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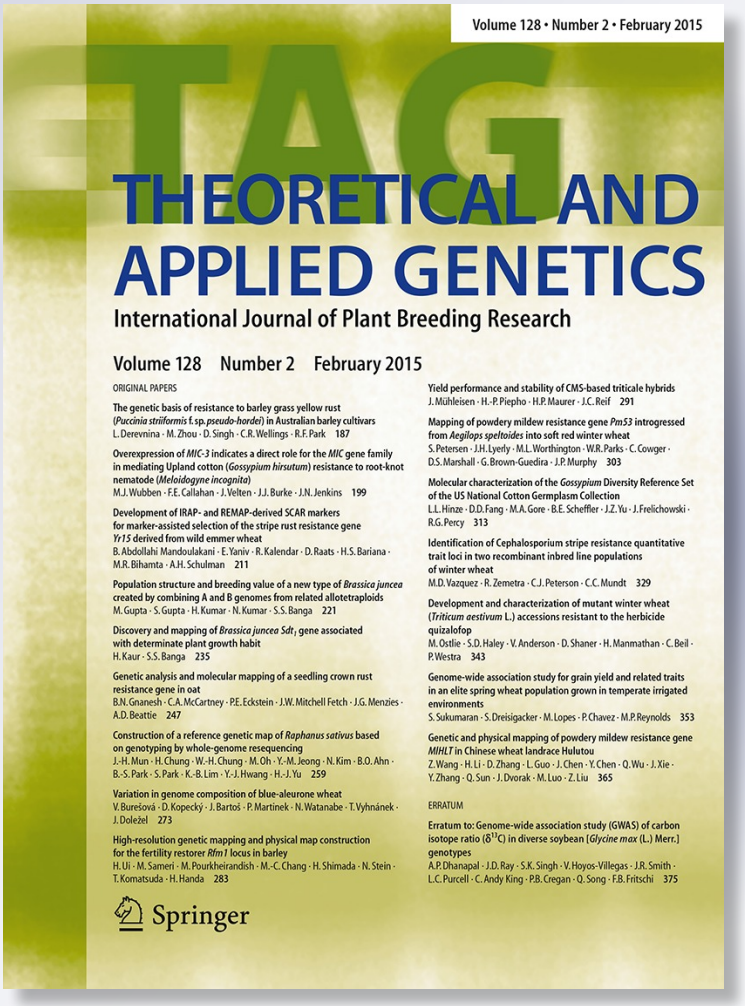
Theoretical and Applied Genetics
International Journal of Plant Breeding Research

ISSN 0040-5752
Volume 128
Number 2

Theor Appl Genet (2015) 128:365-373
DOI 10.1007/s00122-014-2436-2



Springer

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ORIGINAL PAPER

Genetic and physical mapping of powdery mildew resistance gene *MlHLT* in Chinese wheat landrace Hulutou

Zhenzhong Wang · Hanwen Li · Deyun Zhang · Li Guo · Jiaojiao Chen · Yongxing Chen · Qiuhong Wu · Jingzhong Xie · Yan Zhang · Qixin Sun · Jan Dvorak · Ming-cheng Luo · Zhiyong Liu

Received: 14 August 2014 / Accepted: 21 November 2014 / Published online: 4 December 2014

Abstract

Key message **A powdery mildew resistance gene *MlHLT* derived from a Chinese wheat landrace maps within a 3.6 centimorgan (cM) genetic interval spanning a 13.4 megabase (Mb) physical genomic region on chromosome 1DS.**

Abstract Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a devastating disease that can cause severe yield losses. Chinese wheat landrace Hulutou confers nearly immune resistance against prevailing *Bgt* isolate E09 in Beijing. Genetic analysis indicate that the powdery mildew resistance of Hulutou is controlled by a single dominant gene, provisionally designated *MlHLT*. Bulked segregant analysis(BSA) and simple sequence repeat (SSR) mapping showed that *MlHLT* is located on chromosome arm 1DS between markers *Xgwm337* and *Xcfd83*/*Xcfd72*. By applying comparative genomics analysis, collinearity genomic regions of the *MlHLT* locus on *Aegilops tauschii* chromosome 1DS were identified in *Brachypodium distachyon* chromosome 2, rice chromosome 5 and sorghum chromosome 9, respectively. Three new polymorphic markers were developed using the draft genome sequences and the extended single nucleotide polymorphism (SNP) marker sequences of *Ae. tauschii* accession AL8/78, as well as the *Triticum aestivum* cv. Chinese Spring 454 contig sequences and the International Wheat Genome Sequencing Consortium (IWGSC) survey sequences. *MlHLT* mapped into a 3.6 cM genetic interval spanning 13.4 Mb physical genomic region containing seven contigs (ctg220, ctg4623, ctg1063, ctg5929, ctg3163, ctg699 and ctg1065) on 1DS that has synteny with a 369.8 kb genomic region in *Brachypodium*, a 380.8 kb genomic region in rice and a 298.4 kb genomic region in sorghum. The genetic and physical maps of *MlHLT* provide framework for map-based cloning and marker-assisted selection (MAS) of the powdery mildew resistance gene *MlHLT* in Hulutou.

Introduction

Bread wheat is one of the major food crops for humans, but wheat yields are continuously challenged by various wheat diseases. Wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is a common wheat disease and is widespread in the world. In addition to pesticides and biological control, breeding and using resistant cultivars is urgently needed to reduce the prevalence of powdery mildew. Up to date, more than 60 genes/alleles for resistance to wheat powdery mildew have been identified at 49 loci (*Pm1* to *Pm53*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, *Pm31* = *Pm21*) (McIntosh et al. 2013, 2014), some of these genes have been applied for varietal breeding. However, continuing evolution of the pathogens

Communicated by Thomas Miedaner.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-014-2436-2) contains supplementary material, which is available to authorized users.

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often overcomes the resistance of available resistance genes, so searches for diversified resistance is an ongoing process.

Chinese wheat landraces harbor rich genetic diversity for wheat powdery mildew resistance. Six powdery mildew resistance genes, *Pm5d* (Nematollahi et al. 2008), *Pm5e* (Huang et al. 2003b), *Pm24* (Huang and Röder 2011), *Pm24b* (Xue et al. 2012), *Pm45* (Ma et al. 2011) and *Pm47* (Xiao et al. 2013), on 4 loci have been identified from Chinese landraces. Moreover, powdery mildew resistance genes provisionally designated *PmTm4* (Hu et al. 2008), *Mlxbd* (Huang et al. 2000a), *PmH* (Zhou et al. 2005), and *pmX* (Fu et al. 2013) exhibit sound resistance to prevalent *Bgt* isolates in China.

Fine mapping and map-based cloning in common wheat are tedious because of the large wheat genome size (17 giga base), allohexaploid nature (AABBDD), and highly repetitive DNA (90 %). The availability of wheat expressed sequence tags (EST) (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) and the rice (International Rice Genome Sequencing project 2005), sorghum (Paterson et al. 2009), and *Brachypodium distachyon* (The International *Brachypodium* Initiative 2010) genome sequences provide comparative genomics tools for wheat gene mapping and map-based cloning (Fu et al. 2009; Liu et al. 2012; Qin et al. 2011; Saintenac et al. 2013; Wang et al. 2014; Wu et al. 2013; Zhang et al. 2010). The recently released draft genome sequences of *Triticum aestivum* cv. Chinese Spring, *T. urartu* accession G1812 and *Aegilops tauschii* accession AL8/78 provide nearly complete gene sets of the wheat A, B and D genomes (Brenchley et al. 2012; Jia et al. 2013; Ling et al. 2013) for marker development and gene identification.

The physical map of *Ae. tauschii*, the diploid donor of the D genome in hexaploid wheat, has been constructed (Luo et al. 2013). Although the sequences of bacterial artificial chromosome (BAC) contigs are still unavailable, the extended sequences of mapped SNP markers (Luo et al. 2013) combined with the draft genome sequences of *Ae. tauschii* (Jia et al. 2013) provide an efficient tool for comparative genomics analyses among grass families and marker development for fine mapping and map-based cloning of genes from the D genome.

Hulutou is a Chinese wheat landrace collected from Shaanxi province that is highly resistant to the prevalent *Bgt* isolate E09 in Beijing. In this paper, we report: (1) the identification and genetic mapping of the Hulutou powdery mildew resistance gene *MlHLT* (2) and comparative genomics analysis of the genomic region of *MlHLT* locus and physical mapping of *MlHLT* using the physical map of *Ae. tauschii*.

Materials and methods

Plant materials

The Chinese wheat landrace Hulutou was used as the powdery mildew-resistant parent to make cross with a highly susceptible Chinese elite common wheat variety Shi4185. F_1 hybrids, F_2 segregating population and F_2-derived F_3 families were evaluated for powdery mildew resistance with *Bgt* isolate E09. A highly susceptible common wheat line Xuezao was used as the susceptible control.

Chinese Spring (CS) and its nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 1 (kindly provided by Drs. WJ Raupp and BS Gill, Wheat Genetics Resource Centre, Kansas State University, USA) were used for chromosomal arm assignment and bin mapping of molecular markers flanking the powdery mildew resistance gene in Hulutou.

Powdery mildew evaluations

The prevailing *Bgt* isolate E09 was kindly provided by Dr. Xiayu Duan, Institute of Plant Protection, Chinese Academy of Agricultural Science. The parental lines Hulutou, Shi4185, F_1, F_2 and $F_{2:3}$ families were evaluated for powdery mildew resistance under controlled greenhouse conditions. The infection type (IT) was recorded 15 days after inoculation and reconfirmed after 20 days with a scale of 0–4, with 0 representing an immune reaction without visible symptoms, 0; for necrotic flecks without uredia, and 1–4 for highly resistant, moderately resistant, moderately susceptible and highly susceptible, respectively. The values of 0–2 were classified as resistant, and those of 3–4 were classified as susceptible (Liu et al. 1999).

Genomic DNA isolation and SSR marker analysis

Genomic DNA isolations from seedling leaves of the parental lines and $F_{2:3}$ families were performed using the CTAB protocol (Saghai-Maroof et al. 1984). Resistant and susceptible DNA bulks were produced by separately mixing equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible $F_{2:3}$ families for bulked segregant analysis (Michelmore et al. 1991). Wheat genomic SSRs (*Xgwm*, *Xwmc*, *Xbarc*, *Xcfa*, and *Xcfd* series, http://wheat.pw.usda.gov) were used for polymorphism surveys between the two DNA bulks, and the polymorphic markers were subsequently genotyped in the mapping populations.

PCRs were carried out in a 10 μl reaction volume with the following conditions: one denaturation cycle at 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, 50–60 °C (depending on specific primers) for 45 s, and

72 °C for 1 min, followed by an extension step of 72°C for 10 min. Fragment analyses of PCR products were carried out on 8 % non-denaturing polyacrylamide gels (39 acrylamide:1 bisacrylamide). After electrophoresis, the gels were silver stained and photographed.

Chromosome arm assignment and physical bin mapping

Polymorphic markers flanking the resistance gene were located with a set of Chinese Spring nullisomic-tetrasomics, ditelosomics and deletion lines of homoeologous group 1. Polymorphic markers were mapped to chromosome bins by determining the smallest deletion bin possessing them.

EST-STS marker screening and comparative genomics analysis

EST-STS (sequence-tagged site derived from expressed sequence tag) primer pairs (http://wheat.pw.usda.gov/SNP/primers/contig_primer_list.xls) mapped on chromosome 1DS were screened for polymorphisms between Hulutou and Shi4185 as well as the resistant and susceptible DNA bulks. The polymorphic EST-STS markers were validated in the mapping population to confirm the genetic linkage relationship between markers and the powdery mildew resistance gene. The corresponding EST sequences of polymorphic EST-STS markers flanking the resistance gene were subsequently used as queries to search the *Ae. tauschii* SNP database (http://probes.pw.usda.gov/WheatDMarker/), and the *Brachypodium* (http://mips.helmholtz-muenchen.de/plant/brachypodium/), rice (http://rice.plantbiology.msu.edu/), and sorghum (http://mips.helmholtz-muenchen.de/plant/sorghum/) genome sequences with an e-value cutoff of 10e-10. The orthologous genomic regions were identified through comparative genomics analysis of the putative highly conserved gene pairs in *Ae. tauschii*, *Brachypodium*, rice, and sorghum.

Polymorphic SSR and STS markers development

The orthologous gene pairs were used to search the draft genome sequences of *Ae. tauschii* accession AL8/78 (Jia et al. 2013), the hexaploid wheat cv. Chinese Spring 454 contigs (Brenchley et al. 2012) and IWGSC survey sequences (http://www.wheatgenome.org/) to find homologous contig or scaffold sequences for polymorphic marker development. These contig and scaffold sequences were first used to screen simple sequence repeat (SSR) motifs using BatchPrimer3 (You et al. 2008). If no SSR polymorphisms were detected between the parental lines and the resistant and susceptible DNA bulks, the Chinese Spring orthologous contigs of 454 assembly and IWGSC survey sequences were used to design STS (sequence-tagged site) primer pairs to identify length polymorphisms using DNAMAN software with the following parameters: amplification product size of 200–800 bp with the optimum 500 bp, primer length of 18–22 bp, Tm of 55–65 °C, GC content of 40–60 %.

Genetic and physical mapping

Polymorphic markers between the parental lines and the resistant and susceptible DNA bulks were genotyped on the mapping population to develop a linkage map using Mapmaker 3.0 and Kosambi map function, with an LOD (likelihood of odd) score threshold of 3.0 (Lincoln et al. 1992). The genetic map was constructed with the software Mapdraw V2. 1 (Liu and Meng 2003). The corresponding contig and scaffold of the polymorphic markers linked to the powdery mildew resistance gene in Hulutou were then used to search the *Ae. tauschii* physical map database (http://probes.pw.usda.gov/WheatDMarker/) to find assembled BAC contigs to delimit the powdery mildew resistance gene into a specific physical interval.

Results

Genetic analysis of the powdery mildew resistance gene in Hulutou

Chinese wheat landrace Hulutou, wheat cultivar Shi4185, the F_1 hybrid plants, 310 F_2 plants and F_2-derived F_3 families from the cross between Shi4185 and Hulutou were challenged with *Bgt* isolate E09. Hulutou is highly resistant (IT = 0, 1) and Shi4185 is highly susceptible (IT = 4) to E09 (Fig. 1). The F_1 hybrids are highly resistant to E09, indicating the dominant nature of the powdery mildew resistance in Hulutou. The F_2 plants segregated as 221 resistant: 89 susceptible, as expected of a 3:1 ratio ($\chi^2 = 2.28$, $P > 0.05$). The $F_{2:3}$ families segregated as 71 homozygous resistant, 150 segregating and 89 homozygous

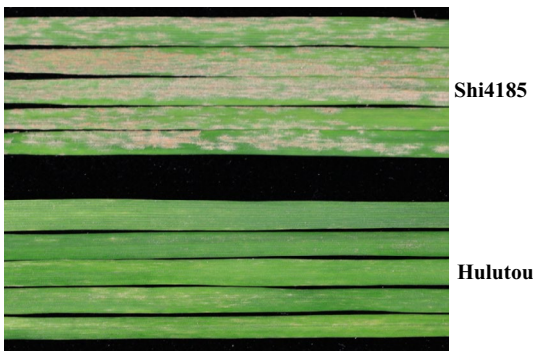

Fig. 1 Phenotype of resistant parent Hulutou and susceptible parent Shi4185 at 15 days post inoculation (DPI) with *Bgt* isolate E09

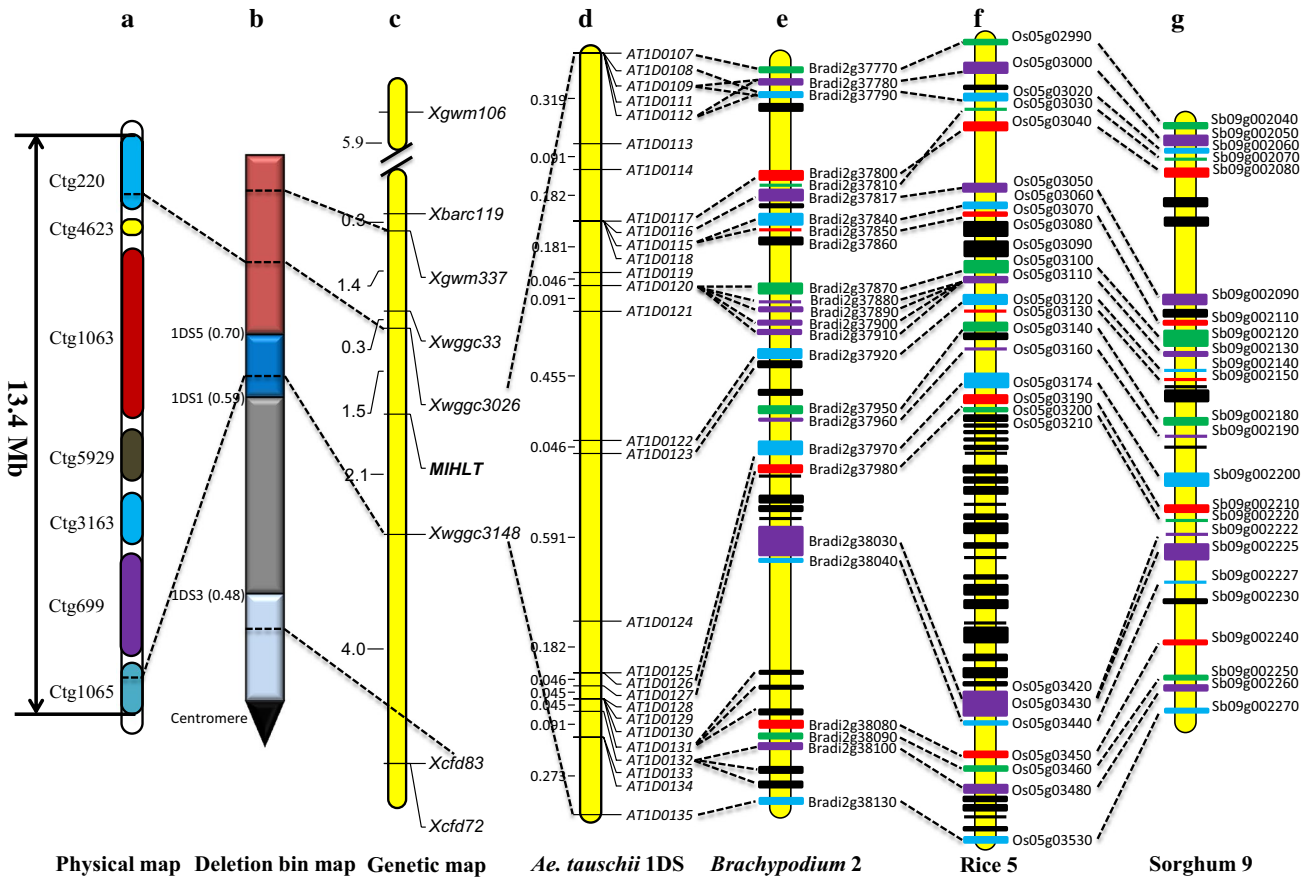

Fig. 2 Comparative genetic linkage and physical maps of the powdery mildew resistance gene *MlHLT*. **a** Physical map of *MlHLT*. **b** Deletion bin map of *MlHLT*. **c** Genetic linkage map of *MlHLT*. **d** Genetic linkage map of *MlHLT* orthologous genomic region on chromosome 1DS of *Aegilops tauschii* (Luo et al. 2013). **e** Orthologous genomic region of *MlHLT* on *Brachypodium* chromosome 2. **f** Orthologous genomic region of *MlHLT* on rice chromosome 5. **g** Orthologous genomic region of *MlHLT* on sorghum chromosome 9

susceptible fitting a 1:2:1 segregation ratio ($\chi^2 = 2.41$, $P > 0.05$). These results suggested that the powdery mildew resistance in Hulutou is controlled by a single dominant gene, provisionally designated *MlHLT*.

Molecular mapping of *MlHLT*

To localize the powdery mildew resistance gene *MlHLT* in the wheat genome, 194 SSR primer pairs distributed randomly throughout the whole genome were screened for polymorphisms between the parental lines as well as the resistant and susceptible DNA bulks. Only one marker, *Xgwm337*, was found to be polymorphic between the parental lines as well as the DNA bulks, and this marker mapped 3.2 cM away from the *MlHLT* after genotyping the mapping population (Fig. 2). Since *Xgwm337* was mapped on chromosome 1DS, SSR markers linked to *Xgwm337* on chromosome 1DS were subsequently surveyed for polymorphisms. Four markers, *Xgwm106*, *Xbarc119*, *Xcfd83*, and *Xcfd72* revealed polymorphisms and localized the *MlHLT* gene into a 9.3 cM genetic interval between markers *Xgwm337* and *Xcfd83/Xcfd72* (Fig. 2).

Identification of polymorphic EST-STS markers linked to *MlHLT*

A total of 43 wheat EST-STS markers that mapped on chromosome 1DS were used for polymorphism surveys between the parental lines Shi4185 and Hulutou as well as the resistant and susceptible DNA bulks. One EST-STS marker *Xwggc33*, developed from wheat EST BE405518, showed polymorphism and was located 1.8 cM distal to *MlHLT* after genotyping the mapping population (Figs. 2, 3a).

Identifying collinearity of genomic regions of *MlHLT* in *Ae. tauschii*, *Brachypodium*, rice and sorghum and comparative genomics analysis

The sequence of BE405518 was used to search the *Ae. tauschii* SNP marker-extended sequence database and the

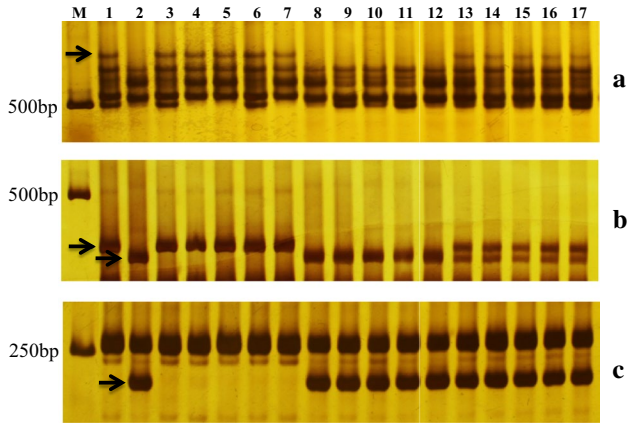

Fig. 3 PCR amplification patterns of polymorphic markers, *Xwggc33* (**a**), *Xwggc3026* (**b**), and *Xwggc3148* (**c**). The *black arrows* show DNA fragments that are polymorphic between resistant and susceptible lines. *Lanes 1* and *2* are Hulutou and Shi4185, *lanes 3–7* represent homozygous resistant $F_{2:3}$ families, *lanes 8–12* represent homozygous susceptible $F_{2:3}$ families and *lanes 13–17* represent heterozygous $F_{2:3}$ families, respectively

genome sequence databases of *Brachypodium*, rice and sorghum to identify orthologous gene pairs. Homologs of BE405518 were not found in the *Ae. tauschii* SNP marker-extended sequence database, but were identified in *Brachypodium* chromosome 2 (Bradi2g37640), rice chromosome 5 (Os05g02880) and sorghum chromosome 9 (Sb09g001920) (Table 1). Since only one EST-STS marker *Xwggc33* was identified that mapped distal to *MlHLT*, additional EST sequences mapping around *Xwggc33* (BE405518) on chromosome 1DS (Luo et al. 2009) were used as queries to search the *Ae. tauschii* SNP marker-extended sequence database, and the genome sequence databases of *Brachypodium*, rice and sorghum. More orthologous gene pairs were identified in the genomic region of *MlHLT* in *Ae. tauschii* and collinearity regions in *Brachypodium*, rice and sorghum, respectively (Table 1). The coding sequences of putative gene pairs with high synteny were used as queries to search the draft genome sequences of *Ae. tauschii* accession AL8/78, the Chinese Spring 454 contigs and IWGSC individual chromosome survey sequences which then were

Table 1 Comparative genomics analysis among wheat ESTs, *Aegilops tauschii* extended SNP marker sequences, and *Brachypodium*, rice, sorghum genome sequences

Wheat ESTs	Extended SNP marker sequences	*Brachypodium*	Rice	Sorghum
BF473056	BF473056/AT1D0066/AT1D0067	Bradi2g37310	Os05g02250	Sb09g001500
BE446624	–	Bradi2g37430	Os05g02390	Sb09g001560
BE406989	–	–	–	–
KSuE18	–	–	–	–
BE499070	–	–	–	–
BE591682	–	Bradi2g37440	Os05g02490	Sb09g001650
BF484606	BF484606/AT1D0090	Bradi2g37517	Os05g02640	Sb09g001790
BE293621	–	–	–	–
BE495292	AT1D0088	Bradi2g37530	Os05g02650	Sb09g001810
BG313657	AT1D0088	Bradi2g37530	Os05g02650	Sb09g001810
BG275046	AT1D0088	Bradi2g37530	Os05g02650	Sb09g001810
BG314086	–	–	–	–
BE405414	–	–	–	–
BF201480	AT1D0096	–	–	–
BE405518	–	Bradi2g37640	Os05g02880	Sb09g001920
BE426265	AT1D0099/AT1D0101	Bradi2g37720	Os05g02820	–
BE404396	AT1D0110	–	–	–
BF292046	AT1D0102/AT1D0105	Bradi2g37675	Os05g02740	Sb09g002005
BE489565	AT1D0114	–	–	–
BE490041	AT1D0117	Bradi2g37800	Os05g03040	Sb09g002080
BE494541	–	–	–	–
BE489313	–	–	–	–
BE604778	–	–	–	–
BE497795	–	–	–	–
BG604768	AT1D0131	Bradi2g38070	–	–
BE604778	–	–	–	–
BE445121	BE445121	Bradi2g38340	Os05g03820	Sb09g002470
BF291549	BF291549	Bradi2g38590	Os05g04340	Sb09g002710

– indicated no orthologous genes in the collinearity genomic regions of *Aegilops tauschii*, *Brachypodium*, rice and sorghum

Table 2 Polymorphic markers linked to powdery mildew resistance gene *MlHLT*

SNP	Markers	Marker type	Annealing temperature (°C)	Forward primer (5′–3′)	Reverse primer (5′–3′)
–	*Xwggc33*	EST-STS	57	AAGGCTGGTGACTGGAAGAA	GCTGATGCTCCTTGATCTCC
AT1D0107	*Xwggc3026*	SSR	59	GGAGTTGACCACAGACCTTT	ACTGGCACCAATCTACCG
AT1D0135	*Xwggc3148*	STS	55	CCTGGCTGGAGATGCTCTAA	CTGACAACCGGTTTCCTTGT

used as templates to design primers. Out of 130 designed primer pairs, two polymorphic markers, *Xwggc3026* (developed from Bradi2g37770) and *Xwggc3148* (developed from Bradi2g38130), were developed and mapped 1.5 cM distal and 2.1 cM proximal to *MlHLT*, respectively (Figs. 2, 3b, c; Table 2). Thus, orthologous genomic regions of the locus were identified on *Ae. tauschii* chromosome 1DS (29 SNP markers from AT1D0107 to AT1D0135, 13.4 Mb), *Brachypodium* chromosome 2 (38 predicted genes from Bradi2g37770 to Bradi2g38130, 369.8 kb), rice chromosome 5 (53 predicted genes from Os05g02990 to Os05g03530, 380.8 kb) and sorghum chromosome 9 (30 predicted genes from Sb09g002040 to Sb09g002270, 298.4 kb) (Fig. 2).

Out of the 29 *Ae. tauschii* SNP marker-extended sequences, 15 are orthologous to 23 predicted genes in *Brachypodium*, whereas 13 *Ae. tauschii* SNP markers are orthologous to 14 predicted genes in rice and 13 *Ae. tauschii* SNP markers are orthologous to 13 predicted genes in sorghum. In the 38 predicted *Brachypodium* genes, 24 are orthologs of 21 predicted rice genes and 23 predicted *Brachypodium* genes are orthologs of 21 predicted sorghum genes. Out of 53 predicted rice genes, 22 are orthologs of 23 predicted sorghum genes. Moreover, a 123.6 kb segmental deletion in rice chromosome 5 containing 22 predicted genes (Os05g03210–Os05g03420) was identified (Fig. 2; Table S1). Gene annotation revealed that 5 transposons (Os05g03220, Os05g03270, Os05g03280, Os05g03290, and Os05g03370) and 5 retrotransposons (Os05g03230, Os05g03250, Os05g03260, Os05g03350, and Os05g03360) were widely distributed in this region and these transposons account for 45.5 % of the DNA sequences (Table S1).

Physical bin mapping of *MlHLT*

To locate the *MlHLT* gene in the deletion bins on chromosome 1DS, the nullisomic-tetrasomics, ditelosomics, and deletion lines of Chinese Spring homoeologous group 1 were selected to determine the chromosomal and physical bin locations of the *MlHLT*-linked markers. *Xgwm337* was not detected in N1D-T1B, Dt1DL, 1DS-5, 1DS-1, 1DS-3 (Fig. 4a), and *Xcfd83* was also absent in N1D-T1B, Dt1DL, but present in 1DS-5, 1DS-1, 1DS-3 (Fig. 4b). Both *Xwggc3026* and *Xwggc3148* were absent in N1D-T1B, Dt1DL, 1DS-1, 1DS-3. However, marker *Xwggc3148* was present in 1DS-5 and marker *Xwggc3026* was absent in 1DS-5 (Fig. 4c, d), indicating *MlHLT* is located in the distal bin 1DS-0.59-1.00 (Fig. 2).

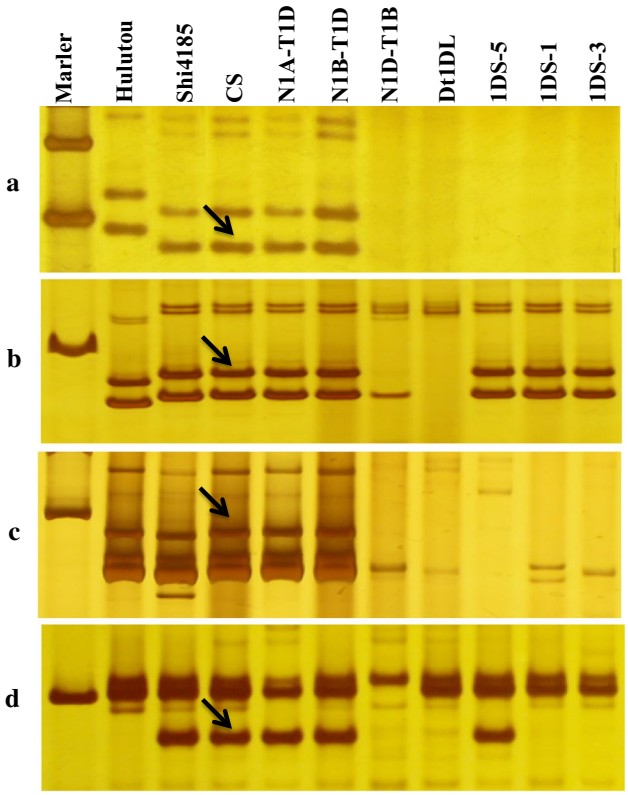

Fig. 4 Amplification patterns of markers *Xgwm337* **a**, *Xcfd83* **b**, *Xwggc3026* **c** and *Xwggc3148* **d** in the parental lines Hulutou and Shi4185, Chinese Spring (CS) and its homoeologous group 1 nullisomic-tetrasomics, ditelosomics, and deletion lines

Physical mapping of *MlHLT*

Xwggc3026 and *Xwggc3148* corresponding to the extended sequences of markers AT1D0107 and AT1D0135 on chromosome 1DS are anchored to the assembled BAC contigs ctg220 and ctg1065 in the physical map of *Ae. tauschii*, respectively. Therefore, the powdery mildew resistance gene *MlHLT* could be delimited into a 13.4 Mb genomic region containing seven contigs, ctg220, ctg4623, ctg1063, ctg5929, ctg3163, ctg699 and ctg1065, on chromosome 1DS (Fig. 2).

Discussion

Wheat landraces are valuable germplasms for wheat breeding and Chinese landraces are extremely diversified for powdery mildew resistance (Huang et al. 1997b). Up to date, six powdery mildew resistance genes have been identified from Chinese wheat landraces. *Pm5e* was identified from Fuzhuang 30, a derivative of a cross between two Chinese landraces Liquan-Heshangtou/Huaxianqishifeng (Huang et al. 2003b; Zhuang 2003). *Pm5d* was found in IGV1-455 and IGV2-556, derivatives of the powdery mildew resistance line CI10904 that was originally introduced from Jinling University, Nanjing, China (Hsam et al. 2001). *Pm5e* is likely allelic to *Pm5d* (Hsam et al. 2001; Huang et al. 2000a; Huang et al. 2003b; Nematollahi et al. 2008). In addition to *Pm5e* and *Pm5d*, several powdery mildew resistance alleles have been identified from Chinese landraces Tangmai4 (*PmTm4*), Xiaobaidongmai (*Mlxbd*), Hongquanmang(*PmH*), Mazhamai (*PmMZ*), Youbailan (*PmYBL*), Baiyouyantiao (*PmBYYT*), and Bensanyuehuang (*PmBSYH*) and these alleles map on the same genetic region as *Pm5* on 7BL (Hu et al. 2008; Huang et al. 2000a; Sheng et al. 1992; Zhou et al. 2005).

More recently, two other broad-spectrum powdery mildew resistance genes, *Pm45* and *Pm47,* have been identified from the Chinese wheat landraces D57 and Hongyanglazi and these genes map on chromosomes 6DS and 7BS, respectively (Ma et al. 2011; Xiao et al. 2013).

So far, two powdery mildew resistance genes, *Pm24* and *Pm24b*, have been located on the short arm of chromosome 1D. *Pm24* was identified from the Chinese landrace Chiyacao collected from Henan province, China (Huang et al. 1997a) and maps on chromosome 1D (Huang et al. 2000b). Recently, *Pm24* was shown to map in a gene-rich region with high recombination rates between the markers *Xgwm789/Xgwm603* (2.4 cM) and *Xbarc229* (3.6 cM) and to co-segregate with *Xgwm1291* on 1DS bin 1DS 5-0.70-1.00 (Huang and Röder 2011). When studying the powdery mildew resistance gene in Chinese landrace Baihulu collected from Shaanxi Province, China, Xue et al. (2012) found that the resistance locus *Pm24b* mapped within a 2.5 cM interval between markers *Xgwm603/Xgwm789* and *Xbarc229*on chromosome 1DSbin 1DS-0.59-1.00. Phytopathology analyses and allelism tests indicated that *Pm24b* in Baihulu was either allelic or tightly linked with *Pm24* in Chiyacao (Huang and Röder 2011; Xue et al. 2012).

Hulutou is a common wheat landrace collected from Shaanxi province, China. Genetic mapping results indicated that the powdery mildew resistance gene *MlHLT* in Hulutou was located within a 3.6 cM interval between markers *Xwggc3026* and *Xwggc3148* on chromosome 1DSbin 1DS-0.59-1.00, as is *Pm24* and *Pm24b*. It is not known if Baihulu and Hulutou were collected from same area of Shaanxi Province, China, hence phytopathology and allelism tests need to be conducted in the future to clarify whether *MlHLT* is allelic or is closely linked to *Pm24* and *Pm24b*.

High degrees of macro- and micro-collinearities are found among the genomes of wheat, *Brachypodium*, rice and sorghum, because the grass family originated from a common ancestor (Bossolini et al. 2007; Gu et al. 2009; The International Brachypodium Initiative 2010). Comparative genomics has been widely applied for wheat gene mapping using the genome sequences of rice, sorghum and *Brachypodium*. By applying comparative genomics analyses, the stripe rust resistance gene *Yr36* was shown to map to a 0.14 cM genomic region using the rice collinear sequences (Fu et al. 2009); and the stem rust resistance gene *Sr35* was delimited within a 1.0 cM interval in the collinear region of *B. distachyon* (Saintenac et al. 2013). In the current study, we applied comparative genomics analysis and used the recently released Chinese Spring 454 contigs (Brenchley et al. 2012) and IWGSC survey sequences (http://www.wheatgenome.org), together with the *Ae. tauschii* physical map (Luo et al. 2013) and draft genome sequences (Jia et al. 2013) to develop markers linked to the powdery mildew resistance gene in Hulutou (Fig. 2).

Common wheat (*T. aestivum*) was derived about 8,000 years ago from a natural hybridization between cultivated emmer wheat (AABB) and the wild diploid relative *Ae. tauschii* (DD) (Jia et al. 2013; Petersen et al. 2006). Relatively low genetic diversity has reported for the D genome in common wheat, compared to the A and B genomes (Cavanagh et al. 2013; Chao et al. 2009). In our study, out of 173 primer pairs designed, only three (1.7 %) polymorphic molecular markers were developed and integrated into the genetic linkage map of *MlHLT* on 1DS. Finally, *MlHLT* was mapped within a 3.6 cM genetic interval between markers *Xwggc3026* and *Xwggc3148*, corresponding to a 13.4 Mb physical genomic region within 1DS that can be used as a framework for map-based cloning.

Several wheat resistance genes have been identified on chromosome 1DS. Both stem rust resistance gene *Sr33* and leaf rust resistance gene *Lr21* are located on 1DS and have been successfully cloned (Huang et al. 2003a; Periyannan et al. 2013). Another stem rust resistance gene *Sr45* was found not to be allelic, but tightly linked with *Sr33*, and the order of these resistance loci was probably: centromere–*Sr45*–*Sr33*–*Lr21* (Marais et al. 1998). Recently, a PCR-based co-dominant marker *cssu45* linked with *Sr45* at a distance of 0.39 cM was developed, which could facilitate selecting *Sr45* and *Lr21* in breeding populations combined with *Lr21*-specific marker (Periyannan et al. 2014). Another leaf rust resistance gene *Lr60* has been found 8.4 cM distal to molecular marker *Xbarc149* and linked to *Lr21* (Hiebert et al. 2008). The powdery

mildew resistance *Pm24*, *Pm24b* and *MlHLT* genes also map within the same genetic interval and are tightly linked to marker *Xgwm337* (Huang and Röder 2011; Xue et al. 2012). These findings thus permit pyramiding of the leaf rust, stem rust and powdery mildew resistance genes in 1DS via marker-assisted selection and will benefit development of durable and broad-spectrum resistance wheat varieties.

Author contribution statement ZW and ZL designed the experiments; ZW, HL, DZ, LG, JC, YC, QW, JX, YZ performed the experiments; QS, JD, ML, provided materials; ZW and ZL wrote the paper.

Acknowledgments We are grateful to Professor Andrew Jackson, University of California at Berkeley for editing the manuscript. This work was financially supported by the Ministry of Science and Technology of China (2011AA100104, 2013CB127705), National Science Foundation of China (31030056) and Ministry of Education (MOE) of China (111-2-03).

Conflict of interest The authors have declared that there is no conflict of interest.

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