Mol Breeding (2013) 31:777–784
DOI 10.1007/s11032-012-9832-6

Efficient and fine mapping of *RMES1* conferring resistance to sorghum aphid *Melanaphis sacchari*

Faming Wang · Songmin Zhao · Yonghua Han · Yutao Shao · Zhenying Dong · Yang Gao · Kunpu Zhang · Xin Liu · Dawei Li · Jinhua Chang · Daowen Wang

Received: 15 September 2012 / Accepted: 28 December 2012 / Published online: 20 January 2013

Abstract *Melanaphis sacchari* causes serious damage to sorghum (*Sorghum bicolor* (L.) Moench) growth, development and productivity in many countries. A dominant gene (*RMES1*) conferring resistance to *M. sacchari* has been found in the grain sorghum variety Henong 16 (HN16), but fine mapping of the *RMES1* locus remains to be reported. In this study, genetic populations segregating for *RMES1* were prepared with HN16 and BTx623 as parental lines. The latter had been used for sorghum genome sequencing but was found to be susceptible to *M. sacchari* in this work. A total of 11 molecular markers were mapped to the short arm of chromosome 6 harboring *RMES1*. The closest markers flanking the *RMES1* locus were *Sb6m2650* and *Sb6rj2776*, which delimited a chromosomal region of about 126 kb containing five predicted genes. The utility of the newly identified DNA markers for tagging *RMES1* in molecular breeding of *M. sacchari* resistance, and further efforts in cloning *RMES1*, are discussed.

Keywords Fine mapping · *RMES1* · Molecular marker · *Melanaphis sacchari* · Aphid · Sorghum

Faming Wang and Songmin Zhao contributed equally to this work.

F. Wang · Y. Han · Y. Shao · Z. Dong · Y. Gao · K. Zhang · X. Liu · D. Wang (✉)
The State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
e-mail: dwwang@genetics.ac.cn

S. Zhao · D. Li
The State Key Laboratory of Agro-Biotechnology, China Agricultural University, Beijing 100193, China

Y. Shao
Graduate University of Chinese Academy of Sciences, Yuquan Road, Beijing 100039, China

J. Chang (✉)
College of Agronomy, Agricultural University of Hebei, Baoding 071001, China
e-mail: changjinhua@hebau.edu.cn

Introduction

Among the major cereal crops cultivated worldwide, sorghum (*Sorghum bicolor* (L.) Moench) is unique in being used efficiently for food, fuel, feed and fibre production (Paterson et al. 2009). These multiple usages, together with its highly efficient C4 photosynthesis system, strong tolerance to abiotic stresses and high yield potential, make sorghum an increasingly important crop for many countries in dealing with shortages of natural resources and climate changes (Palmer 1992; Jackson et al. 2008; Paterson et al. 2009). Consequently, more and more genetics and genomics studies are being conducted on sorghum, with an aim of further improving the productivity of this crop through molecular breeding (Carpita

and McCann 2008; Li et al. 2009; Mace et al. 2009; Paterson et al. 2009; Dugas et al. 2011; Vermerris 2011; Zheng et al. 2011; Bouchet et al. 2012; Zou et al. 2012).

One of the most potent threats to further raising sorghum yield potential is insect damage, as more than 150 insect species have been found to negatively affect worldwide sorghum production (Young and Teetes 1977; Sharma 1993). The major insect pests of sorghum include three aphid species, *Melanaphis sacchari*, *Schizaphis graminum* and *Rhopalosiphum maidis*. While *M. sacchari* causes serious damage to sorghum and sugarcane (van den Berg 2002; Singh et al. 2004), *Schizaphis graminum* is destructive of both wheat and sorghum production (Teetes 1980; Eddleman et al. 1999; Blackman and Eastop 2000; Kindler et al. 2002; Punnuri et al. 2012). In order to control the damage from *M. sacchari*, a number of investigations have been conducted to identify sorghum genes conferring resistance to this pest (Lu and Dahlberg 2001; Singh et al. 2004; Chang et al. 2006, 2012). A dominant resistance gene against *M. sacchari*, designated as *RMES1* (Resistance to *Melanaphis sacchari*) hereafter, was found in the Chinese grain sorghum variety Henong 16 (HN16) (Chang et al. 2006). Using the segregating populations derived from HN16 and another grain sorghum variety Qianshan that was susceptible to *M. sacchari* infestation, *RMES1* was genetically mapped to chromosome 6 (Chang et al. 2006, 2012). However, the genetic distance values of the mapped microsatellite and amplified fragment length polymorphism markers to *RMES1* were generally quite large ($\geq$6 cM), and their efficiencies in selecting aphid resistant progenies in segregating sorghum populations were generally less than 90 % (Chang et al. 2012).

For more effective use of *RMES1* in molecular breeding, it is necessary to identify additional markers with closer genetic distances to *RMES1*, and to finally isolate the gene through map-based cloning. Thus, the major objective of this study was to fine-map *RMES1* by developing new DNA markers. Considering that the genome in the sorghum line BTx623 has been sequenced (Paterson et al. 2009), we tested whether this line could be employed for efficiently mapping *RMES1*. Towards this end, the response of BTx623 to *M. sacchari* infestation was investigated, and the inheritance character of *RMES1* in the BTx623 $\times$ HN16 cross and derivative populations was investigated. New DNA markers were developed, which permitted the mapping of *RMES1* to a region of about 126 kb on chromosome 6. Finally, the efficiencies of two mapped markers (*Sb6m2650* and *Sb6rj2776*) for selecting *RMES1* and aphid resistance in BC2F2 progenies were investigated.

Materials and methods

Plant materials and *M. sacchari* culture

HN16 possessing *RMES1* has been described previously (Chang et al. 2006, 2012). BTx623 is the line used for international sorghum genome sequencing (Paterson et al. 2009). By crossing BTx623 with HN16 and selfing F1 and F2 progenies, 312 F3 families were obtained in October, 2010. The F3 families, together with 571 F4 seedlings derived from five F3 plants (F3-92, 131, 147, 183, 211) heterozygous for *RMES1*, provided the main materials for mapping *RMES1*. *M. sacchari* was cultured on the seedlings of the susceptible variety Qianshan in a growth chamber at 30 °C with a 16-h light/8-h dark photoperiod.

Inoculation of *M. sacchari* and phenotyping

For investigating the response of BTx623 to *M. sacchari*, the seeds of BTx623 and HN16 were germinated and grown in vermiculite in a greenhouse with the temperature set at 30 °C with a 16-h light/8-h dark photoperiod. At 7 days after sowing, 30 uniform seedlings (at two-leaf stage) were selected from each variety. Each seedling was then inoculated with 10 apterous adult aphids. The number of aphids on each inoculated seedling was recorded daily for 7 days.

To verify the dominance of *RMES1*, 30 uniform seedlings were raised for each of the two parental lines (BTx623 and HN16) and the F1, followed by mass inoculation with 300–400 nymphs and apterous adults. These aphids came from 3 to 5 detached leaves of Qianshan seedlings that had been co-cultivated with *M. sacchari*. The responses of the three genotypes to aphid infestation were recorded at 10 days post-inoculation (DPI). For evaluating the responses of individual F3 families to *M. sacchari*, 30–35 uniform seedlings were raised for each family, and were inoculated with 300–400 nymphs and

apterous adults as described above. The responses to aphid infestation were recorded at 10 DPI. Using the same method, the 571 F4 seedlings were also tested for their responses to *M. sacchari*. Prior to the test, leaf samples were collected from each of the 571 F4 seedlings for subsequent DNA extraction and mapping experiments.

Extraction of genomic DNA samples and PCR conditions

Genomic DNA samples were extracted from the relevant sorghum materials as described previously (Saghai-Maroof et al. 1984). PCR was conducted as described by Chang et al. (2012). The main cycling conditions included a pre-denaturation step at 94 °C for 5 min, 30 cycles of denaturation (94 °C for 45 s), primer annealing, and extension (72 °C for 1 min), and a final extension at 72 °C for 10 min. The temperature for primer annealing was adjusted for individual markers.

Marker development and evaluation

Previous study suggested that the microsatellite marker *Xtxp006* was located 8.7 cM away from *RMES1* on the short arm of chromosome 6 (Chang et al. 2006). Thus, the chromosomal region containing *Xtxp006* (from 1.90 to 4.90 Mb) was downloaded from the sorghum genomic database (www.phytozome.net/sorghum), and was searched for microsatellites using the SSRIT program (www.gramene.org/gramene/searches/ssrtool). The primer pairs flanking each microsatellite motif were designed with the program Primer Premier 5.0 (Premier Biosoft International, CA, USA). Aided by the program RJPrimers (http://probes.pw.usda.gov/RJPrimers/; You et al. 2010), a series of repeat junction (RJ) markers were also developed using the downloaded sequence. All primer pairs were first screened with the two parental lines (BTx623 and HN16). The polymorphic and co-dominant markers were selected for the mapping experiment.

The sorghum genetic markers have been named in different ways by different studies (Mace et al. 2009 and references therein). In this work, we designed an alternative scheme for naming sorghum markers. For example, Li et al. (2009) mapped five microsatellite markers (*sam72772*, *sam71839*, *sam71307*, *sam46174* and *sam43054*) in the chromosomal region (1.90–4.90 Mb) harboring *RMES1* on the short arm of chromosome 6. In our scheme, the five markers were renamed as *Sb6m1954*, *Sb6m2600*, *Sb6m3291*, *Sb6m4667* and *Sb6m4892*, respectively. The prefix "*Sb6m*" stands for *Sorghum bicolor* chromosome 6 microsatellite, whereas the suffix indicates the approximate physical position of the marker on the specific chromosome. For naming the RJ markers developed in this study, the prefix was accordingly changed to "*Sb6rj*".

Genetic mapping

The 64 F3 families homozygously susceptible to *M. sacchari*, together with the 571 F4 seedlings segregating for the response to *M. sacchari*, were employed for fine mapping of *RMES1*. The mapping data were analyzed using MAPMAKER/Exp version 3.0b (Lincoln et al. 1993). The map positions of the markers were visualized using the software Mapchart version 2.1 (Voorrips 2002).

Analysis of selection efficiency for *RMES1* by the markers flanking *RMES1*

The efficiency of selecting *RMES1* by the markers flanking, and with the shortest genetic distances to, the resistance gene locus was investigated using BC2F2 progenies. Briefly, a backcrossing program was conducted using BTx623 as recurrent parent and HN16 as pollen donor. Twenty BC2F1 plants were selfed to produce the BC2F2 population. Two random samples of BC2F2 seedlings were genotyped using the left and right flanking markers, respectively. Subsequently, the seedlings with HN16 marker allele (putatively containing *RMES1*) were subject to inoculation with *M. sacchari* as described above. The selection efficiency for a given marker was calculated using the following formula: (the number of BC2F2 seedlings resistant to *M. sacchari*/the number of BC2F2 seedlings with HN16 marker allele) × 100 %.

Statistical analysis

Statistical analysis of the data (mean ± SD) depicted in Fig. 1 was conducted using PASW statistics 18 for Windows (SPSS Inc., Chicago, IL, USA). The Chi squared goodness-of-fit test was conducted as described previously (Chernoff and Lehmann 1954).

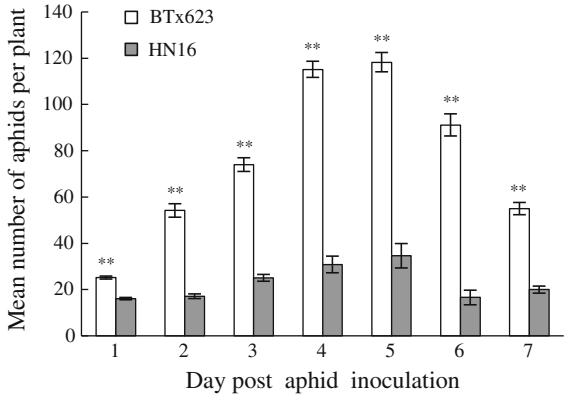

Fig. 1 Comparison of the mean numbers of aphids in BTx623 or HN16 seedlings at different days post-inoculation. The data set displayed is representative of five separate experiments. **indicates significant difference at $P < 0.01$

Results

Response of BTx623 to *M. sacchari* infestation

In both BTx623 and HN16, the mean number of nymphs and adults per plant increased for the first 5 days after *M. sacchari* inoculation, but declined on days 6 and 7 (Fig. 1). However, the scale of the increase was significantly larger in BTx623, and the mean number of aphids per plant was consistently and substantially higher in BTx623 than in HN16 from day 1 to day 7 (Fig. 1). By day 7, the mean number of aphids per plant was approximately six times more than the initial inoculum in BTx623, whereas the number of aphids feeding on HN16 was only twice of the initial inoculum (Fig. 1). Moreover, after 7 days of *M. sacchari* infestation, BTx623 seedlings, but not those of HN16, became wilted and later died. The resistant response of HN16 seedlings to aphid inoculation observed here agreed well with its strong resistance to *M. sacchari* at adult stage in the field (Chang et al. 2006, 2012). The experiment depicted in Fig. 1 was repeated five times, obtaining very similar results.

Inheritance of *RMES1* in the BTx623 × HN16 cross

Following the experiment above, F1 to F4 populations were developed by crossing BTx623 with HN16. After mass inoculation of *M. sacchari*, the F1 plants behaved like HN16 in being resistant to aphid infestation (Fig. 2). On the other hand, the inoculated BTx623 seedlings all died after 7 DPI (Fig. 2). Systematic screening of F3 families from self-pollinated F2 plants by mass inoculation of aphids identified 82 families homozygously resistant, and 64 families homozygously susceptible, to *M. sacchari*, with the remaining 166 families containing both aphid resistant and susceptible progenies (Table 1). The Chi squared test indicated that the responses to *M. sacchari* of the F3 families fitted the segregation ratio of 1:2:1, suggesting that *RMES1* derived from HN16 segregated as a single dominant nuclear gene. Further to the above test, 571 F4 seedlings, germinated from a randomly selected sample of the F4 seeds of five self-pollinated heterozygous F3 plants, were examined for responses to *M. sacchari*. Among the 571 F4 seedlings, 411 and 160 were found to be resistant and susceptible to *M. sacchari*, respectively. The Chi squared test showed that the segregation of resistant and susceptible seedlings in this F4 population occurred at a ratio of 3–1, again suggesting that *RMES1* behaved as a single dominant nuclear gene.

Genetic mapping of *RMES1*

As a first step in the mapping experiment, we investigated the polymorphisms of the molecular markers that resided in the target chromosomal region (chromosome 6, 1.90–4.90 Mb) between HN16 and BTx623. Of the five microsatellite markers previously mapped (Li et al. 2009), three were polymorphic and

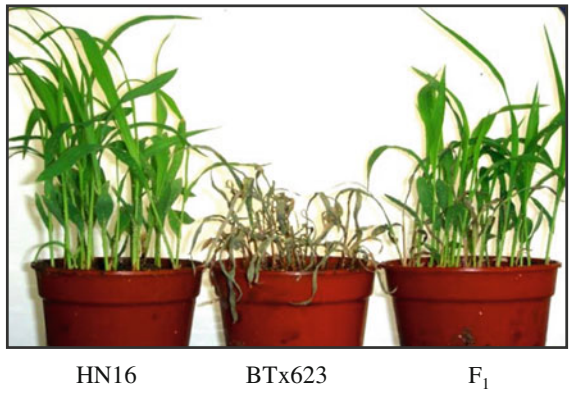

Fig. 2 Comparison of the responses to *M. sacchari* feeding among HN16, BTx623 and F1 seedlings. The graph was taken at 8 days post aphid inoculation, and is representative of three independent experiments

Table 1 Chi squared test of the segregation ratio of F3 families

F3 family	Observed frequency	Expected frequency	χ^2
Homozygously resistant	82	78	3.36
Homozygously susceptible	64	78	
Heterozygous	166	156	

$\chi^2 = 3.36 < \chi^2_{0.05,\ 2} = 5.99$

co-dominant (Table 2). Concomitantly, six new polymorphic and co-dominant microsatellite markers were identified by this work (Table 2). Furthermore, two polymorphic and co-dominant RJ markers were discovered (Table 2). After screening the F3 and F4 populations with known phenotypes to *M. sacchari* with the 11 markers, the genotype and phenotype data were analyzed by the MAPMAKER program. The results showed that the 11 markers covered a genetic distance of 49 cM on the short arm of chromosome 6 (Fig. 3). *Sb6m2650* and *Sb6rj2776* were the two closest markers flanking *RMES1*, and their genetic distances to *RMES1* were 2 and 1 cM, respectively (Fig. 3).

According to the genomic sequence of chromosome 6 (Paterson et al. 2009; www. phytozome.net/sorghum), the 11 mapped markers covered approximately 2,940 kb, with about 126 kb found between *Sb6m2650* and *Sb6rj2776* (Fig. 4). Five genes, namely *Sb06g001620*, *Sb06g001630*, *Sb06g001640*, *Sb06g001645* and *Sb06g001650*, had been predicted between *Sb6m2650* and *Sb6rj2776* by the sorghum genome sequencing project (Fig. 4).

Efficiencies of *Sb6m2650* and *Sb6rj2776* in tagging *RMES1* in BC2F2 progenies

After genotyping 435 BC2F2 seedlings with the co-dominant marker *Sb6m2650*, 320 were found to contain the HN16 marker allele. Of the 320 seedlings, 317 survived after *M. sacchari* attack. Thus, the selection efficiency (accuracy) for *RMES1* by *Sb6m2650* was about 99.1 %. For the co-dominant marker *Sb6rj2776*, a different sample of BC2F2 seedlings

Table 2 List of 11 markers mapped to the region hosting *RMES1*

Marker	Type	Primer sequence (5′–3′)
Sb6m1954	Published microsatellite marker	F, GTAGAGAAGAGAATTGGGAGC
(*sam72772*)		R, AATGTGGTGAAGTTTGCTCT
Sb6m2600		F, TTTAAAAATATTGTATAACCCAA
(*sam71839*)		R, ATTTTCTTATTCCTTCTAGAATTA
Sb6m4892		F, CCAGCACCATAGTTCCAG
(*sam43054*)		R, TCAGAATTCACACACATGCT
Sb6m2388	Newly developed microsatellite marker	F, AAATCGTGTATTACGTTCCCTG
		R, CCCAAGCCAACTCCCTCA
Sb6m2463		F, CAAGGCAATTTCCCATAGT
		R, CATTAGCTCCGGCATCAAC
Sb6m2650		F, CACATCAAATCTTGCGGTAT
		R, TTAAATTCGCCTTGTTCG
Sb6m3174		F, TAGCGGATTCAATGTTGC
		R, TCCACATCATCTTCCACAA
Sb6m3500		F, TCGTGCTGCTTGCCTTCA
		R, CCGAGCGTTGTTGTCTTCA
Sb6m3610		F, GAAAAGGTTGCTTCGTAA
		R, GAACATCCGTCCCATAAA
Sb6rj2776	Newly developed RJ marker	F, CAGCATGGTCGAACTGAAGA
		R, TCGCAAATTACAGCCAACTG
Sb6rj2880		F, ATCGAGCCATCCATCTCAAC
		R, TGGTCGAAATTTACGAGACAAA

The markers *sam72772*, *sam71839* and *sam43054* were from Li et al. (2009)

F, forward primer; R, reverse primer

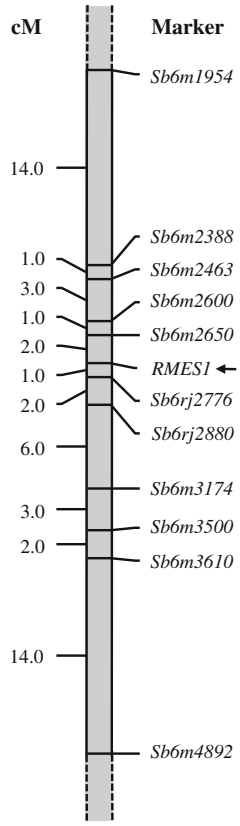

Fig. 3 Linkage map of the region harboring *RMES1* on sorghum chromosome 6. The genetic distances (cM) between adjacent markers are shown on the *left*, whereas the names of mapped markers are on the *right*. The genetic position of *RMES1* is indicated by an *arrow*

(440 in total) was genotyped, leading to the finding of 318 individuals with the HN16 marker allele. After inoculating the 318 seedlings with *M. sacchari*, 316 individuals survived. Therefore, the efficiency (accuracy) for selecting *RMES1* by *Sb6rj2776* was approximately 99.4 %.

Discussion

Previous studies suggest that *RMES1* in HN16 is a major dominant gene conferring effective resistance to *M. sacchari* (Chang et al. 2006, 2012). In this study, we confirmed previous findings using a different genetic cross and derivative populations. More importantly, we succeeded in further mapping *RMES1* to a discrete region on chromosome 6. Genetically, the region harboring *RMES1* is now filled with 11 molecular markers, with the nearest flanking markers (*Sb6rj2776* and *Sb6m2650*) being 1 and 2 cM, respectively, away from *RMES1*. Physically, it is now clear that *RMES1* resides in a chromosomal segment of about 126 kb containing only five predicted genes. Compared to previous studies (Chang et al. 2006, 2012), this work represents a significant advance on understanding the genetic and physical features of the *RMES1* locus.

The major reason behind the efficient mapping of *RMES1* by this work is the use of BTx623 as a parent line for developing the genetic populations. The strong susceptibility of BTx623 to *M. sacchari* facilitated the identification of F3 and F4 progenies lacking *RMES1*. The draft genome sequence of BTx623 aided the finding of new molecular markers required for fine mapping of *RMES1*. Both microsatellite and RJ markers were useful for delineating the genetic position of *RMES1*. The usefulness of microsatellite markers in mapping sorghum genes has been well documented (for example, Chang et al. 2006; Magalhaes et al. 2007; Apotikar et al. 2011; Kawahigashi et al. 2011; Lin et al. 2012). By contrast, the deployment

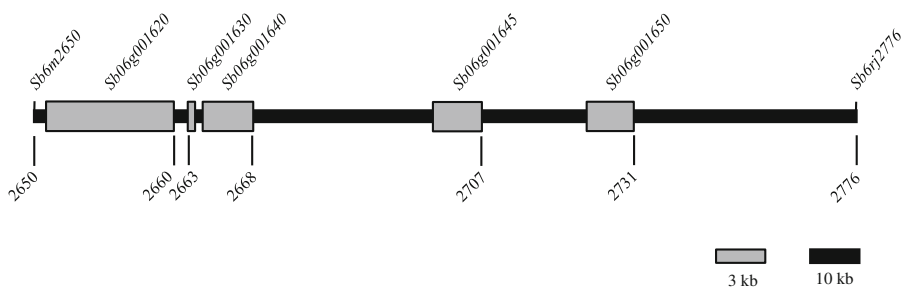

Fig. 4 A diagram illustrating the five predicted genes (*Sb06g001620*, *Sb06g001630*, *Sb06g001640*, *Sb06g001645* and *Sb06g001650*) present in the 126-kb genomic segment bordered by *Sb6m2650* and *Sb6rj2776*. The *grey box* represents the open reading frame (ORF) of the gene. The approximate physical positions of two flanking markers (*Sb6m2650* and *Sb6rj2776*) and the start codons in the ORFs of the five genes on sorghum chromosome 6 are indicated. The products deduced for *Sb06g001620* and *Sb06g001630* are WD domain-containing and ribosome L18/L5e proteins, respectively. Those deduced for *Sb06g001640*, *Sb06g001645* and *Sb06g001650* are leucine-rich repeat proteins

of RJ primers in plant genetic mapping studies has been reported only recently (Paux et al. 2010; You et al. 2010), and this work represents the first demonstration that RJ markers can be used successfully for mapping target genes in sorghum.

In previous mapping studies in sorghum, the genetic markers were named in several different ways (reviewed by Mace et al. 2009), none of which took into account the physical position of the marker on the sorghum chromosome sequence. To facilitate cross-comparisons of future mapping data, we propose a new scheme for naming sorghum genetic markers, which provides information on the approximate physical location of the marker on the specific chromosome. This scheme is likely efficient for designating microsatellite, RJ and indel markers after appropriate modifications.

The efficiencies for selecting *M. sacchari*-resistant plants by *Sb6m2650* and *Sb6rj2776* were all above 99 %. These high efficiencies are consistent with their close genetic linkages with *RMES1*. Therefore, *Sb6m2650* and *Sb6rj2776* are more efficient than previously reported markers in tagging *RMES1* (Chang et al. 2012), which should accelerate the use of this important gene in marker-assisted selection for breeding *M. sacchari* resistance in sorghum.

Despite the availability of a draft genome sequence, the understanding of the molecular genetic basis underlying sorghum traits is currently lagging behind that of other model plants (e.g., *Arabidopsis* and rice). Map-based cloning, frequently and efficiently used for investigating important genes in *Arabidopsis* and rice, has been employed only recently for studying sorghum genes controlling aluminum tolerance (Magalhaes et al. 2007), resistance to the fungal pathogen *Bipolaris sorghicola* (Kawahigashi et al. 2011), or seed shattering (Lin et al. 2012). Further to the work reported here, we are now in the process of developing more markers both within and among the five predicted genes in the 126-kb region hosting *RMES1*, which should finally lead to the isolation of *RMES1* by map-based cloning.

Acknowledgments This study was supported by grants from Hebei Province Natural Science Foundation (C2010000758) and the State Key Laboratory of Plant Cell and Chromosome Engineering (PCCE-2008-KF-02). We thank Professor Yinghua Huang (USDA-ARS Plant Science Research Laboratory, USA), Drs Ruiheng Du and Guoqing Liu (Institute of Millet Crops, Hebei Academy of Agricultural and Forestry Sciences, China), Jianghui Cui (Agricultural University of Hebei, China), and Wei Xue (Baoding Vocational and Technical College, China) for constructive suggestions on our work.

References

Apotikar DB, Venkateswarlu D, Ghorade RB, Wadaskar RM, Patil JV, Kulwal PL (2011) Mapping of shoot fly tolerance loci in sorghum using SSR markers. J Genet 90:59–66

Blackman RL, Eastop VF (2000) Aphids on the world crop pests: an identification and information guide. Wiley, London, pp 1–8

Bouchet S, Pot D, Deu M, Rami JF, Billot C, Perrier X, Rivallan R, Gardes L, Xia L, Wenzl P, Kilian A, Glaszmann JC (2012) Genetic structure, linkage disequilibrium and signature of selection in sorghum: lessons from physically anchored DArT markers. PLoS ONE 3:1–15

Carpita NC, McCann MC (2008) Maize and sorghum: genetic resources for bioenergy grasses. Trends Plant Sci 13:415–520

Chang JH, Xia XY, Zhang L, Li RG, Liu GQ, Luo YW (2006) Analysis of the resistance gene to the sorghum aphid, *Melanaphis sacchari*, with SSR marker in *Sorghum bicolor*. Acta Pratacult Sin 115:113–118 (in Chinese)

Chang JH, Cui JH, Xue W, Zhang QW (2012) Identification of molecular markers for an aphid resistance gene in sorghum and selective efficiency using these markers. J Integr Agric 11:1086–1092

Chernoff H, Lehmann EL (1954) The use of maximum likelihood estimates in χ^2 tests for goodness-of-fit. Ann Math Stat 25:579–586

Dugas DV, Monaco MK, Olsen A, Klein RR, Kumari S, Ware D, Klein PE (2011) Functional annotation of the transcriptome of *Sorghum bicolor* in response to osmotic stress and abscisic acid. BMC Genomics 12:514

Eddleman BR, Chang CC, McCarl BA (1999) Economic benefits from grain sorghum variety improvement in the United States. In: Wiseman BR, Webster JA (eds) Economic, environmental, and social benefits of resistance in field crops. Thomas Say Publications, Maryland, pp 35–37

Jackson DR, Arthur MF, Davis M, Kresovich S, Lawhon WT, Lipinsky ES, Price M, Rudolph A (2008) Research report on development of sweet sorghum as an energy crop. Battelle Press, Columbus, pp 277–290

Kawahigashi H, Kasuga S, Ando T, Kanamori H, Wu J, Yonemaru J, Sazuka T, Matsumoto T (2011) Positional cloning of *ds1*, the target leaf spot resistance gene against *Bipolaris sorghicola* in sorghum. Theor Appl Genet 123:131–142

Kindler SD, Elliott NC, Royer TA, Giles KL, Tao F, Fuentes R (2002) Effect of greenbugs on winter wheat yield. J Econ Entomol 95:89–95

Li ML, Yuyama N, Luo L, Hirata M, Cai HW (2009) *In silico* mapping of 1758 new SSR markers developed from public genomic sequences for sorghum. Mol Breed 24:41–47

Lin ZW, Li XR, Shannon LM, Yeh CT, Wang ML, Bai GH, Peng Z, Li JR, Tric HN, Clemente TE, Doebley J, Schnable PS, Tuinstra MR, Tesso TT, White F, Yu JM (2012) Parallel domestication of the *Shattering1* genes in cereals. Nat Genet 44:720–724

Lincoln SE, Daly MJ, Lander ES (1993) Constructing linkage maps with MapMaker/Exp version 3.0. A tutorial reference

manual, 3rd edn. Whitehead Institute for Medical Research, Cambridge

Lu QS, Dahlberg JA (2001) Chinese sorghum genetic resources. Econ Bot 55:401–425

Mace ES, Rami JF, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzl P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC Plant Biol 9:13

Magalhaes JV, Liu J, Guimaraes CT, Lana UGP, Alves VM, Wang YH, Schaffert RE, Hoekenga OA, Pineros MA, Shaff JE, Klein PE, Carneiro NP, Coelho CM, Trick HN, Kochian LV (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. Nat Genet 39:1156–1161

Palmer GH (1992) Sorghum-food, beverage and brewing potentials. Process Biochem 27:145–153

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberer G, Hellsten U, Mitros T, Poliakov A, Schmutz J, Spannagl M, Tang H, Wang X, Wicker T, Bharti AK, Chapman J, Feltus FA, Gowik U, Grigoriev IV, Lyons E, Maher CA, Martis M, Narechania A, Otillar RP, Penning BW, Salamov AA, Wang Y, Zhang L, Carpita NC, Freeling M, Gingle AR, Hash CT, Keller B, Klein P, Kresovich S, McCann MC, Ming R, Peterson DG, Mehboob-ur-Rahman WD, Westhoff P, Mayer KFX, Messing J, Rokhsar DS (2009) The *Sorghum bicolor* genome and the diversification of grasses. Nature 457:551–556

Paux E, Faure S, Choulet F, Roger D, Gauthier V, Martinant JP, Sourdille P, Balfourier F, Le Paslier MC, Chauveau A, Cakir M, Gandon B, Feuillet C (2010) Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat. Plant Biotechnol J 8:196–210

Punnuri S, Huang Y, Steets J, Wu Y (2012) Developing new markers and QTL mapping for greenbug resistance in sorghum [*Sorghum bicolor* (L.) Moench]. Euphytica. doi:10.1007/s10681-012-0755-4

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

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

Singh BU, Padmaja PG, Seetharama N (2004) Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Prot 23:739–755

Teetes GL (1980) Breeding sorghums resistant to insects. In: Maxwell FG, Jennings PR (eds) Breeding plants resistant to insects. Wiley, New York, pp 457–485

van den Berg J (2002) Status of resistance of sorghum hybrids to the aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae). J Plant Soil (South Africa) 19:151–155

Vermerris W (2011) Survey of genomics approaches to improve bioenergy traits in maize, sorghum and sugarcane. J Integr Plant Biol 53:105–119

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

You FM, Wanjugi H, Huo NX, Lazo GR, Luo MC, Anderson OD, Dvorak J, Gu YQ (2010) RJPrimers: unique transposable element insertion junction discovery and PCR primer design for marker development. Nucleic Acids Res 38:313–320

Young WR, Teetes GL (1977) Sorghum entomology. Annu Rev Entomol 22:193–218

Zheng LY, Guo XS, He B, Sun LJ, Peng Y, Dong SS, Liu TF, Jiang S, Ramachandran S, Liu CM, Jing HC (2011) Genome-wide patterns of genetic variation in sweet and grain sorghum (*Sorghum bicolor*). Genome Biol 12:R114

Zou G, Zhai G, Feng Q, Yan S, Wang A, Zhao Q, Shao J, Zhang Z, Zou J, Han B, Tao YZ (2012) Identification of QTLs for eight agronomically important traits using an ultra-high-density map based on SNPs generated from high-throughput sequencing in sorghum under contrasting photoperiods. J Exp Bot. doi:10.1093/jxb/ers205