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Identification and mapping of *MLIW30*, a novel powdery mildew resistance gene derived from wild emmer wheat

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Abstract Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*), is a destructive foliar disease of common wheat in areas with cool or maritime climates. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, the progenitor of both domesticated tetraploid durum wheat and hexaploid bread wheat, harbors abundant genetic diversity related to resistance to powdery mildew that can be utilized for wheat improvement. An F_2 segregating population was obtained from a cross between resistant bread wheat line 2L6 and susceptible cultivar Liaochun 10, after which genetic analysis of F_2 and F_2-derived F_3 families was performed by inoculating plants with isolate *Bgt* E09. The results of this experiment demonstrated that powdery mildew resistance in 2L6, which was derived from wild emmer wheat accession IW30, was controlled by a single dominant gene, temporarily designated *MLIW30*. Nineteen SSR markers and two STS markers linked with *MLIW30* were acquired by applying bulked segregant analysis. Finally, *MLIW30* was located to the long arm of chromosome 4A and found to be flanked by simple sequence repeat markers *XB1g2000.2* and *XB1g2020.2* at 0.1 cM. Because no powdery mildew resistance gene in or derived from wild emmer wheat has been reported in wheat chromosome 4A, *MLIW30* might be a novel *Pm* gene.

Keywords Powdery mildew resistance gene · Wild emmer wheat · Molecular marker · Comparative genomic analysis

Introduction

Wheat is a major staple food crop in many regions of the world and provides approximately one-fifth of the calories consumed by humans (FAOstat 2012). Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*), is a destructive foliar disease of common wheat in areas with cool or maritime climates, which has led to severe yield losses ranging from 13 to 34 % (Conner et al. 2003; Griffey et al. 1993; Oerke 2006). In recent years, many types of chemical agents have been applied to control *Bgt* infection; however, such agents cause serious environmental problems. Therefore, cultivating disease-resistant varieties is an urgent and significant undertaking because it will reduce the need for chemical agents that damage the environment and increase crop yields.

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Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* ($2n = 4x = 28$; genome AABB), the progenitor of domesticated tetraploid durum wheat (*Triticum turgidum* ssp. *durum*) and hexaploid (*Triticum aestivum* L., $2n = 6x = 42$; AABBDD) bread wheat (Feldman 2001), harbors abundant genetic diversity that is related to resistance to powdery mildew (Moseman et al. 1984). Approximately 78 powdery mildew resistance genes have been mapped to specific chromosomes and chromosomal regions, which have been designated *Pm1–Pm54* (Zhang et al. 2010; McIntosh et al. 2011; Xiao et al. 2013; Mohler et al. 2013). Eleven powdery mildew resistance genes have been identified in wild emmer: *Pm16* (Reader and Miller 1991), *Pm26* (Rong et al. 2000), *Pm30* (Liu et al. 2002), *MlZec1* (Mohler et al. 2005), *MlIW72* (Ji et al. 2008), *Pm36* (Blanco et al. 2008), *Pm41* (Li et al. 2009), *Pm42* (Hua et al. 2009), *PmG16* (Ben-David et al. 2010), *Ml3D232* (Zhang et al. 2010), and *MlIW170* (Liu et al. 2012).

Over the past decade, molecular markers have accelerated the discovery of new powdery mildew genes and facilitated their utilization in breeding programs. Molecular markers, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), sequence tagged sites (STS), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs), have been used to identify and map powdery mildew resistance genes. SSRs are powerful tools for genetic mapping and marker-assisted selection of disease resistance genes because of their even chromosomal distribution and the development of high-density wheat SSR maps (Röder et al. 1998; Somers et al. 2004; Song et al. 2005; http://wheat.pw.usda.gov).

In the present study, we report (1) the identification and genetic mapping of a novel powdery mildew resistance gene, designated *MLIW30*, derived from wild emmer wheat IW30, as well as (2) the development of PCR-based markers suitable for marker-assisted selection (MAS) in wheat breeding programs.

Materials and methods

Plant materials

F_1 hybrids, an F_2 segregating population, and 189 F_3 families derived from crossing of susceptible bread wheat cultivar Liaochun 10 and powdery mildew-resistant line 2L6 (a wild emmer wheat IW30 introgression line created by crossing of IW30/Zheng98//87-1*3) were used to test powdery mildew resistance and analyze genetic linkage. For fine mapping, a new independent F_2 segregating population ($n = 974$) and F_3 families (30 seedlings were used to test powdery mildew from each F_3 family to genotype the corresponding F_2 plants) were generated by crossing Liaochun 10 and 2L6. Common wheat lines Zheng 98, 87-1, and Liaochun 10 are highly susceptible to local prevailing *Bgt* isolate E09 at the seedling stage. *Bgt* isolate E09 is virulent to *Pm1*, *Pm3a*, *Pm3c*, *Pm5*, *Pm7*, *Pm8*, *Pm17*, *and Pm19* (Zhou et al. 2005). 2L6 showed moderate resistance to *Bgt* isolate E09 in the seedling and adult stages. Wild emmer wheat IW30, kindly provided by Drs. T. Fahima and E. Nevo, University of Haifa, Israel, is immune to E09 (kindly donated by Dr. Xiayu Duan, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, China).

Six French cultivars (Apache, Isengrain, Soisson, Crousty, Victo, Cracklin), four Hungarian cultivars (Mv14-85, Gk Cipó, GK Délibáb, Mv18), four Japanese cultivars (Asakaseko komugi, Hokushin, Haruminon, Daichino Minori), three Polish cultivars (Smuga, Bogatka, Fineya), ten Chinese cultivars (Zhongmai 302, Huapei 6, Luomai 23, Jingdong 8, Zhoumai 22, Nongda 3205, Linfen 138, Yangmai 158, Shannong 229, Gaocheng 8901), four American cultivars (TAM202, Jagger, Karl, Custer), three Canadian cultivars (Laura, Olaeta Calandria, Wild cat), one Mexican cultivar (Bajio), and one Argentinian cultivar (Calidad Victoria) were used to validate markers linked to the powdery mildew resistance gene *MLIW30*.

Chinese Spring (CS) and its selected nullisomic-tetrasomic and deletion lines of homeologous group 4, kindly provided by Drs. W. J. Raupp and Dr. B. S. Gill (Wheat Genetics Resource Centre, Kansas State University, Manhattan, KS, USA), were used for chromosomal assignment of the molecular markers.

Powdery mildew evaluation

Powdery mildew testing was conducted in a greenhouse in the spring of 2013 and 2015. F_2 descendants and F_3 families tested to confirm the phenotypes and to establish the resistance genotype of each F_2 plant, as

well as susceptible control cultivar Xuezao, were planted in rectangular pots (60 × 40 cm) with 5 rows of 20 plants in each pot. Seedlings were inoculated with *Bgt* E09 when the first leaf was fully expanded. Infection types (IT) were scored 15 days after inoculation when the susceptible control was heavily infected. E09 infection was classified on a scale of 0–4 according to the scale of Liu et al. (1999): "0" for no visible symptoms or the presence of necrotic flecks; "1" for high resistance (necrosis with low sporulation); "2" for resistance (necrosis with medium sporulation); "3" for susceptible (no necrosis with medium to high sporulation); "4" for highly susceptible (no necrosis with full sporulation). Phenotypes were pooled into two groups: resistant (R, IT 0–2) and susceptible (S, IT 3–4).

Molecular marker analysis

After the powdery mildew test, the leaves of individual F_2 seedling plants were collected for the extraction of genomic DNA. On the basis of bulked segregant analysis (BSA), DNA bulks were constructed by separately combining equal amounts of DNA from ten homozygous resistant and ten homozygous susceptible $F_{2:3}$ families (Michelmore et al. 1991). Wheat SSRs (*Xbarc*, *Xgwm*, *Xwmc*, *Xgpw*, and *Xcfd* series) covering the A and B genomes were chosen from a consensus map (Somers et al. 2004) for polymorphism analyses of parents and BSA screening. The resulting polymorphic markers were used for analysis of the F_2 populations and resistance gene mapping.

PCR amplification was conducted in a 10-μl reaction volume consisting of 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μl of l M dNTPs, 20 ng of each primer, 50 ng genomic DNA, and 0.75 U Taq DNA polymerase (Takara Taq, Takara Bio). The PCR conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 40 s, 50–60 °C (depending on the specific SSR primers; see Table 1) for 40 s, and 72 °C for 70 s; and a final extension at 72 °C for 10 min. Each PCR product was mixed with 2.5–3.0 μl loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, and 0.25 % xylene cyanol) and separated in 8 % non-denaturing polyacrylamide gels (acrylamide:bisacrylamide, 39:1), which were silver-stained and photographed.

Table 1 SSR primers and EST-STS primer generated from SNP sequences and homologous CDSs of *Brachypodium*

Primer	Source	Type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)	Product size of 2L6 (bp)	Product size of Liaochun 10 (bp)
XRF345.2	RFL_Contig2512_345	SSR	GTTGTGCTTATCTGCTCTTCTG	TTGTCCCTTTCAAGCCG	55	179	0
XBs281.1	BS00110281_51	SSR	ACAAGTGGTCTTCGTGCTC	CATCTTTCTTTCCCGCCT	56	259	0
XRAC714.2	RAC875_c8714_1139	SSR	TAATCGGGAAGGAAGGC	CAGAATGCCCAATGAATC	52	195	0
XGE923	GENE-4923_84	SSR	ATCCCGATTGCCTCAT	CAGTGGACAGGTAGTTTCG	53	210	180
XKu972.1	Kukri_c13972_795	SSR	TCCCATACATAACTTGCCG	ACGAAGAGCCCGCTTACCT	58	0	163
XBs758.2	BS00110758_51	SSR	AAGGTCAGCATCTCGTCG	TCTCGTTCCCACCACCAT	56	0	163
XTd712.2	Tdurum_contig8712_129	SSR	AATACAGAAACCCTGGGAAT	CTGGTGCCCTCAAGTTG	55	129	0
XB1g50220.1	Bradi1g50220.1	SSR	TTTGCGAGTTCATTCACC	GATGGTATTCTTCGTCTGTAGG	55	0	531
XB1g51880.2	Bradi1g51880.1	SSR	CCGGAAGAGTCGATGGGTTA	AGGTGGACTGGAAACGAAGA	60	201	0
XB1g2020.2	Bradi1g52020.1	SSR	TGTTGTGTGTGTGTGAGA	GAGAAGAGGAGAGGAGAGGAT	58	150	0
XB1g2000.2	Bradi1g52000.1	SSR	TGTCCGAGCAAATACCCT	CAGCCAAGTGGTGTTTCAT	55	328	308
XB1g060.2	Bradi1g52060.1	SSR	TGCCCTCGTCTTTCTCTGT	CGTCCTCCATCCCATCTAT	58	180	170

Table 1 continued

Primer	Source	Type	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (°C)	Product size of 2L6 (bp)	Product size of Liaochun 10 (bp)
XB1g2070.1	Bradi1g52070.1	SSR	TTTCTCCTTCTCCCGCCA	CACTGCCCTCGTCTTTCT	56	204	193
XB1g2180.1	Bradi1g52180.1	SSR	TCATCCTATCCCGTTCTGCC	GCTGATTGTTGGCTGCTTCT	60	224	248
XB1g52230.1	Bradi1g52230.1	SSR	TGCCGAGAATGATGAATG	GTGTGATTGTATTTCCTTGCTC	54	159	0
XBE1.1	Bradi1g52030.1	EST-STS	TGCCTAAAGACCCAAATGC	TGTCACAAGAAACACACTGGAG	57	0	465

Single-nucleotide polymorphism (SNP) genotyping using the Illumina 90 k iSelect SNP chip and primer design

We constructed resistant and susceptible DNA bulks according to BSA for SNP genotyping using the Illumina 90 k iSelect SNP chip. Differences in the SNP profiles of resistant and susceptible DNA bulks were analyzed. Polymorphic SNPs were mapped to chromosomes according to Wang et al. 2014.

The sequences of SNPs mapped to chromosome 4A were used to search the IWGSC survey sequences (International Wheat Genome Sequencing Consortium IWGSC; http://www.wheatgenome.org/) to find homologous contig or scaffold sequences on chromosome 4AL (Wang et al.2014). SSR primers based on the obtained contig or scaffold sequences were designed with BatchPrimer3 (http://probes.pw.usda.gov/batchprimer3/) and used to detect polymorphisms in resistant and susceptible plants (You et al. 2008). In cases where SSR primers were not available or showed no polymorphisms between resistant and susceptible DNA bulks, the contig or scaffold sequences were used to design STS (sequence-tagged site) primers. SNP sequences were used as Basic Local Alignment Search Tool (BLAST) queries for 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. Subsequently, the collinearity of *Brachypodium*, rice, and sorghum was analyzed. The coding DNA sequences (CDS) of *Brachypodium* were used to blast the IWGSC survey sequences (http://www.wheatgenome.org/) to find homologous contig or scaffold sequences on chromosome 4AL. We designed SSR primers and EST-STS primers according to the obtained sequences.

Chromosome arm assignment and physical mapping

The use of CS wheat and nullisomic-tetrasomic and deletion lines of homeologous group 4 enabled markers to be assigned to chromosomal locations at which genes conferring powdery mildew resistance were physically located.

Statistical analysis

Chi-squared (χ^2) tests were used to determine the goodness-of-fit of observed data with expected segregation ratios. Linkages between molecular markers and resistance genes in 189 F_2 population plants and 974 F_2 population plants were estimated separately using Mapmaker 3.0 with the LOD threshold score set at 3.0 (Lincoln et al. 1992) and Joinmap (Stam and Van Ooijen 1995). The genetic map was drawn using Mapdraw 2.1 software (Liu and Meng 2003).

Results

Inheritance of powdery mildew resistance derived from IW30 wild emmer wheat

Following inoculation resistant line 2L6, F_1 hybrids and 189 F_3 families with E09 expressed moderate resistance (IT of 1–2), whereas susceptible cultivars Liaochun 10 and 87-1 showed high susceptibility (IT of 4) (Fig. 1). The partially infected leaves of the 2L6, F1 hybrids, and resistant plants were dead and dry by necrosis with the extension of infection time, whereas susceptible plants were alive and heavily sporulating. The resistance of the F_1 hybrids was similar to that of 2L6, indicating that resistance was dominant. Segregation analysis of the F_3 families exhibited a homozygous resistant (RR): segregating (Rr): homozygous susceptible (rr) ratio of 42:94:53, coinciding with the expected 1:2:1 genotypic ratio ($\chi^2_{1:2:1} = 1.28$, $P > 0.05$). These results demonstrate that powdery mildew resistance was controlled by a single dominant gene, temporarily designated *MLIW30*.

The new F_2 population and $F_{2:3}$ families ($n = 974$) were inoculated with *Bgt* isolate E09, resulting in the identification of 250 homozygous resistant, 485 segregating, and 239 homozygous susceptible ($\chi^2_{1:2:1} = 0.26$, $P > 0.05$) plants in accordance with the expected 1:2:1 proportion of Mendel's segregation ratio.

Fig. 1 Phenotype of resistant parent 2L6, susceptible parent Liaochun 10, and 87-1 15 days after inoculation with *Bgt* isolate E09

Molecular mapping of the powdery mildew resistance gene

A total of 344 wheat SSR markers (barc, gwm, wmc, gpw, and cfd) mapped onto the A and B chromosomes were used to identify polymorphisms in the parental lines (2L6 and Liaochun 10) and the resistant and susceptible DNA bulks. Four SSR markers, *Xgpw356*, *Xbarc78*, *Xgwm160*, and *Xgwm350*, were linked to the powdery mildew gene (Fig. S1a) and mapped close to *MLIW30* by 189 F_2 segregating population plants (Fig. S2). Among these markers, only *Xbarc78* was co-dominant, whereas the others were dominant.

To saturate the genetic map containing *MLIW30*, 28 STS primers were designed and tested on the basis of wheat ESTs located in regions of chromosome 4AL. However, only one STS, *XBQ2* from EST BQ169038 (forward primer, 5′-GCCCTCACTGTTCTGTC-3′; reverse primer, 5′-ACCTCTCCACCACCATC-3′, 692 and 764 bp in parents 2L6 and Liaochun 10), which was polymorphic between the two parents (2L6 and Liaochun10) and between the DNA bulks, was linked to *MLIW30* as a co-dominant marker and mapped 2.8 cM from *MLIW30* (Fig. S2).

SNP genotyping and comparative genomics analysis

The analysis showed that 378 SNPs were polymorphic between the resistant and susceptible DNA bulks, of which 158 were mapped onto chromosome 4A (Fig. S3). Fifty-four adjacent SNPs were chosen from 158 SNPs by considering the genetic location of the polymorphic SNPs in the consensus SNP genetic linkage map and used to search the IWGSC survey sequences (IWGSC; http://www.wheatgenome.org/) to find homologous contig or scaffold sequences on chromosome 4AL, which were used to design SSR primers with BatchPrimer3 (http://probes.pw.usda.gov/batchprimer3/). Seven SSR primers, *XRF345.2*, *XBs281.1*, *XRAC714.2*, *XGE923*, *XKu972.1*, *XBs758.2*, and *XTd712.2*, were polymorphic between the resistant and susceptible DNA bulks (Table 1). The resistance gene *MLIW30* was flanked by *XRF345.2* (derived from the probe sequence of SNP

RFL_Contig2512_345) and *XBs281.1* (derived from SNP BS00110281_51).

In order to develop new primers, we referred to orthologous genes uniquely tagged in *Brachypodium*, rice, and sorghum by using the SNP probe sequences as BLAST queries (Wang et al. 2014). Orthologous genomic regions of the SNP probe sequences were identified on *Brachypodium* chromosome 1, rice chromosome 6, and sorghum chromosome 10. The collinearity region on *Brachypodium* chromosome 1 was limited to a region extending from *Bradi1g50220.1* to *Bradi1g52230.1*. We exploited the CDSs of collinear *Brachypodium* genes to search the IWGSC survey sequences (IWGSC; http://www.wheatgenome.org/) for homologous contig or scaffold sequences on chromosome 4AL, which were used to design SSR primers or EST-STS primers. Finally, we found that eight SSR markers, *XB1g50220.1*, *XB1g51880.2*, *XB1g2020.2*, *XB1g2000.2*, *XB1g060.2*, *XB1g2070.1*, *XB1g2180.1*, and *XB1g52230.1*, as well as one EST-STS marker, *XBE1.1*, were polymorphic between the resistant and susceptible DNA bulks (Fig. 2b–d; Table 1).

Linkage analysis of the 974 F_2 plants showed that 15 SSR markers, *XRF345.2*, *XB1g50220.1*, *XB1g51880.2*, *XB1g2020.2*, *XB1g2000.2*, *XB1g060.2*, *XB1g2070.1*, *XBs281.1*, *XB1g2180.1*, *XB1g52230.1*, *XRAC714.2*, *XGE923*, *XKu972.1*, *XBs758.2*, and *XTd712.2*, as well as EST-STS marker *XBE1.1*, were closely linked with *MLIW30* and allowed us to establish a linkage map (Table 1). The nearest makers were *XB1g2020.2* and *XB1g2000.2*, oppositely flanking *MLIW30* at a distance 0.1 cM (Fig. 2).

Physical bin mapping of powdery mildew resistance gene *MLIW30*

SSR *Xbarc78* was previously located on the long arm of chromosome 4A (Xue et al. 2008; http://wheat.pw.usda.gov); therefore, *MLIW30* was putatively mapped onto the same chromosome arm. Chinese Spring and its corresponding nullisomic-tetrasomic and deletion lines of homeologous group 4 were used to determine the chromosomal location of the SSR markers linked to *MLIW30*. *Xbarc78, XB1g2000.2*, and *XB1g060.2* were not detected in the PCR products of N4A-T4D, 4AL-4, 4AL-5, 4AL-12, and 4AL-13 (Fig. 3), indicating that *MLIW30* was located in terminal chromosome bin 4AL-0.8-1.00.

Marker assessment

In order to validate the usefulness of the markers in breeding, four markers *XB1g51880.2*, *XB1g2020.2*, *XB1g2000.2*, and *XB1g060.2*, which were nearest to *MLIW30*, were tested in six French cultivars, four Hungarian cultivars, four Japanese cultivars, three Polish cultivars, ten Chinese cultivars, four American cultivars, three Canadian cultivars, one Mexican cultivar, and one Argentinian cultivar. We found that the PCR product size of *XB1g2000.2* and *XB1g060.2* in 2L6 containing *MLIW30* was unique and not detected in the other cultivars; therefore, *XB1g2000.2* and *XB1g060.2* (Fig. 4) are useful as diagnostic markers in marker-assisted selection of powdery mildew resistance gene *MLIW30*. However, the same lengths of fragments of *XB1g51880.2* and *XB1g2020.2* in 2L6 were also found in other cultivars (Fig. 4). The specific marker of *XB1g51880.2* in 2L6 was equal to that of three French cultivars, one Japanese cultivar, three Polish cultivars, one Chinese cultivar, two American cultivars, two Canadian cultivars, and one Argentinian cultivar. The marker of *XB1g2020.2* in 2L6 was the same size as in four Hungarian cultivars, one Japanese cultivar, four Chinese cultivars, one American cultivar, and one Argentinian cultivar. Therefore, we should be cautious about the use of *XB1g51880.2* and *XB1g2020.2* in practical breeding.

Discussion

Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, is distributed in Israel, Jordan, Lebanon, Syria, eastern Turkey, northern Iraq, and western Iran (Ozkan et al. 2011). It harbors abundant genetic resources that can be utilized for wheat improvement and are related to abiotic stress tolerance (salt, drought, and heat), biotic stress tolerance (powdery mildew, rusts, and Fusarium head blight), grain protein quality and quantity, and micronutrient (Zn, Fe, and Mn) concentrations (Chen et al. 2015; Shavrukov et al. 2010; Yaniv et al. 2015). Therefore, genetic studies of wild emmer wheat are beneficial for wheat breeding and cultivation (Xie and Nevo 2008).

Until now, 11 powdery mildew resistance genes have been identified in or derived from wild emmer

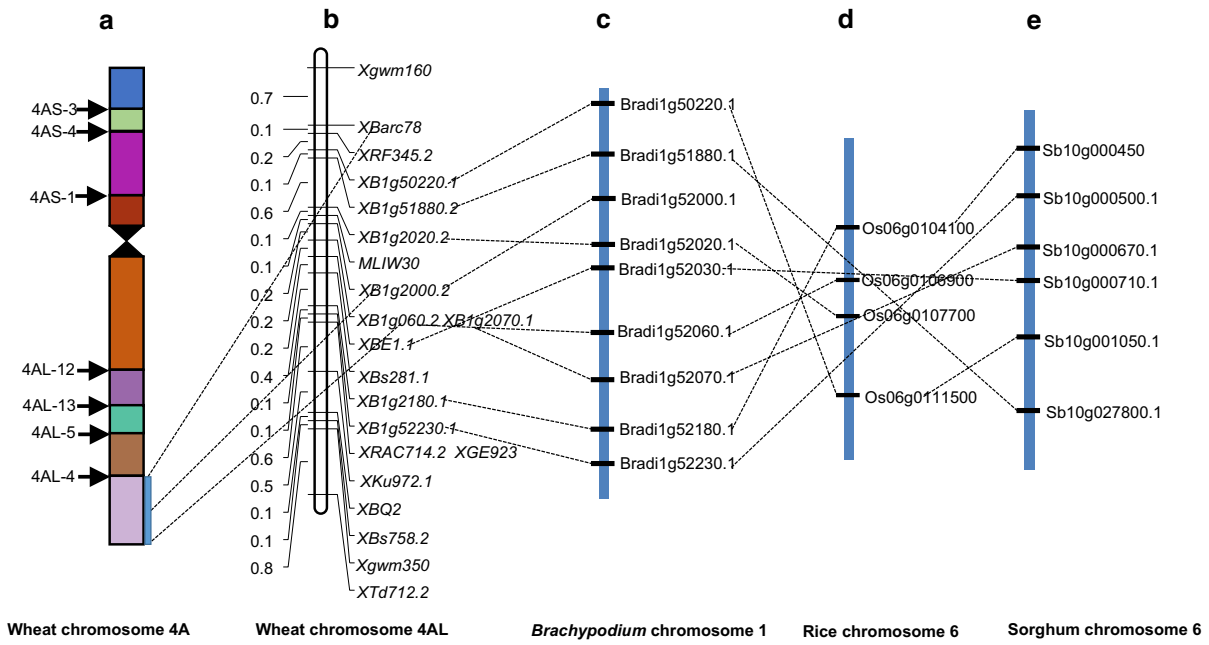

Fig. 2 Comparative genetic linkage maps of powdery mildew resistance gene *MLIW30*. **a** Physical bin map of *MLIW30*. *MLIW30* was mapped to distal bin 4AL-4-0.8-1.00. **b** Genetic linkage map of *MLIW30*. **c** Genetic linkage map of the *MLIW30* orthologous genomic region on chromosome 1 of *Brachypodium*. **d** Orthologous genomic region of *MLIW30* on rice chromosome 6. **e** Orthologous genomic region of *MLIW30* on sorghum chromosome 6

wheat. Powdery mildew resistance genes *Pm26, Pm42,* and *MlIW170* were mapped onto chromosome 2BS (Rong et al. 2000; Hua et al. 2009; Liu et al. 2012). *Pm36*, *Ml3D232*, and *PmAS846* were located in chromosome 5BL (Blanco et al. 2008; Zhang et al. 2010; Xue et al. 2012). Two genes, *MlIW72* and *PmG16*, were mapped to chromosome 7AL (Ji et al. 2008; Ben-David et al. 2010). *Pm30*, *Pm41*, and *MlZec1* were located on chromosomes 5BS, 3BL, and 2BL, respectively (Liu et al. 2002; Li et al. 2009; Mohler et al. 2005). *Pm16* was originally mapped onto chromosome 4A (Reader and Miller 1991). Chen et al. (2005) located *Pm16* on the short chromosome of 5B using molecular markers. Because no powdery mildew resistance gene in or derived from wild emmer wheat has been identified on wheat chromosome 4AL, *MLIW30* might be a novel *Pm* gene.

Several powdery mildew resistance genes/QTLs have been identified on the long arm of wheat chromosome 4A, including a minor QTL *QPm.osu-4A*, which is identified in US hard winter wheat cultivar 2174 and localized on chromosome 4A (Chen et al. 2009). However, *QPm.osu-4A* is only detected in the field. According to Hao et al. 2015, *QPm.uga-4A* was detected in chromosome 4AS from soft red winter wheat AGS 2000 and likely represented adult plant resistance. Other minor QTLs were detected on chromosome 4A in the French wheat cultivar 'Courtot,' French wheat line 'RE714,' and Swiss wheat cultivar 'Forno,' all effective as adult plant resistance to powdery mildew (Bougot et al. 2006; Chantret et al. 2001; Keller et al. 1999; Mingeot et al. 2002). Because *MLIW30* was effective at both seedling and adult plant stages, we think that *MLIW30* is different from these 4A-located *QPm*s. A major non-race-specific powdery mildew resistance QTL *QPm.tut-4A* was identified in a wheat–*T. militinae* introgression line (Jakobson et al. 2006, 2012). *T. militinae* is a tetraploid wheat (A^tA^tGG) of the timopheevii group (Dorofeyev et al. 1976). *QPm.tut-4A* and *MLIW30* were mapped to the same region of chromosome 4AL with the shared common marker *Xgwm160*. *QPm.tut-4A* confers partial resistance to powdery mildew in a manner similar to that of *MLIW30*, which is incomplete resistant rather than immune to powdery mildew. However, *QPm.tut-4A* was located to loci proximal to *Xgwm160*, whereas *MLIW30* was distal to *Xgwm160*. We think

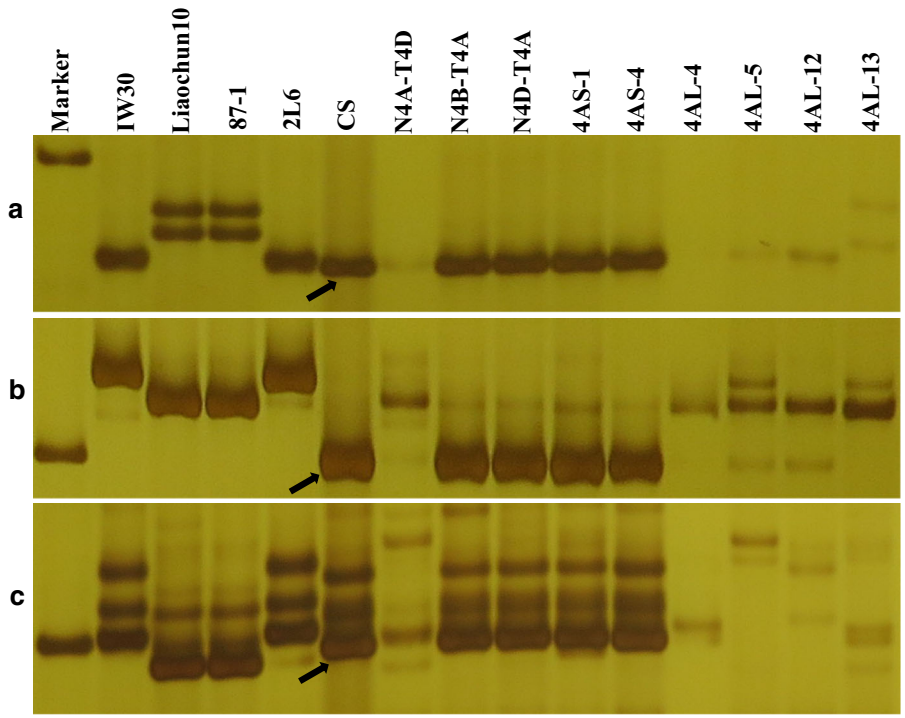

Fig. 3 Amplification patterns of markers *Xbarc78* (**a**), *XB1g2000.2* (**b**), and *XB1g060.2* (**c**) in parental lines 2L6 and Liaochun10, IW30, 87-1, Chinese Spring (CS), and CS homologous group 4 nulli-tetrasomic and deletion lines. The *arrow* indicates the specific amplification in CS

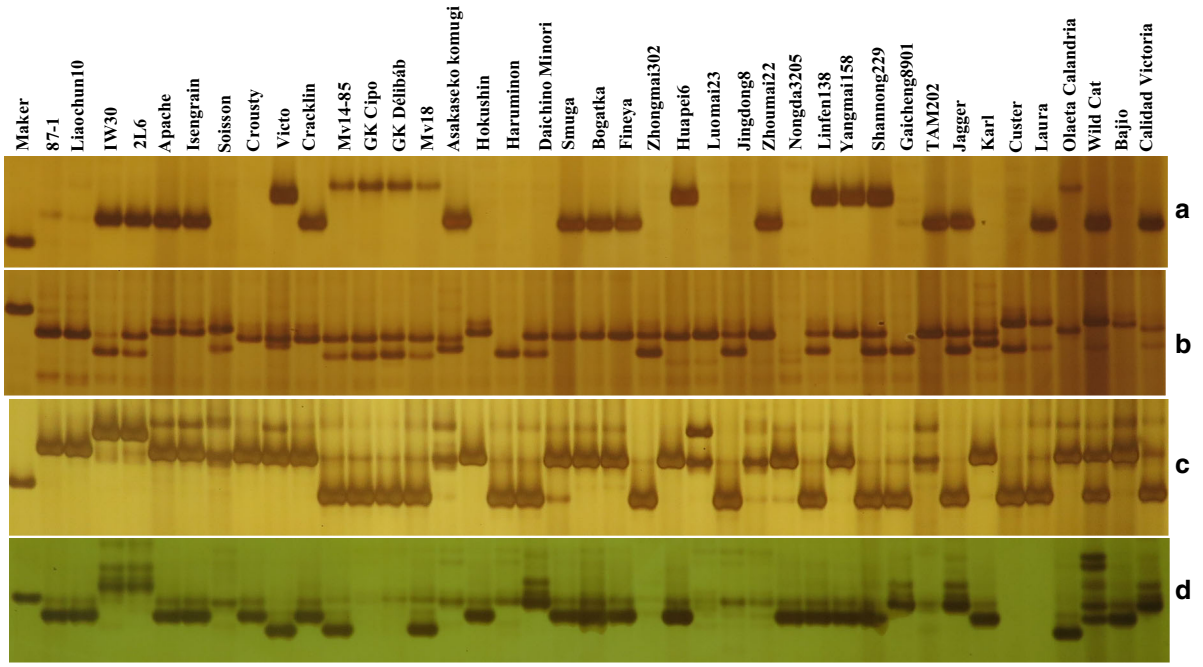

Fig. 4 SSR markers *XB1g51880.2* (**a**), *XB1g2020.2* (**b**), *XB1g2000.2* (**c**), and *XB1g060.2* (**d**) in parental lines 2L6 and Liaochun10, IW30, 87-1, and 36 cultivars from different countries (M, 100-bp DNA ladder)

MLIW30 and *QPm.tut-4A* are at different loci. Still we cannot exclude the possibility that these two genes were from the same locus, but mapped differently because of the impreciseness of genetic analysis of wide hybridization of *T. aestivum* (AABBDD) with *T. militinae*. (A^tA^tGG) or *T. diccoides* (AABB).

MLIW30 was located in terminal chromosome bin 4AL-0.8-1.00. Many studies have confirmed that the distal region of chromosome arm 4AL was translocated from chromosome arm 7BS during the evolution of *T. turgidum* and *T. aestivum* (Hernandez et al. 2012; Hossain et al. 2004; Ishikawa et al. 2009; Miftahudin et al. 2004). This means that the true homeologous region of the *MLIW30* locus is on chromosome arms 7AS and 7DS. For example, three wheat homeologous *Wx* genes, *Wx-A1*, *Wx-B1*, and *Wx-D1*, were located on chromosome arms 7AS, 4AL, and 7DS, respectively (Miura and Sugawara 1996). To date, no powdery mildew resistance gene has been identified on chromosome arm 7AS. Six wheat *Pm* resistance genes on chromosome arm 7DS were previously reported: *Pm15*, *Pm19*, *Pm29*, *Pm38*, *MlNCD1*, and *PmAF7DS* (Lutz et al. 1995; Maxwell et al. 2012; Reddy et al. 2016; Spielmeyer et al. 2005; Tosa and Sakai 1990; Zeller et al. 2002). According to Reddy et al. (2016), these genes were different. *MLIW30* might be a homeologous allele to one of these genes or represent a novel homeolocus.

High-density SNP genotyping chips are an effective tool for analyzing genomic polymorphisms (Barker and Edwards 2009). The Illumina 90 k iSelect SNP chip can directly assess polymorphic SNPs to develop SSR or EST-STS molecular markers by genotyping resistant and susceptible DNA bulks. In the present study, 378 SNPs were found to be polymorphic between the resistant and susceptible DNA bulks. According to the consensus SNP genetic linkage map provided by Wang et al. (2014), 158 SNPs were mapped to chromosome 4A, mainly from 111.274351CM to 164.1295399CM. The information of the probe sequences of these identified SNPs in chromosome 4A provided guidance for the development of new markers using comparative genomics.

In this study, a dominant powdery mildew resistance gene, *MLIW30*, was identified in wild emmer wheat IW30. Genetic analysis and molecular mapping demonstrated that *MLIW30* was located on the distal region of chromosome arm 4AL and flanked by markers *XB1g2020.2* and *XB1g2000.2*. Nineteen SSR markers and two STS markers were found to be linked to *MLIW30* with the closest markers only 0.1 cM from *MLIW30*. These markers are suitable for studies using marker-assisted selection to improve powdery mildew resistance in wheat.

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