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ORIGINAL ARTICLE

Molecular mapping of major QTL conferring resistance to orange wheat blossom midge (*Sitodiplosis mosellana*) in Chinese wheat varieties with selective populations

Lijing Zhang[1] · Miaomiao Geng[1] · Zhe Zhang[1] · Yue Zhang[1] · Guijun Yan[2] · Shumin Wen[1] · Guiru Liu[1] · Ruihui Wang[1]

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Abstract

Key message **Two novel midge resistance QTL were mapped to a 4.9-Mb interval on chromosome arm 4AL based on the genetic maps constructed with SNP markers.**

Abstract Orange wheat blossom midge (OWBM) is a devastating insect pest affecting wheat production. In order to detect OWBM resistance genes and quantitative trait loci (QTL) for wheat breeding, two recombinant inbred line (RIL) populations were established and used for molecular mapping. A total of seven QTL were detected on chromosomes 2D, 4A, 4D and 7D, respectively, of which positive alleles were all from the resistant parents except for the QTL on 7D. Two stable QTL (*QSm.hbau-4A.2-1* and *QSm.hbau-4A.2-2*) were detected in both populations with the LOD scores ranging from 5.58 to 29.22 under all three environments, and they explained a combined phenotypic variation of 24.4–44.8%. These two novel QTL were mapped to a 4.9-Mb physical interval. The single-nucleotide polymorphism (SNP) markers *AX-109543456*, *AX-108942696* and *AX-110928325* were closely linked to the QTL and could be used for marker-assisted selection (MAS) for OWBM resistance in wheat breeding programs.

Abbreviations

ANOVA	Analysis of variance
EST	Expressed sequence tag
ICIM	Inclusive composite interval mapping
KASP	Kompetitive allele specific PCR
LOD	Logarithm of odds
MAS	Marker-assisted selection
NIL	Near-isogenic line
QTL	Quantitative trait loci
RIL	Recombinant inbred line
SNP	Single-nucleotide polymorphism
SSR	Simple sequence repeat
OWBM	Orange wheat blossom midge

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✉ Guiru Liu
nxlgr@hebau.edu.cn

✉ Ruihui Wang
wangrh@hebau.edu.cn

[1] North China Key Laboratory for Crop Germplasm Resources of Education Ministry, College of Agronomy, Hebei Agricultural University, Baoding 071000, Hebei, People's Republic of China

[2] School of Agriculture and Environment, Faculty of Science, and the Institute of Agriculture, The University of Western Australia, Perth 6009, Australia

Introduction

Orange wheat blossom midge (OWBM), *Sitodiplosis mosellana* (Géhin), is one of the economically important insect pests and has caused serious yield losses in most wheat-growing areas worldwide (Berzonsky et al. 2003; Thomas et al. 2005; Bruce et al. 2007; Wen et al. 2007; Gaafar et al. 2011a; Jacquemin et al. 2014; https://gd.eppo.int/taxon/SITDMO/distribution). In Canada and the UK, annual wheat losses caused by OWBM exceed C$60,000,000 (Kassa et al. 2016) and £60,000,000 (Oakley et al. 2005), respectively. In China, two serious outbreaks caused by OWBM occurred in the 1950s and 1980s, resulting in approximately 50% yield reduction in wheat production (Duan et al. 2013). During the past two decades, about 10% of the wheat-growing areas in China were affected annually by OWBM (Wen et al. 2007).

Resistant wheat varieties have been successfully used to manage OWBM (DePauw et al. 2009; Vera et al. 2013; https://ahdb.org.uk/). The first identified antibiosis gene *Sm1* mapped on chromosome 2BS from American wheat variety 'Augusta'(McKenzie et al. 2002; Berzonsky et al. 2003) has been widely utilized to develop varieties resistant to OWBM. To date, about 30 bread and durum wheat varieties with *Sm1* have been released in Europe and North America (Gaafar et al. 2011b; Lamb et al. 2001; Blake et al. 2014). However, the released varieties in these regions are almost all spring wheat (Gaafar et al. 2011b). Most winter wheat varieties with *Sm1* were registered in Canada in the 1920s or even earlier in the USA (Lamb et al. 2016), which cannot fulfill the needs of current winter wheat breeding. In one study carried out by Gaafar et al. (2011b) in Germany, six of 50 winter wheat varieties were immune to OWBM and two varieties were believed to carry *Sm1* gene. The wide application of *Sm1* gene has caused a narrow genetic basis for midge resistance. Thereby, OWBM biotypes with new virulence might emerge. To solve this problem, a 'varietal blending' strategy has been proposed and adopted in the targeted regions of Canada, thus providing a refuge to insure predominance of the susceptible type (Fox et al. 2012; Smith et al. 2014; http://midgetolerantwheat.ca).

Traditional wheat breeding for OWBM resistance relies heavily on large populations and precise phenotypic evaluations. Since the amount of OWBM occurring naturally in midge nurseries is environmentally sensitive, it is necessary to evaluate the midge resistance of wheat breeding lines for consecutive years. The evaluation of midge resistance is labor intensive and time-consuming (Sun et al. 1995; Wen et al. 2007; Wise et al. 2015) and limits the number of accessions to be evaluated. Managing OWBM is also difficult as they are hard to be reared and evaluated in laboratory conditions. Marker-assisted selection (MAS) based on tagging genes or QTL is an alternative way for selecting midge resistance in wheat breeding programs (Collard and Mackill 2008) because it uses markers to screen wheat accessions directly in the laboratory. Once markers are identified which are tightly linked to or co-segregated with the target genes or QTL, they can be used to identify resistant accessions quickly and accurately. The linked markers of *Sm1* (*Xgwm210*, *Xbarc35* and *XWM1*) have been successfully developed and used for MAS breeding to identify wheat varieties having the *Sm1* locus (Thomas et al. 2005; Randhawa et al. 2013). In addition to *Sm1*, another major QTL *QSm.mst-1A* from spring wheat variety 'Reeder' was mapped on chromosome 1A (Blake et al. 2011), but the markers linked to this QTL have not been applied in MAS breeding. Similarly, we previously identified a major QTL *QSm.hbau-4A.1* linked to *Xwmc262* and *Xbarc343* on chromosome arm 4AL using a Chinese wheat population, the genetic interval between the two closest flanking markers was 2.5 cM, and these two markers were used for discriminating near-isogenic lines (NILs) derived from the progeny of residual heterozygous lines (Hao et al. 2017). Although these two markers have been mapped to the QTL region, the density of the markers is still limited, and more closely linked markers are needed for more efficient MAS.

Biparental populations are commonly used to detect QTL/genes related to target traits of interest in plants. In most cases, the number of individuals from a biparental population is limited by the cost of genotyping and phenotyping. Therefore, selective population has been adopted as a cost-efficient strategy to identify the QTL/genes with power equivalent to using the entire population (Darvasi and Soller 1992; Gallais et al. 2007; Mysków and Stojalowski 2016). Selective populations have been widely used in association analysis (Fontanesi et al. 2012; Fowler et al. 2013; Yan et al. 2017; Zongo et al. 2017; Lu et al. 2018) and linkage mapping (Foolad et al. 1997; Zhang et al. 2003; Wingbermuehle et al. 2004; Navabi et al. 2009; Masojć et al. 2011; Reinprecht et al. 2015). Cui et al. (2015) concluded that QTL can still be detected under strong selection intensity with a selective population as small as 25 individuals. Other studies confirmed that genotyping selected individuals could replace genotyping the entire population for mapping major QTL (Sun et al. 2010; Zou et al. 2016).

In this study, two selective RIL populations were used to construct genetic maps and detect major QTL conferring OWBM resistance. This research detected new genomic regions for OWBM resistance and provided more closely linked molecular markers for MAS in wheat breeding programs.

Materials and method

Plant materials and field trials

Two RIL populations (F_{6-8} generation) were used in this study, and each population was developed using a single-seed descent (SSD) method (Knott and Kumar 1975) from a F_2 generation. One population, named as HY-RIL, has 351 lines derived from the cross of Henong215 (HN215) and Yanyou361 (YY361). HN215 was a winter wheat variety with superior OWBM resistance and widely grown in Northern China during the 1990s. YY361 was also a winter wheat variety with good grain quality but susceptible to OWBM and widely grown throughout China from 2000 to 2010 (Qu et al. 2011). Another population called 6J-RIL contained 280 lines which were derived from the cross of 6218 and Jimai24 (JM24). JM24 shared the same pedigree with HN215 and was highly resistant to OWBM, while 6218 was extremely susceptible to OWBM.

All the lines were planted in a midge nursery during 2014–2015, 2015–2016 and 2016–2017 growing seasons at an experimental station of Hebei Agricultural University, Baoding, China. These three environments were represented by E1, E2 and E3, respectively. A completely randomized block design was used with three replications per line under each environment. Each line was planted in a single row of 20 cm length and 20-cm row spacing. No pesticides were applied to the midge nursery, and normal field management was implemented.

Phenotypic evaluation and statistical analysis

All the lines from populations HY-RIL and 6J-RIL and their parents were evaluated for OWBM resistance. Resistance levels of all the materials were assessed according to the number of larvae per spike. At the milky stage (Zadoks stage 73) (Zadoks et al. 1974), 10 or 15 spikes from each line were collected to manually count the number of larvae on each kernel. Five scales were assigned to each kernel according to the number of larvae on it, i.e., grade 0 indicating zero or no larva on the kernel; grade 1 indicating one larva per kernel; grade 2 indicating two larvae per kernel; grade 3 indicating three larvae; and grade 4 indicating at least four larvae. The total number of kernels corresponding to each grade was counted. Resistance level of each wheat line was expressed as the estimated loss rate (*L*) based on the following formula (Hao et al. 2017).

$$L(\%) = \Sigma(xf)/4\Sigma f \times 100\%$$

where x is the resistance grade for each kernel and f is the number of kernels at that grade. The average of three replicates was used to infer the final estimated loss rate (*FL*) for each line. If an outlier existed in the three replications of one wheat line, the maximum *L* value was taken as the *FL* for that line. Finally, the resistance level for each line was estimated by the resistance index (*RI*), which was the ratio of *FL* to the average estimated loss rate (*ML*) of all tested lines (Table 1).

SPSS18.0 software was used to perform analysis of variance (ANOVA). Broad-sense heritability (H^2) of the trait was calculated based on the following formula:

$$H^2 = V_G/V_P$$

where V_G and V_P represent genetic variance and phenotypic variance, respectively (Wu et al. 2016).

SNP assay

Two selective populations were used for SNP genotyping and further linkage map construction. One selective population called HY-S contained 62 resistant and 31 susceptible lines selected from the HY-RIL population. Another selective population named 6J-S contained 54 resistant lines and 39 susceptible lines selected from the population 6J-RIL.

Table 1 Classification criteria of resistance level to OWBM in wheat lines based on resistance index (*RI*)

Resistance level	Resistance index (*RI*)	Resistance evaluation
0	0	Immune
1	0.01–0.19	Highly resistant
2	0.20–0.49	Moderately resistant
3	0.5–0.99	Low resistant
4	1.0–1.50	Susceptible
5	> 1.5	Highly susceptible

Genomic DNA was extracted from mature seeds using the CTAB method (Saghai-Maroof et al. 1984). One hundred and ninety lines including HY-S, 6J-S and their four parents were genotyped using the Affymetrix Axiom wheat 55 K SNP array containing 53,063 wheat SNPs provided by China Golden Markers Biotechnology Co., Ltd. (Beijing, China). Having been filtrated with high stringency, only the markers falling in Poly High Resolution (PHR) group with highest reliability were kept. SNPs heterozygous in the parents or SNPs with a missing rate higher than 10% were removed. The remaining polymorphic SNPs were used for further QTL mapping. BIN function of QTL IciMapping4.1 software was used to remove redundant markers based on their segregation patterns among RILs (Meng et al. 2015). One marker of each bin was randomly selected for map construction (Liu et al. 2018). The haplotype identical to the resistant parent was denoted as A, while the haplotype identical to the susceptible parent was denoted as B, heterozygous haplotype was denoted as H. If no haplotype was detected, the locus was denoted as '-'.

Linkage map construction and QTL analysis

Genetic linkage maps were constructed with JoinMap4.0 software (Van Ooijen 2006) with a LOD threshold of 3 after preliminary trials using LOD scores ranging from 2 to 10. Marker order on the same linkage group was determined with regression mapping algorithm (Zhai et al. 2016). Recombination rates among markers were converted to genetic distance using the Kosambi function (Kosambi 1943). Chromosomal arms were determined based on the physical locations of SNP markers on the reference genome (IWGSC RefSeq v1.0, hereafter abbreviated as IWGSCv1.0).

Inclusive composite interval mapping (ICIM) in QTL IciMapping4.1 software was used to detect the QTL related to the OWBM resistance and to estimate their additive

effects. Missing phenotypic data were deleted using the 'Deletion' command. The scanning step for all putative QTL was 1.0 cM, and the *P* value inclusion threshold was 0.001. Threshold of LOD score was calculated using 1000 permutations test at $P \leq 0.05$. Only the QTL detected under at least two environments was considered a stable QTL. The QTL with a LOD score larger than 3.0 and with explained phenotypic variance greater than 10% was defined as a major QTL. MapChart2.3 software was used to draw the linkage map with the results of QTL mapping (Su et al. 2018).

Comparison of gene annotations within QTL mapping interval

Flanking sequences of SNP markers confining the major QTL were aligned to the reference genome (IWGSCv1.0) for obtaining their physical location information, which can facilitate gene identification between the two flanking markers. Sequences of all the genes located in the QTL interval were used in a Blastn query to search against the genomes of *T. turgidum* ssp. *dicoccoides*, *Aegilops tauschii* and *T. urartu* (http://202.194.139.32/blast/viroblast.php; https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies; http://plants.ensembl.org/Triticum_aestivum/Info/Index) to obtain the homologous relationship among them. According to their function annotation, genes with resistance-related function were identified from the mapped QTL interval.

Results

Phenotypic evaluation

Phenotypic values of the two selective populations and their parents are shown in Table 2. Under three environments, HN215 and JM24 were highly resistant to OWBM with the *RI* values ranging from 0.09 to 0.12 and 0.02 to 0.13, respectively, while YY361 and 6218 were extremely susceptible to OWBM with the *RI* larger than 3.00. The *RI* values of the HY-S and 6J-S populations across the three environments ranged from 0 to 13.23 and 0 to 5.59, respectively. The skewness of the two selective populations deviated from zero, indicating that the phenotypic data did not follow a normal distribution. The reason may be that minor QTL with larger effects existed in the two selective populations. Broad-sense heritability (H^2) for OWBM resistance was 0.57 in HY-S and 0.82 in 6J-S, respectively, suggesting that genetic factors other than environmental factors played an important role in OWBM resistance.

Details of the linkage maps

After filtration, 18,123 high-quality SNPs were obtained and screened for polymorphism between parents of the selective populations. A total of 9154 polymorphic SNPs were used for linkage map construction in the HY-S population. These markers were grouped into 1716 bins. After removing unlinked markers, a linkage map with 8994 SNP markers (within 1631 bins) was constructed, spanning 2840.29 cM in length with an average marker density of 1.74 cM/locus covering the 21 wheat chromosomes (Table 3, Fig. S1). Of the 8994 polymorphic SNP markers, 3095 SNPs (34.4%) were mapped to the A genome spanning 801.58 cM with an average marker density of 1.37 cM/locus, 3239 (36.0%) were mapped to the B genome covering 638.16 cM with an average marker density of 1.50 cM/locus, and 2660 (29.6%) were mapped to the D genome spanning 1400.55 cM with an average marker density of 2.26 cM/locus. The number of markers distributed on chromosomes ranged from 176 markers for chromosome 4A to 1010 markers for 4B (Fig. S1).

In the 6J-S population, a total of 10,599 polymorphic SNPs were grouped into 1517 bins for linkage map construction. After removing unlinked markers, the final linkage map contained 9709 SNP markers (within 1371 bins) on 21 chromosomes, spanning 3129.19 cM in length with an average marker density of 2.28 cM/locus (Table S1, Fig.

Table 2 Distribution of phenotypic values (*RI*) for the OWBM resistance of two selective mapping populations and their parents under different environments

Population	En[a]	Parents				RILs						H^2
		HN215	YY361	6218	JM24	Min.	Max.	Average	SD	Skewness	Kurtosis	
HY-S	E1	0.11	4.35**			0	7.48	1.54	1.57	2.21	5.15	0.57
	E2	0.09	4.72**			0	13.23	1.47	2.03	3.47	15.86	
	E3	0.12	4.20**			0	5.55	2.13	1.47	1.81	2.16	
6J-S	E1			4.43	0.02**	0.01	4.33	1.04	1.14	0.93	-0.29	0.82
	E2			6.67	0.02**	0	5.59	1.36	1.32	1.50	1.65	
	E3			3.08	0.13**	0	3.11	1.37	1.86	0.95	-0.12	

[a]E1, E2 and E3 indicate the experiments conducted in 2014–2015; 2015–2016; 2016–2017, respectively

**Significance level at $P = 0.01$

Table 3 Mapping information of the linkage map constructed with HY-S RILs using SNP markers

Chromosome	No. of SNPs	BINs	Length (cM)	cM/bin marker
1A	471	66	76.36	1.16
2A	330	67	85.49	1.28
3A	542	132	107.89	0.82
4A	176	58	148.56	2.56
5A	485	88	155.90	1.77
6A	563	72	120.02	1.67
7A	528	101	107.36	1.06
1B	529	45	63.10	1.40
2B	448	83	72.44	0.87
3B	466	99	110.91	1.12
4B	1010	58	88.97	1.53
5B	236	52	132.22	2.54
6B	201	32	74.29	2.32
7B	349	57	96.23	1.69
1D	607	82	231.48	2.82
2D	208	73	180.08	2.47
3D	341	95	243.20	2.56
4D	220	68	139.84	2.06
5D	517	107	179.74	1.68
6D	313	81	234.62	2.90
7D	454	115	191.59	1.67
A Genome	3095	584	801.58	1.37
B Genome	3239	426	638.16	1.50
D Genome	2660	621	1400.55	2.26
Total	8994	1631	2840.29	1.74

S2). Of the 9709 polymorphic markers, 3070 SNPs (31.6%) were mapped to the A genome spanning 923.09 cM, 3885 (40.0%) were mapped to the B genome covering 736.72 cM, and 2754 (28.4%) were mapped to the D genome spanning 1469.38 cM. These markers were unevenly distributed on the 21 chromosomes, and the marker numbers ranged from 127 on chromosome 4D to 964 on chromosome 3B.

QTL mapping with selective populations

A total of seven QTL related to OWBM resistance were detected in the two populations. Two of them were detected in the HY-S population under at least one environment (Table 4). QTL *QSm.hbau-4A.2-1* on chromosome 4A was detected under all three environments with additive effects ranging from −1.05 to −0.92, LOD scores ranging from 5.58 to 11.54, and with the phenotypic variance explained from 24.40 to 39.73%. This QTL was confined to an interval of 0.89 cM between markers *AX-109543456* and *AX-108942696* (Fig. 1). QTL *QSm.hbau-4D.1* on chromosome 4D was only detected in E3 with additive effect of −0.58 and a LOD score of 5.01, which explained 14.57% of the phenotypic variation. The resistant wheat parent HN215 contributed additive effects for enhancing OWBM resistance at all QTL loci.

In the 6J-S population, five QTL associated with OWBM resistance on chromosomes 2D, 4A, 4D and 7D were identified under at least one environment (Table 4). Two QTL, *QSm.hbau-2D.1* and *QSm.hbau-2D.2*, were mapped 6 cM apart on chromosome 2D. *QSm.hbau-2D.1* was detected under E1 and E2 with LOD scores of 6.83 and 7.48, respectively. QTL *QSm.hbau-2D.2* was only found under E3 with a

Table 4 QTL for OWBM resistance in the two selective populations

QTL	Populations	Position (cM)	Interval markers	Environments[a]	LOD[b]	PVE[c]	Add[d]
QSm.hbau-2D.1	6J-S	10	*AX-111692223-AX-108897340*	E1/E2	6.83/7.48	19.72/5.23	−0.46/−0.54
QSm.hbau-2D.2	6J-S	16	*AX-111416794-AX-111574926*	E3	8.35	20.59	−0.37
QSm.hbau-4A.2-1	HN-S	4	*AX-109543456-AX-108942696*	E1/E2/E3	8.38/5.58/11.54	35.03/24.40/39.73	−0.96/−1.05/−0.92
QSm.hbau-4A.2-2	6J-S	143	*AX-110928325-AX-108942696*	E1/E2/E3	13.70/29.22/10.62	44.75/35.09/27.39	−0.69/−1.40/−0.43
QSm.hbau-4D.1	HN-S	28	*AX-110572006-AX-111071805*	E3	5.01	14.57	−0.58
QSm.hbau-4D.2	6J-S	7	*AX-89398511-AX-110774143*	E3	6.81	17.14	−0.34
QSm.hbau-7D.1	6J-S	13	*AX-109858991-AX-94588651*	E2	16.87	16.53	0.96

[a]E1, 2014–2015 Baoding; E2, 2015–2016 Baoding; E3, 2016–2017 Baoding

[b]LOD, logarithm of odds value

[c]PVE, percentage of the phenotypic variance explained by individual QTL

[d]Add, additive effect of resistance allele

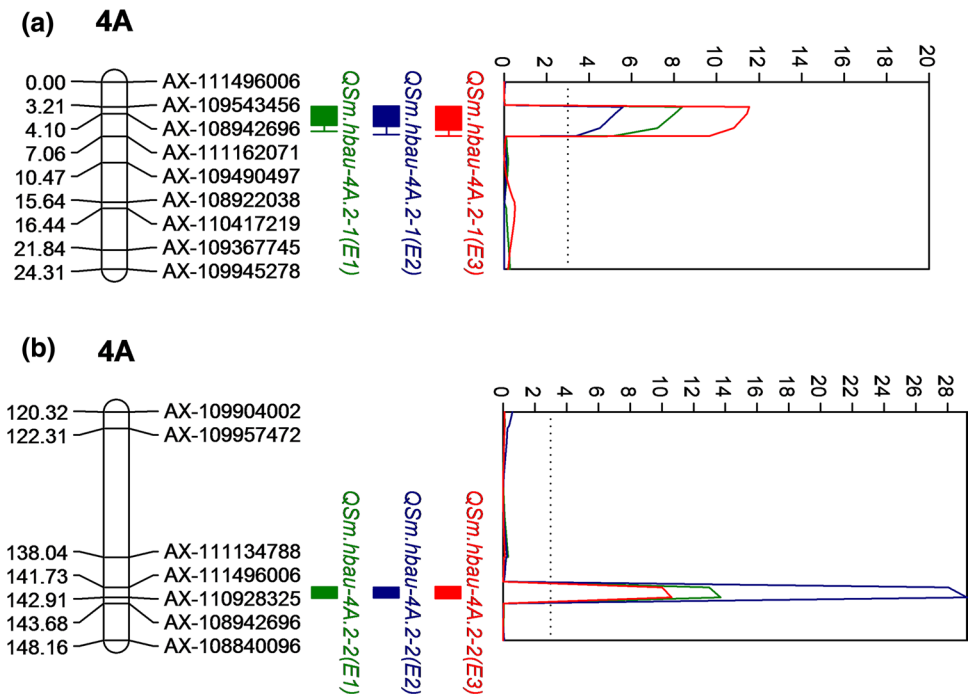

Fig. 1 The LOD values of major QTL in two selective populations. The names of the marker loci and the QTL interval are listed on the right side of the corresponding chromosomes. Environments where each corresponding QTL was detected are shown in parentheses. The positions (cM) of the marker loci are listed on the left side of the corresponding chromosomes. The LOD scores of the markers are also shown. Green, blue and red represent the QTL locations and LOD score distributions of markers in E1, E2 and E3, respectively. E1, E2 and E3 indicate the experiments conducted in 2014–2015, 2015–2016 and 2016–2017, respectively. **a** *QSm.hbau-4A.2-1* in HY-S population; **b** *QSm.hbau-4A.2-2* in 6J-S population

LOD score of 8.35. QTL *QSm.hbau-4A.2-2* on chromosome 4A was detected under all three environments with LOD scores ranging from 10.62 to 29.22, with additive effects ranging from − 1.40 to − 0.43, and with the phenotypic variance explained being from 27.39 to 44.75%. This QTL was confined to a 0.77-cM region with two flanking SNP markers *AX-110928325* and *AX-108942696* (Fig. 1). QTL *QSm.hbau-4D.2* on chromosome 4D was only detected in E3 and explained 17.14% of the phenotypic variation with a LOD score of 6.81. QTL *QSm.hbau-7D.1* on chromosome 7D was detected in E2 with a LOD score of 16.87. The resistant parent JM24 contributed positive effects for enhancing OWBM resistance at all QTL loci with an exception for QTL on 7D.

Comparison of QTL on 4AL among two populations

Two major QTL with high LOD scores and flanked by a same marker (*AX-108942696*) were mapped to an overlapped interval on 4A in two populations, separately. In the HY-S population, the QTL was mapped to a 0.89-cM interval with flanking SNP markers *AX-109543456* and *AX-108942696*, while the other QTL was mapped to a 0.77-cM interval with flanking markers *AX-110928325* and *AX-108942696* in the 6J-S population. The physical regions of *QSm.hbau-4A.2-1* and *QSm.hbau-4A.2-2* were 4.9 Mb (703,434,395–708,327,301 bp) in HY-S and 1.2 Mb (703,434,395–704,647,631 bp) in 6J-S, respectively, showing an overlapped physical region (1.2 Mb) existed. The additive effects of these two QTL came from the OWBM-resistant parents, HN215 and JM24, respectively. Considering these two resistant wheat parents share the same pedigree, we compared all SNP alleles within the target region of the QTL to determine whether there were differences between HN215 and JM24 or not. However, no difference was found in the target region between the two parents. This indicated that the differences in mapping positions for OWBM resistance are probably due to the differences in crossing orientation and genetic background between the two RIL populations. But we cannot determine whether these two QTL are the same in the present study, we only confirmed that the genomic region for OWBM resistance may be within a 4.9-Mb physical interval.

Comparative genomic analysis and gene annotation of the QTL mapping interval

When projected to the reference genome of Chinese Spring (IWGSCv1.0), the 4.9-Mb genomic region on 4AL contained 58 genes. Based on gene function annotation and related reports (Schuler et al. 1998; Feuillet et al. 2003; Huang et al. 2003; Mindrebo et al. 2016), nine of 58 genes (*TraesCS4A01G436000*, *TraesCS4A01G436100*, *TraesCS4A01G436300*, *TraesCS4A01G436500*, *TraesCS4A01G436800*, *TraesCS4A01G437400*, *TraesCS4A01G437700*, *TraesCS4A01G437800* and *TraesCS4A01G438000*) may be related to OWBM resistance (Table S2). According to our previous study, five differentially expressed genes, *TraesCS4A01G436000*, *TraesCS4A01G436100*, *TraesCS4A01G436500*, *TraesCS4A01G437300* and *TraesCS4A01G437800*, were revealed

as putative candidates for OWBM resistance by using BSR-Seq analysis (Hao et al. 2019), four of which were consistent with those identified in this study.

Reference sequences of the 58 genes were aligned against different wheat species, i.e., *T. turgidum* ssp. *dicoccoides*, *T. urartu* and *Ae. tauschii* (Fig. 2). For *T. turgidum* ssp. *dicoccoides*, there were 34 orthologous genes, of which two were located on 7AL and 32 were located on 4AL. A total of 16 homologous genes were found in *T. urartu*, of which nine were located on 7AS, one was located on 6A, and the other six were located on 4A and 2A. In the *Ae. tauschii* genome, homologous genes of the 4A-interval were mostly located on 7DS (27 of 33), and the remaining six genes were mapped to 1D (two genes), 4D (two genes) and one unknown chromosomal location.

Comparisons of homologous genes in genomes of hexaploid, tetraploid and diploid wheat revealed complex collinearity relationships (Fig. 2). Homologous genes from hexaploid wheat showed good collinearity with those in the corresponding regions between *T. dicoccoides* and *Ae. tauschii*, even though some genes were reversed and rearranged. No significant collinearity was revealed between hexaploid wheat and *T. urartu*, possibly due to the low number of homologous genes in the corresponding region of *T. urartu*.

Discussion

Two novel QTL with OWBM resistance

Though OWBM is an economically important insect pest, few gene resources are reported for midge management (Thomas et al. 2005; Gharalari et al. 2009; Blake et al. 2011; Kassa et al. 2016; Hao et al. 2017). Almost all the resistant varieties released from 2010 to 2015 in Canada, UK and USA had the gene *Sm1* (Fox et al. 2012; Blake et al. 2014; Pozniak and Clarke 2015; http://www.bcpc.org/; https://www.usda.gov/; http://midgetolerantwheat.ca/). Such a situation may render the opportunity for emergence of a new OWBM biotype, leading researchers to find gene resources other than *Sm1* (Lamb et al. 2001; Gharalari et al. 2009; Chavalle et al. 2017; Echegaray et al. 2018). Blake et al. (2011) detected a new major QTL (*QSm.mst-1A*) on

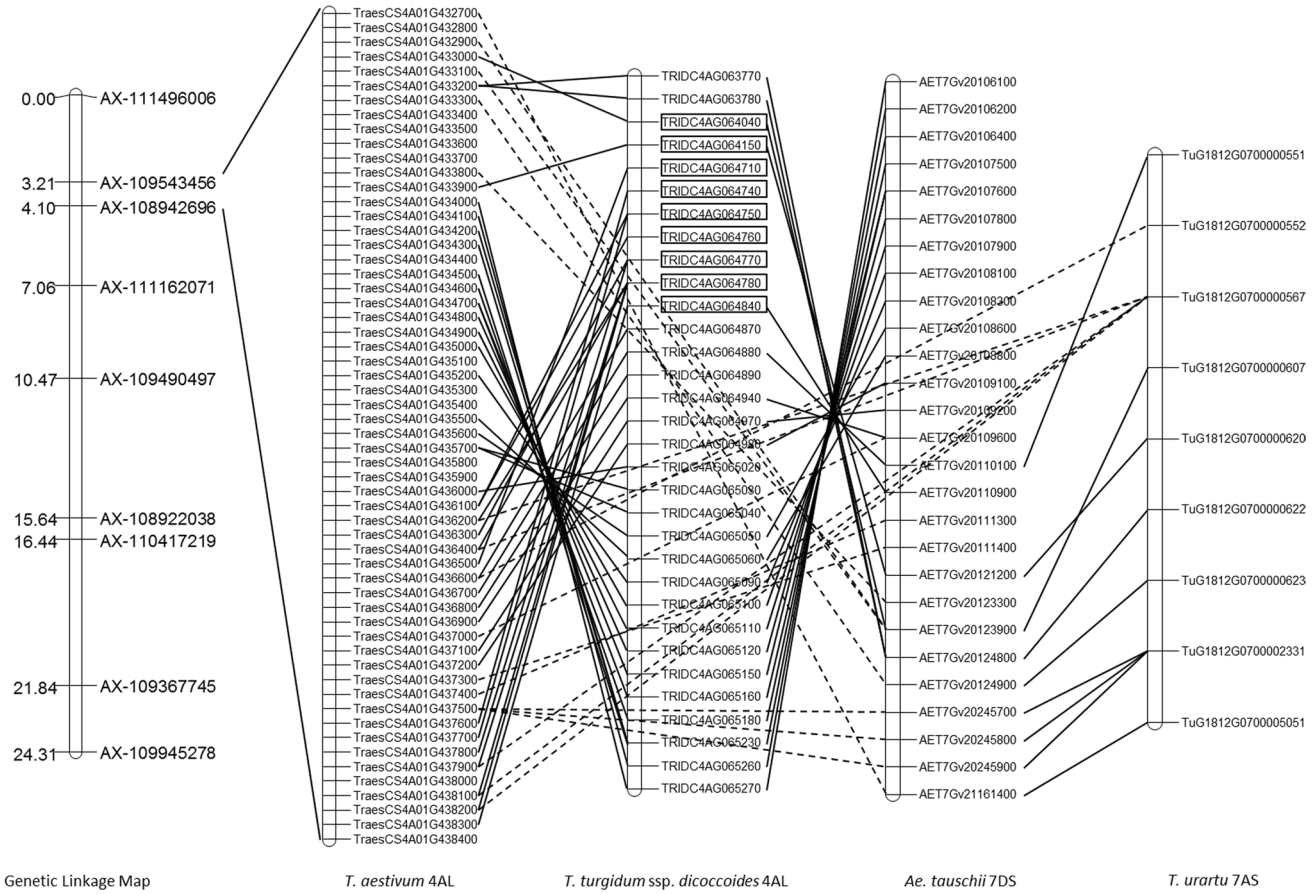

Fig. 2 Comparison of linkage maps for the major QTL and homologous genes among Chinese Spring wheat, *T. turgidum* ssp. *dicoccoides*, *Ae. tauschii*, and *T. urartu*. The boxed genes represent the gene located in the corresponding genomic intervals between the QTL-flanking markers

chromosome 1A in the bread wheat variety 'Reeder.' In this study, two major QTL were detected on chromosome 4AL, which are different from either the location of *Sm1* (Thomas et al. 2005) or the one of *QSm.mst-1A* (Blake et al. 2011).

OWBM resistance in Chinese wheat may be different from that conferred by *Sm1*. Lamb et al. (2016) found that two resistant lines from China showed a lower resistance level than that of *Sm1* origin reflected by low levels of larval antibiosis and oviposition deterrence. This probably implies that either virulence of OWBM biotypes may be different between Chinese and Canadian populations or a totally different resistance mechanism may exist in wheat varieties from both countries (Duan et al. 2013). A panel of Chinese winter wheat accessions including 35 resistant and 32 susceptible varieties was screened using *Sm1*-linked marker *Xbarc35* in our group (Table S3). Among them, 31 resistant and 30 susceptible varieties were positive to *Xbarc35*. It suggests that *Xbarc35* may not be useful for detecting midge resistance in Chinese wheat germplasm. In addition, average *RI* values for the lines with resistant alleles at *QSm.hbau-4A.2-1* and *QSm.hbau-4A.2-2* loci were different from those of *Sm1*, and no dead larvae were discovered on spikes of resistant lines or parents, indicating that resistance conditioned by major QTL in Chinese wheat in this study may just reduce infestation rather than having antibiotic effects conditioned by *Sm1* (Table S4). Such a different resistance mechanism conferred by these QTL can complement the role of the *Sm1* gene.

Ferulic acid content in wheat kernels was believed as one biochemical component for OWBM resistance (Ding et al. 2000; Abdel-Aal et al. 2001). Resistant varieties with *Sm1* were believed to express high content of *p*-ferulic acid in kernels (Ding et al. 2000; Thomas et al. 2005; Kassa et al. 2016). However, Hao et al. (2019) found that the expression levels of homologous genes encoding caffeic acid *O*-methyltransferase (COMT), an enzyme catalyzing synthesis of ferulic acid, did not present significant differences between resistant and susceptible wheat varieties. It could also be deduced that the resistance mechanism of QTL in Chinese wheat varieties may be different from that of *Sm1* (Thomas et al. 2005).

Comparison with previous studies

Several studies showed that OWBM resistance was related to agronomic traits of wheat varieties (Shi et al. 2003; Wu et al. 2015). Previously, we carried out correlation and conditional QTL analysis between OWBM resistance and agronomic traits, such as plant height, stem length under spike, heading date and spikelet density (An et al. 2014; An 2015). Resistance conditioned by QTL on chromosome 4D may be ascribed to plant height and stem length under spike, while resistance conferred by QTL on 4A was not affected by these agronomic traits.

In other studies conducted by our group, we have identified two QTL related to OWBM resistance. In the F_6-derived RILs of the 6218/JM24 population, *QSm.hbau-4A.1* was mapped to a 2.5-cM interval on 4AL with flanking markers *Xbarc343* and *Xwmc262* (Hao et al. 2017). The other QTL on 4AL was mapped to a 2.9- or 2.6-cM interval between markers *Xwmc497* and *Xwmc313* (in 2013) or *Xwmc313* and *Xwmc776* (in 2014), respectively, by using the F_6-derived RILs of HN215/YY361 population (An 2015). The precise locations of QTL on chromosome 4A in the two studies were different, and such differences may be related to the difference of genetic background between these two populations. But we can confirm that genomic regions conferring OWBM resistance were all located on the long arm of chromosome 4A. In one study designed for BSR-Seq analysis with 6218/JM24 RILs, one candidate genomic region with high confidence was mapped to the physical interval (15-Mb, 699,000,000–714,000,000 bp) of 4AL (Hao et al. 2019), which encompassed the 4A-interval (4.9-Mb) region identified in this study.

Two major QTL were mapped to the similar interval on 4AL by using two RIL populations in the present study. Although the locations of these two QTL (*QSm.hbau-4A.2-1* and *QSm.hbau-4A.2-2*) were slightly different from those detected by Hao et al. (2017) and An (2015), the physical interval of the major QTL overlapped with Hao et al. (2019). Based on the results of this study, we conclude that QTL conferring OWBM resistance do exist in the 4A-interval from Chinese wheat. QTL conferring resistance to OWBM in Chinese wheat may represent a novel gene resource and has been getting adapted to Chinese wheat breeding programs.

Mapping major QTL with selective or entire population

To verify whether the QTL mapping results of the selective population were consistent with those of the entire population, new markers were developed (Table S5) to detect QTL in the whole HY-RIL population (351 RILs) in coupling with simple sequence repeat (SSR) markers. Six QTL were detected, of which one stably expressed major QTL was detected on chromosome 4A under all environments (Table S6, Fig. S3). This QTL was mapped to a 3.1-cM interval corresponding to a 2.8-Mb (704,444,188–707,248,000 bp) physical region, which is included within the major QTL interval (4.9 Mb, 703,434,395–708,327,301 bp) of *QSm.hbau-4A.2-1* identified by using the selective population in this study.

In previous studies of our group, Hao et al. (2017) detected a major QTL, *QSm.hbau-4A.1*, by using the whole

F_6-derived 6J-RIL population. This QTL was mapped to a 3.8-Mb (691,143,653–694,903,746 bp) physical region which is close to, but not overlapped with the interval of *QSm.hbau-4A.2-2* in this study due to the lower density of linkage map. In addition, Hao et al. (2019) integrated two expressed sequence tag (EST) and four kompetitive allele specific PCR (KASP) markers onto chromosome 4A in the genetic map constructed by Hao et al. (2017), using another 92 selected RILs from the population 6J-RIL. One major QTL on chromosome 4A was mapped to a 24.88-cM interval corresponding to a 12.3-Mb (694,903,746–707,248,000 bp) physical region which covered the physical interval (1.2 Mb, 703,434,395–704647631 bp) obtained by SNP markers in this study. These studies showed that whether we use the whole genotypes or selected genotypes, the detection of the major QTL is not affected.

In addition, the selective genotyping approach can largely reduce the cost and time paid on genotyping and phenotyping work and can still maintain the detection power in major QTL identification. However, the limitations for selective genotyping strategy are that it cannot achieve in multiple trait analysis, and mapping the QTL by environmental interaction, and it is hard to estimate the genetic effects. Therefore, it is a good choice to use the selective population rather than using the entire population if the objectives are to find the major QTL, to map only one trait and to screen markers for MAS.

Possible source of OWBM resistance in Chinese wheat varieties

In this study, the resistant wheat parents HN215 and JM24 are sister lines. Both were selected from the same parental combination (Anyang10/Aifeng1//Lovrin10/70-114). Additionally, another winter wheat variety, Jimai23 ($RI = 0.16$), also shared this pedigree. Among the lineage, Aifeng1 was bred from the cross Xinong6028/Shuiyuan86//58(18)2. In the 1980s, Jimai23 and JM24 were released as elite varieties, and HN215 was considered as a breeding line. But the striking characteristic for these lines was their stable resistance to OWBM since 1983 (Sun et al. 1995; Qu et al. 2011) and the stable inheritance of the resistance. Shimai12 ($RI = 0.03$) was selected from the pedigree Shi91-5096/Jimai23 and shows as good resistance to OWBM as Jimai23. In this study, two major QTL from HN215 and JM24 were detected in an overlapping physical interval on 4AL. It could be inferred that genes conferring resistance to OWBM from HN215 and JM24 may have the same origin and that the two major QTL from the resistant parents have strong transmission ability in resultant generations. Based on our current discovery and pedigree information (not listed), we speculate that the OWBM resistance in Chinese wheat may have originated from Lovrin10 or Xinong6028.

Homologous relationship of QTL mapping interval

During the evolution of hexaploid wheat, chromosomal rearrangements occurred between 4AL, 5AL and 7BS (Nelson et al.1995; Jorgensen et al. 2017; Dvorak et al. 2018), resulting in a complex homology between specific chromosome fragments on 4AL and corresponding regions of group 7. Based on the results in the present study, most of the genes in the 4A-interval were homologous to genes located on the short arm of chromosome 7 of diploid wheat, implying that the 4A-interval mapped in this study might be included in or overlapped with the 4AL-7BS translocation fragment. Since the accurate bordering between 4AL and 7BS translocation has not been physically determined, the size of the 4A-interval within the translocation cannot be determined yet. If the 4A-interval is indeed related to the 4AL-7BS translocation region, it will increase the difficulty for fine mapping or map-based cloning for OWBM resistance genes within 4A-interval.

Conclusion

Seven QTL related to OWBM resistance were detected using two selective populations with SNP markers in this study, of which two major QTL with additive effects from two resistant wheat parents were mapped to similar interval on chromosome arm 4AL with a common flanking SNP marker *AX-108942696*. A 4.9-Mb physical region on 4AL was proposed for the target region to maximize the possibility of isolating the candidate gene for OWBM resistance. Mapping results of the major QTL on 4AL using two selective populations were consistent with our other results using entire populations.

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Author Contribution statement LZ and RW designed and conducted the study. MG, GY, SW and GL provided advice to the authors. SW, GL and RW performed RIL population construction. GL and RW performed OWBM invasion treatment and analyzed resistance index. LZ and ZZ performed experimental material collection for SNP genotyping. LZ, ZZ and YZ performed resistance evaluation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All research conducted in relation to this publication is in compliance with ethical standards. The authors declare that this study was performed and reported in accordance with the ethical standards of scientific conduct.

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