No doubt, elucidation of the genetic mechanism for the resistance is valuable to the breeding efforts.

Classic genetic analysis using phenotypic data demonstrated the inheritance of sorghum resistance to greenbug

biotypes was relatively simple. Weibel et al. (1972) reported that the inheritance of biotype C resistance probably was controlled by a single incompletely dominant gene. Olonju Dixon et al. (1990) indicated that one to five resistance genes from different sources complemented each other and increased sorghum resistance. The recent studies of sorghum resistance to greenbug biotype I demonstrated that the resistance was incompletely dominant, and probably controlled by two genes which may rely on complementary gene action (Tuinstra et al. 2001).

Recently molecular marker systems and functional genomic technologies have been employed to resolve sorghum resistance to greenbugs into quantitative trait loci (QTLs) and into induced gene expression profiles. The association experiments of sorghum phenotypic resistance to biotypes C, E, I and K with restriction fragment length polymorphisms (RFLP) indicated that nine resistance loci in eight linkage groups in the sorghum genome independently explained 3–49% phenotypic resistance variation, and that epistasis accounted for 3–56% of the variation (Katsar et al. 2002). Agrama et al. (2002) observed nine QTLs in seven linkage groups responsible for resistance to biotypes I and K using simple sequence repeat (SSR) markers. Another similar experiment using a different resistant sorghum source identified eight QTLs on two linkage groups expressing resistance to both biotypes I and K (Nagaraj et al. 2005). Later, microarray analyses from two independent experiments revealed that 82 and 157 sorghum transcripts were responsive to greenbug feeding, and that those differentially expressed genes were probably involved in coordinated plant defense responses in sorghum (Zhu-Salzman et al. 2004; Park et al. 2005).

Greenbug biotype I has been the most damaging insect pest of sorghum in the US and often causes severe crop damage and economic loss (Harvey et al. 1991; Kofoid et al. 1991; Teetes and Pendleton 2000; Burd and Porter 2006). Sorghum sources resistant to the widely dispersed biotype have been identified (Andrews et al. 1993; Wu et al. 2006). Of the resistant sources, PI 550610 is a resistant line, introduced from Syria via Russia to the US (Andrews et al. 1993) and it is genetically distinct from other strong resistant sources, such as PIs 550607 and 607900 as revealed with over 800 AFLP markers (Wu et al. 2006). The specific objectives of this study were to identify SSR markers closely linked to major QTLs conferring resistance to greenbug biotype I in sorghum PI 550610, and to map the chromosomal locations of QTLs. The resultant information will facilitate early selection of breeding lines through marker-assisted selection and cloning of the greenbug resistance genes via the map-based cloning method.

Materials and methods

Plant materials

One F_2 population of 277 plants and one F_3 population of 277 families derived from each of the F_2 individuals by selfing were developed to obtain genotyping and phenotyping data for QTL mapping in the study. The female parent, Westland A line of the F_2 population is susceptible to greenbug biotype I, and the male parent, PI 550610 is a highly resistant line. Westland A line was selected from Wheatland, which originated from an early milo-type introduction from Kenya (Karper 1944; NPGS 2006). The cross of Westland A line by PI 550610 was made in 2003. F_2 seeds were harvested from one selfed F_1 plant. Both Westland A line and PI 550610 were homozygous at all polymorphic SSR marker loci examined in the study.

Phenotypic evaluation of greenbug biotype I resistance in $F_{2:3}$ families

With the aim to use the averaged phenotypic value of each $F_{2:3}$ family to accurately reflect the resistance value of the parental F_2 plant, 12 individuals of each $F_{2:3}$ family and two parents Westland A line and PI 550610 were used for phenotypic evaluation of greenbug damage to the plants. The phenotypic data were collected from two greenhouse experiments. Each experiment used a randomized complete block design with three replications. For each replication, two seedlings of each of the 277 $F_{2:3}$ families and two parents were prepared in one well of three growing flats, with each flat having 70 wells. In order to have all seedlings in similar size, we planted five seeds into each well and then thinned the seedlings to two just before greenbug infestation. The sorghum seedlings were infested with biotype I greenbugs at 2–3 leaf stage and maintained at $28 \pm 2°C$ in a greenhouse set to 14L: 10D photoperiod according to Wu et al. (2006). Foliage damage of the seedlings were visually scored at 3 days (GD3DPI), 7 days (GD7DPI), 10 days (GD10DPI), 14 days (GD14DPI) and 21 days (GD21DPI) post greenbug infestation. A scale of one to six was used with 1, 2, 3, 4, and 5 as <20, 20–40, 40–60, 60–80, 80% or more of foliage area damaged and 6 being dead (Starks and Burton 1977).

Genotyping with SSR markers

Plant genomic DNA was extracted from each of the 277 F_2 plants and parents according to the CTAB procedure of Doyle and Doyle (1990). DNA solutions were adjusted to a concentration of 10 ng/μl as the templates for PCR reactions.

Two parents and two randomly selected F_2 individuals were used in screening for polymorphic markers from a collection of 249 SSR primer pairs reported by Brown et al. (1996), Taramino et al. (1997), Dean et al. (1999), Bhattramakki et al. (2000) and Kong et al. (2000). SSR PCR reactions were performed in 96-well PCR plates in a PTC-220 Dyad Thermal Cycler (MJ Research Inc, MA, USA). The PCR reactions were programmed for 5 min at 94°C, 14 cycles of 20 s at 94°C, 1 min at 58°C and 30 s at 72°C, then 28 cycles of 20 s at 94°C, 1 min at 55°C and 30 s at 72°C, and followed by 10 min extension at 72°C. The SSR PCR reaction mixtures (10.85 μl in total) consisted of 4.47 μl of H_2O, 0.67 μl of 10× reaction buffer, 0.40 μl of 25 mM $MgCl_2$, 0.13 μl of 10 mM dNTP, 1.34 μl of 1 mM SSR forward primer tailed with M13 forward primer sequence (5′-CACGACGTTGTAAAACGACG-3′), 1.34 μl of 1 mM SSR reverse primer, 1 μl of 1 mM M13 forward primer labeled with florescent dye either in 700 or 800 nm (Li-Cor Inc, NE, USA), and 1.5 μl 10 ng/μl template DNA. PCR products of one plate labeled with the 700 nm dye and of another plate labeled with the 800 nm dye (Li-Cor Inc, NE, USA) were pooled and mixed thoroughly, loaded into wells of 6.5% KB^{plus} gel (Li-Cor Inc, NE, USA), and run in a 4300 DNA Analyzer (Li-Cor Inc, NE, USA). For codominant SSR markers, gel images were visually scored for each DNA sample.

Data analysis

SAS/MEANS was used for calculation of means of F_2 families and parents (SAS Institute 2003). SAS/TTEST was used to compare the foliage damage differences of Westland A line and PI 550610 at five time points post greenbug infestation, after the data were transformed by square root to correct non-normality as tested by SAS/UNIVARIATE (SAS Institute 2003). The transformed data were used for ANOVA of $F_{2:3}$ families with entry and replication as random effects in SAS/MIXED (SAS Institute 2003). The SAS/MIXED procedure was also used to obtain REML estimates of the variance components. Broad sense heritabilities (h^2) for the five greenbug damage ratings were calculated as $h^2 = \hat{\sigma}_g^2 / \left[\hat{\sigma}_g^2 + \hat{\sigma}_{ge}^2 + \left(\hat{\sigma}_{error}^2 / r \right) \right]$.

Genetic linkage maps were constructed using MAPMAKER 3.0 (Lander et al. 1987; Lincoln et al. 1992) with a threshold value of LOD (logarithm of odd ratio) $\geq$ 5.0. Kosambi mapping function was used to convert the recombination fractions to genetic distance (cM) (Kosambi 1944). The linkage groups of SSR markers were assigned to sorghum chromosomes following Kim et al. (2005) and Menz et al. (2002).

Averaged phenotypic values of the greenbug damage ratings for each family and parental line at each of the five

time points were used for QTL mapping analyses. For QTL analysis, composite interval mapping (CIM) and multiple interval mapping (MIM) were performed using Windows QTL Cartographer 2.5 (Basten et al. 2003; Wang et al. 2005). The threshold of significant QTLs in CIM was determined by a 1,000 permutation test (Basten et al. 2003; Wang et al. 2005). The significant P value of 0.05 was used for the model selection in MIM.

Results

Greenbug resistance in parental lines and $F_{2:3}$ families

As expected, PI 550610 showed significantly higher resistance to greenbug biotype I than Westland A line ($P < 0.01$ or 0.05) for all five ratings (Table 1). Significant ($P < 0.01$) and continuous variations in resistance to greenbug feeding was observed among the segregating families over the five leaf damage ratings. Broad sense heritabilities for the damage ratings ranged from 0.43 to 0.83.

QTL detection and their effects

Among the 249 SSR primer combinations, 118 (47.39%) were polymorphic. The polymorphic SSRs were mapped to 10 chromosomes and spanned a genetic distance of 1005 cM. CIM and MIM analyses identified four genomic regions (QTLs), significantly affecting the greenbug resistance as shown in Table 2. Among these QTLs, two of them located on SBI-09 consistently correlated to the resistance to greenbugs biotype I over all five leaf damage ratings (Fig. 1). In Fig. 1, the adjacent peaks on both sides of the major peak were probably ghost effects of the major QTL. One QTL on SBI-03 was responsible for the resistance scores at 3 DPI and 21 DPI, while another QTL on SBI-01 showed a significant effect on GD10DPI, which may imply a plant age-related effect on expression of the QTLs.

The major resistance QTL on chromosome nine (Table 2), designated as *QSsgr-09-01*, resided in the interval of 7.3 cM between Xtxp289 and Xtxp358 on the basis of linkage and QTL analyses. The location of *QSsgr-09-01* was 3.1 cM away from the latter marker, and 4.2 cM from the former marker (Fig. 1). This QTL had the highest LOD values, ranging from 14.2 to 39.5 for all five damage ratings. *QSsgr-09-01* accounted for 54.5–80.3% of the phenotypic variation in genetic resistance to greenbug biotype I (Table 2). The intra-locus genetic effects of *QSsgr-09-01* were additive and partially dominant. The second QTL identified on SBI-09 was flanked by Xtxp67 and Xtxp230, and designated as *QSsgr-09-02*. *QSsgr-09-02*

Table 1 The damage rating distributions and estimates of variance components and h^2 of $F_{2:3}$ families, and means and associated standard deviations of two parents and the families at five time points post greenbug biotype I infestation on sorghum seedlings

Trait descriptor	Number of $F_{2:3}$ families						Variance component estimates of $F_{2:3}$ families				Rating scale [0–6]		
	Rating scales										Means and associated standard deviations		
	0–1.00	1.01–2.00	2.01–3.00	3.01–4.00	4.01–5.00	5.01–6.00	$\hat{\sigma}^2_g$	$\hat{\sigma}^2_{ge}$	$\hat{\sigma}^2_{error}$	h^2	$F_{2:3}$ families	Westland A line	PI 550610
GD3DPI[a]	191	81	4	1	0	0	0.13 ± 0.02	0.16 ± 0.09	0.01 ± 0.09	0.43	0.78 ± 0.43	2.42 ± 0.39	0.21 ± 0.07
GD7DPI	7	102	81	36	37	14	1.18 ± 0.12	0.96 ± 0.09	0.04 ± 0.12	0.54	2.67 ± 1.20	5.34 ± 0.29	1.21 ± 0.14
GD10DPI	1	37	83	72	33	51	1.53 ± 0.15	0.34 ± 1.11	0.17 ± 1.11	0.75	3.56 ± 1.35	5.88 ± 0.17	1.74 ± 0.33
GD14DPI	0	4	41	84	79	79	1.10 ± 0.11	0.00	0.22 ± 0.33	0.83	4.27 ± 1.17	6.00 ± 0.00	2.85 ± 0.41
GD21DPI	0	0	4	37	106	130	0.44 ± 0.05	0.00	0.19 ± 0.35	0.70	4.96 ± 0.81	6.00 ± 0.00	4.09 ± 0.45

[a] GD3DPI stands for greenbug damage showing on sorghum seedlings at the 3-day post-infestation

had low LOD values ranging from 2.5 to 4.7, and explained 1.3–5.9% of the resistance variation (Fig. 1; Table 2). The other two putative resistance QTLs expressed a low level resistance with an inconsistent pattern (Table 2), and were located on SBI-01 and SBI-03, respectively. For each of the five greenbug damage ratings, the QTLs identified collectively explained a large portion of the phenotypic resistance variation, ranging from 63.0 to 81.6%.

Selection value for the greenbug resistance using the closely linked SSR markers

The two QTLs showing consistent resistance to greenbug biotype I over the five time points were flanked with the SSR markers Xtxp358 and Xtxp289, and Xtxp67 and Xtxp230, respectively (Fig. 1). The genotypic resistance level of each progeny plant derived from the crosses using PI 550610 as a resistant donor parent can be accurately predicted by the flanking markers. The means and associated standard deviations for three respective genotypes of the four markers are given in Table 3. As expected, the homozygous genotypes of the alleles from PI 550610 consistently had lower damage ratings than the respective genotypes of heterozygote or homozygote of the alleles from the susceptible parent (Table 3). For the five leaf damage ratings, the means of the homozygous genotypes of the four marker alleles from PI 550610 were consistently lower than those of either two markers, which was lower than the mean values of Xtxp358, although the differences between the means of the four markers and two markers were not significant ($P > 0.05$) (Table 3). Thus, the results suggest that the selection for genetic resistance to the greenbug with four markers or two markers be more predicable than using only one marker.

Discussion

The greenbugs collected in Stevens County, Kansas State in 1990, were designated biotype I, because they showed virulence to the sorghum plants resistant to biotype E (Harvey et al. 1991). Later surveys indicated that this biotype was ubiquitous and most damaging biotype on sorghum plants in the Great Plains (Bowling et al. 1994; Burd and Porter 2006). Mapping the sorghum QTLs for resistance to greenbug biotype I will facilitate both identification of greenbug resistance genes in plants and development of marker-assisted selections for breeding greenbug resistant new cultivars.

Recent sorghum QTL mapping studies revealed that multiple genomic regions of sorghum were involved in the resistance to greenbug biotype I (Agrama et al. 2002; Katsar et al. 2002; Nagaraj et al. 2005). A RFLP study on

Table 2 QTLs and their additive, dominant effects on greenbug resistance in a population of $F_{2:3}$ families derived from cross Westland A line (susceptible) × PI 550610 (resistant) identified by composite interval mapping

QTL no.		1		2		3		4		Model
Chromosome		SBI-09		SBI-09		SBI-03		SBI-01		
Marker interval		Xtxp289-Xtxp358	LR[b]	Xtxp67-Xtxp230	LR	Xtxp215-Xtxp216	LR	Xtxp319-Xtxp284	LR	
QTL position[a]		9.3		63.8–65.7		41.2		56.6		
GD3DPI	LOD	39.5		3.1		2.6		n.s.		
	A	−0.48	666	−0.13	8.5	−0.06	7.1			
	D	−0.18	13.8	−0.02	0.6	−0.09	3.7			
	R^2 (%)	54.9		5.9		2.2				63.0
GD7DPI	LOD	25.5		4.7		n.s.		n.s.		
	A	−1.36	279	−0.19	10.1					
	D	−0.55	19.7	−0.00	0.4					
	R^2 (%)	71.2		4.5						75.7
GD10DPI	LOD	29.4		4.5		n.s.		3.9		
	A	−1.68	666	−0.11	17.8			−0.07	37.4	
	D	−0.49	18.0	−0.21	0.0			−0.19	10.5	
	R^2 (%)	77.0		2.5				1.2		80.7
GD14DPI	LOD	31.8		2.9		n.s.		n.s.		
	A	−1.47	666	−0.11	13.2					
	D	−0.27	9.8	0.00	0.1					
	R^2 (%)	80.3		1.3						81.6
GD21DPI	LOD	14.2		2.5		2.8		n.s.		
	A	−0.96	61.4	−0.20	7.6	−0.18				
	D	−0.10	2.1	−0.06	0.1	−0.04				
	R^2 (%)	59.5		2.6		1.2				63.3

A additive effect, *D* dominant effect, *n.s.* non-significant at the LOD value, *LOD* logarithm of the odds

[a] QTL position from the telomere of the short arm

[b] Likelihood ratio test statistic (LR) is two times the negative natural log of the ratio of the likelihoods. Hypotheses are: H0: $a = 0$, $d = 0$; H1: $a \neq 0$, $d = 0$; H2: $a = 0$, $d \neq 0$

the genomic regions of PI 550607 indicated three linkage groups D, H and J were involved in the resistance (Katsar et al. 2002). To date, it is known that the three linkage groups D, H, and J correspond to sorghum chromosomes SBI-06, SBI-05 and SBI-07, based on the nomenclature of chromosome by Kim et al. (2005), and linkage groups reported by Bowers et al. (2003), and Katsar et al. (2002). Another mapping experiment with the mapping population derived from a cross between two sorghum lines Redlan and GBIK resistant to biotype I detected seven QTLs, dispersed on six linkage groups, which collectively affected the resistance variations at the 2-leaf and 8-leaf stages (Agrama et al. 2002). The six linkage groups (B, C, D, F, H and J) correspond to sorghum chromosomes SBI-02, SBI-03, SBI-04, SBI-09, SBI-10 and SBI-05. More recently, Nagaraj et al. (2005), using a population from the cross "96-4121" (resistant) by Redlan (susceptible), identified three QTLs significantly associated with biotype I resistance and tolerance. The linkage groups three and five

carrying the three loci were probably associated with sorghum chromosomes SBI-04 and SBI-01, respectively. Our present experiment using the population developed from two parents Westland A line and PI 550610, allowed identification of two QTLs on SBI-09, one on SBI-01, and one on SBI-05. Together, the results from the four experiments suggested that the genetic resistance to greenbug biotype I from different sources of sorghum may be a consequence of distinct loci.

It is noted that the experiment of Agrama et al. (2002) indicated that a SSR marker SbAGB03 on SBI-09 showed significant association to greenbug biotype I resistance. Although SbAGB03 was polymorphic in our mapping population, single marker and CIM analyses revealed that this marker was not significantly associated with resistance to greenbug biotype I in PI 550610. Thus, the two QTLs (*QSsgr-09-01* and *QSsgr-09-02*) on SBI-09 identified in our mapping population are new QTLs for the resistance to greenbug biotype I. Furthermore, the observation of

Fig. 1 Regional linkage map (*lower part*) and CIM QTL LOD profile (*upper part*) of sorghum chromosome nine (SBI-09) constructed with SSR markers from F_2 and $F_{2:3}$ populations derived from a cross of Westland A line (susceptible to greenbug biotype I) by PI 550610 (resistant) to demonstrate the LOD profiles and the QTL locations for greenbug resistance at five time points, GD3DPI, GD7DPI, GD10DPI, GD14DPI and GD21DPI. The line with LOD value of 2.4 parallel to the *X*-axis indicates the significant threshold of detection of QTLs. Genetic distance in centiMorgan (cM) and SSR marker designations are shown along the linkage map
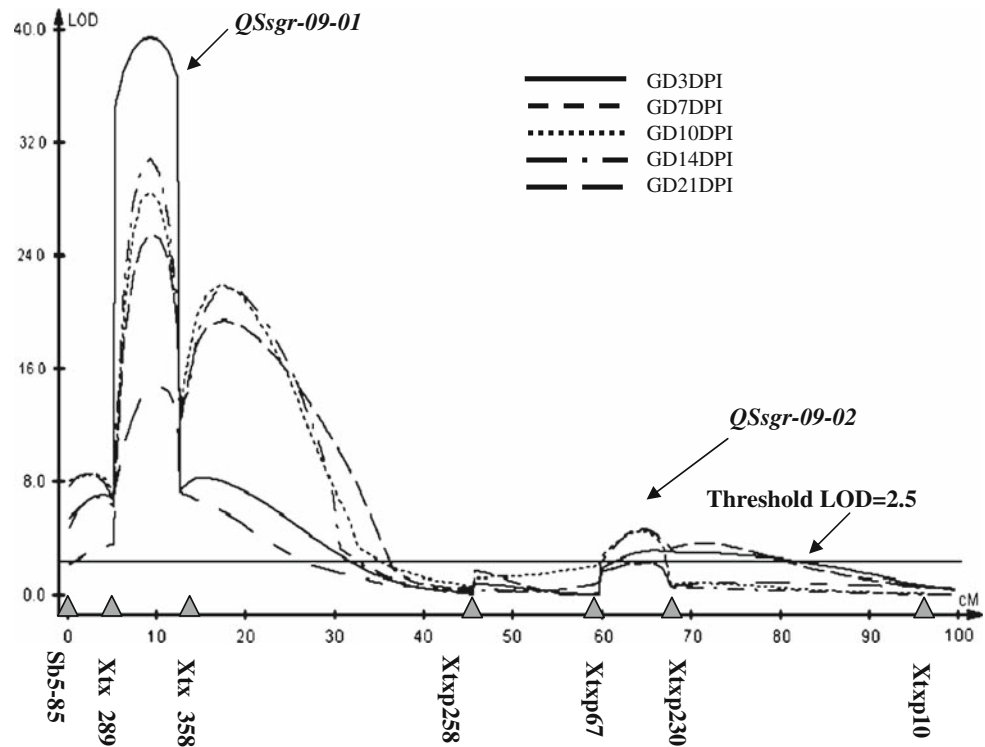
Table 3 Means and associated standard deviations of five greenbug damage ratings in various genotypes carrying SSR markers flanking the two QTLs *QSsgr-09-01* and *QSsgr-09-02*

Marker genotype	Plant no.	GD3DPI Mean ± SD	GD7DPI	GD10DPI	GD14DPI	GD21DPI
a_1a_1	60	1.33 ± 0.38	4.30 ± 0.76	5.44 ± 0.56	5.87 ± 0.39	5.94 ± 0.19
A_1a_1	134	0.72 ± 0.28	2.42 ± 0.73	3.27 ± 0.86	4.05 ± 0.76	4.85 ± 0.58
A_1A_1	76	0.45 ± 0.22[a]	1.64 ± 0.63[a]	2.33 ± 0.74[a]	3.13 ± 0.67[a]	4.16 ± 0.58[a]
$a_1a_1a_2a_2$	28	1.40 ± 0.51	4.29 ± 0.90	5.38 ± 0.71	5.78 ± 0.55	5.91 ± 0.25
$A_1a_1A_2a_2$	67	0.71 ± 0.23	2.41 ± 0.62	3.30 ± 0.76	4.08 ± 0.68	4.87 ± 0.53
$A_1A_1A_2A_2$	32	0.40 ± 0.19[b]	1.54 ± 0.58[b]	2.21 ± 0.75[b]	3.06 ± 0.60[a]	4.12 ± 0.50[a]
$a_1a_1a_2a_2$ $a_3a_3a_4a_4$	22	1.43 ± 0.56	4.37 ± 0.90	5.48 ± 0.67	5.85 ± 0.49	5.94 ± 0.19
$A_1a_1A_2a_2$ $A_3a_3A_4a_4$	51	0.71 ± 0.22	2.43 ± 0.53	3.32 ± 0.67	4.10 ± 0.62	4.88 ± 0.49
$A_1A_1A_2A_2$ $A_3A_3A_4A_4$	26	0.38 ± 0.21[b]	1.52 ± 0.63[b]	2.19 ± 0.82[b]	3.06 ± 0.66[a]	4.13 ± 0.55[a]

Same letter within a column is not significant at the probability level of 0.05

a allele for the susceptibility in parent Westland A line, *A* allele for the resistance in parent PI 550610, *1*, *2*, *3*, and *4* SSR marker Xtxp358, Xtxp230, Xtxp289 and Xtxp67, respectively

additive and partially dominant effects of the QTLs basically substantiated the findings on the biotype I resistance of Tuinstra et al. (2001). Tuinstra et al. (2001) observed 1:3 (resistant : susceptible) segregation ratio for greenbug resistance in three BC1F1 populations derived from biotype I greenbug resistant "KS 97" sorghum and explained that the results may come from two dominant genes with complementary gene action. However, the presence of the male sterile allele in this particular cross could have

prevented authors from detecting a QTL on chromosome 8, where the major restorer locus was mapped on by Klein et al. (2001, 2005).

Among the QTLs identified in PI 550610 from this study, the two QTLs on SBI-09 conferred a major portion of the phenotypic resistance to the greenbug and proved to be the valuable source for improving greenbug resistance of commercial sorghum hybrids. However, effective selection of resistant progeny from the crosses to

incorporate the two resistance QTLs is greatly affected by environment effects (Schweissing and Wilde 1978), tedious procedures of greenhouse screening and culture of true biotype I greenbugs, and the segregation of the two QTLs. We suggest the simultaneous use of the two closest markers Xtxp358 and Xtxp230, or the marker intervals, i.e. four markers Xtxp358-Xtxp289 and Xtxp67-Xtxp230 of the two QTLs will precisely assist the selection by clarifying the inheritance of greenbug resistance in breeding materials. The SSR markers close to the QTLs should also provide a valuable tool for breeding resistant parents of commercial sorghum hybrids.

The results of this study indicated sorghum chromosome nine (SBI-09) carried one major QTL and a minor one, conferring a large portion of the phenotypic variation for the greenbug resistance in the mapping population. The major QTL, *QSsgr-09-01*, in the interval of Xtxp289 and Xtxp358, accounted for 55–80% of the phenotypic resistance, and the minor one, *QSsgr-09-02*, in the interval of Xtxp67 and Xtxp230, explained 1–6% of the resistance. The newly identified SSR markers closely linked to the two QTLs will be useful in marker-assisted selection for developing resistant sorghum hybrids. These newly identified SSR markers and the information on chromosomal location of the greenbug resistance QTLs will facilitate our continued research toward isolation of the greenbug resistance gene through a map-based cloning approach, and subsequent characterization of the resistance mechanisms operating in this resistant sorghum line.

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