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ORIGINAL PAPER

Genetic mapping of two powdery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions

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Abstract Powdery mildew is a severe foliar disease for wheat and could cause great yield loss in epidemic years. To explore new powdery mildew resistance genes, two einkorn accessions including TA2033 and M80, both resistant to this disease, were studied for the inheritance of resistance. Each accession possessed a single but different dominant resistance gene that was designated as *Mlm2033* and *Mlm80*, respectively. Marker mapping indicated that they are both linked to *Xgwm344* on the long arm of chromosome 7A. To establish their genetic relationship with *Pm1* on 7AL, five RFLP markers previously reported to co-segregate with *Pm1a* were converted to STS markers. Three of them detected polymorphism between the mapping parents and were mapped close to *Mlm2033* or *Mlm80* or both. *Xmag2185*, the locus determined by the STS marker derived from PSR680, one of the RFLP markers, was placed less than 2 cM away from them. The allelism test indicated that *Mlm2033* and *Mlm80* are likely allelic to each other. In addition, through comparative and EST mapping, more markers linked to these two genes were identified. The high density mapping of *Mlm2033* and *Mlm80* will contribute to map-based cloning of the *Pm1* locus. The markers for both genes will also facilitate their transfer to wheat.

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Introduction

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* (Bgt), is one of the most widespread wheat (*Triticum aestivum* L.) foliar diseases worldwide, which competes for nutrients and reduces the photosynthetic ability of the leaves. The application of high-yielding dwarf varieties as well as the improved irrigation and fertilization conditions has further increased the threat of this disease. Severe epidemics often occur in cool and humid climates and cause up to 30% or even higher yield loss. Even though cultivation measures and application of fungicides can alleviate the yield loss, deployment of resistant varieties is the preferred choice for the disease control because of its cost savings and environmental friendliness.

Two kinds of powdery mildew resistance, i.e., qualitative and quantitative resistance, have been reported in wheat. The qualitative resistance, controlled by single genes, is most widely employed in wheat breeding programs. Up to now, 49 powdery mildew resistance alleles mapped at 33 loci, including three recessive genes (*Pm5*, *Pm9* and *Pm26*), have been identified (McIntosh et al. 2003, 2004, 2005), most of which show race-specific resistance. Among these genes, *Pm2*, *Pm4a*, *Pm4b*, *Pm5*, *Pm6*, and *Pm8* were commonly used in China (Duan et al. 2002). Race-specific resistance could be overcome in a short period

of time when extensively used in production because of the co-evolution of host resistance and pathogen virulence (McDonald and Linde 2002). For example, virulent Bgt strains have been found for all the commonly used resistance genes (Niewoehner and Leath 1998; Clarkson 2000; Duan et al. 2002). *Pm8* has been overcome by nearly all the races in China and elsewhere (Duan et al. 2002; Clarkson 2000). Thus, the discovery and utilization of new powdery mildew resistance genes have been a long-term objective for wheat geneticists and breeders.

Many of the powdery mildew resistance genes are from either diploid wheat, such as *Pm1b* and *Pm25*, or from tetraploid wheat, such as *Pm3h*, *Pm4a*, *Pm4b*, *Pm5a*, *Pm6*, *Pm16*, *Pm26*, *Pm27*, *Pm30*, *Pm31* and *Pm33* (McIntosh et al. 2003, 2004, 2005). Recently, Srnic et al. (2005) reported the identification of two dominant resistance genes from *Triticum monococcum* L. and *Triticum timopheevii* L. Relative to more distant *Triticeae* species, it is easier to transfer genes from these ancestor species of wheat, which, therefore, have been extensively studied for enrichment of genes useful for the wheat improvement.

The advent of molecular markers in the early 1980s has greatly stimulated researches in genetics and breeding. For exploring alien germplasms, a marker-based approach could be more effective and rapid in identifying and transferring useful genes (Tanksley and McCouch 1997). Currently, molecular markers for more than 29 powdery mildew resistance genes have been reported (McIntosh et al. 2003, 2004, 2005; Srnic et al. 2005). Some of these markers have been successfully used in map-based cloning (Yahiaoui et al. 2004), marker-assisted selection and pyramiding of the resistance genes (Liu et al. 2000) as well as understanding the relationship between different genes (Singrün et al. 2003).

We have identified a number of diploid wheat accessions resistant to powdery mildew. In this study, genetic analysis and molecular marker mapping were conducted to explore for new powdery mildew resistance genes in two of these germplasms. Two powdery mildew resistance genes were found and mapped to chromosome 7A.

Materials and methods

Plant materials

Triticum monococcum accessions TA2033, TA2721, TA2716, TA2725 and TA2025 were introduced from the Wheat Germplasm Resource Center at Kansas State University courtesy of Dr. B. S. Gill. Also included in this research were *Triticum boeoticum* L. accession M80 and *T. monococcum* accession M389. TA2033 and M80 were resistant to powdery mildew disease. The crosses of TA2721 × TA2033, M389*2/TA2033, TA2033 × TA2716, TA2725 × M80 and M80 × TA2025 were made for genetic analysis and mapping. The cross of TA2033 with M80 was made for the allelism test.

Resistance evaluation

F_2 populations derived from TA2721 × TA2033, TA2725 × M80, M80 × TA2025, TA2033 × M80, and the BC_1 population from M389*2/TA2033 were evaluated using the powdery mildew isolate Bgt19. The F_2 population derived from TA2033 × TA2716 was evaluated using the local field Bgt composite collected from Nanjing, Jiangsu. Thirty to forty-four plants of each of the parents were included in the evaluation. The F_3 progeny test was conducted using Bgt19 for the crosses of TA2721 × TA2033, TA2725 × M80 and M80 × TA2025 to confirm the F_2 plant phenotypes or determine the genotypes. All plants were inoculated at the one-leaf stage in the greenhouse with Sumai No. 3 and the susceptible parent of each cross as the susceptible control and the resistant parent as the resistant control.

Data were collected 7d after inoculation when the susceptible controls showed distinct disease symptoms and the resistant controls were still clear from the disease. Resistance performance was scored on a 0–5 scale, representing no visible symptom, necrosis without sporulation, sparse sporulation, moderate sporulation, abundant sporulation, and abundant sporulation with more than 80% of the leaf area covered with mycelia, respectively. Score of 0–2 was viewed as resistant and 3–5 as susceptible.

Marker analysis

DNA was extracted from young seedling tissues following the procedures described by Ma et al. (1994). For bulked segregant analysis, two DNA bulks were prepared for each of the two F_2 populations derived from TA2721 × TA2033 and TA2725 × M80 by combining equal amounts of DNA from six resistant or six susceptible F_2 plants. All the plants used in the bulks but three that were dead after the tissue was collected were progeny-tested. The susceptible plants did not segregate in resistance in their F_3 progenies, but some of the resistant plants did.

Fifty-four SSR markers chosen from gwm series (Röder et al. 1998), cfa and cfd series (Guyomarc'h

et al. 2002; Sourdille et al. 2003) and barc and wmc series (http://www.wheat.pw.usda.gov), including 53 mapped to the A genome of bread wheat and gwm344, the one closely linked to *Pm1e* on chromosome 7AL (Singrün et al. 2003), were used for the marker analysis. The PCR was performed in a PE9600 thermal cycler (Perkin Elmer, Norwalk, CT, USA) or PTC-225 thermal cycler (MJ research) in a volume of 10 μl containing 10–20 ng of template, 2 pmol of each of the primers, 2 nmol of each of the dNTPs, 15 nmol of MgCl2, 0.1 U Taq DNA polymerase and 1 × PCR buffer. The PCR profile was as follows: one cycle of 94°C 3 min, followed by 35 cycles of 94°C 30 s, 50–60°C (depending on the specific primers) 30 s and 72°C 50 s, and a final extension at 72°C for 5 min. The PCR products were separated in 8% non-denaturing polyacrylamide gels with a 19:1 or 25:1 acrylamide/bisacrylamide ratio, and then silver-stained as described by Santos et al. (1993).

To saturate the regions carrying the powdery mildew resistance genes with molecular markers, five RFLP markers linked to *Pm1a* (Ma et al. 1994; Neu et al. 2002), including C607, PSR121, PSR148, CDO347 and PSR680, were converted to sequence-tagged site (STS) markers MAG1702, MAG1704, MAG1714, MAG2166 and MAG2185, respectively. The sequences for primer design were obtained through end-sequencing the RFLP probes or retrieved from the NCBI nucleotide database. Additional sequences for STS marker development came from wheat ESTs (expressed sequence tags) homologous ($E < 8 \times 10^{-8}$) to the coding DNA sequences (CDS) of the corresponding syntenic rice contig. To identify the syntenic rice PAC or BAC clones, sequences of CDO347, C607, PSR121, PSR148 and PSR680 were used in the BLASTn analysis against the rice sequences deposited in http://www.tigr.org. In addition, 14 ESTs mapped to the 0.86–1.0 interval of 7AL, 0.63–1.0 interval of 7BL and 0.61–1.0 interval of 7DL in the deletion bin map (Hossain et al. 2004) that were not represented in the hits in the rice–wheat homology search were also converted to STS markers. These ESTs include BE405003, BE405531, BQ171700, BE405507, BF200008, BF484317, BM140366, BE605194, BE498985, BE591497, BQ161734, BE403180, BE406627 and BE424236, among which the first seven code for resistance gene analogs (RGA), BE605194, BE498985 and BE591497 for putative protein kinases and BQ161734 for a putative plasma membrane protein family member. The STS markers corresponding to BE405003, BQ171700, BE591497 and BE405531 are MAG1705, MAG1708, MAG1754 and MAG1757, respectively.

All the STS primers were designed using MACVECTOR V8.0 (Accelrys, UK). The polymorphic survey was performed in the procedure similar to the SSR analysis mentioned above. A few STS markers key to this study were listed in Table 1. When the STS markers of interest detected no polymorphism between the parents, restriction enzymes *Afa*I, *Alu*I, *Mbo*I, *Msp*I, *Hae*III, *Hind*III, *Sal*I and *Taq*I (TaKaRa Bio. Co. Ltd., Dalian, China) were selectively employed in digesting the PCR products. Each digestion was performed according to the supplier's manual in a 5 μl reaction mix with 0.05 μg DNA. The digested products were separated in 8% non-denaturing polyacrylamide gels.

Linkage analysis

Recombination frequency in the allelism test was estimated using the formula $r = 2\sqrt{f/N}$, where N is the total number of plants and f is the number of susceptible plants. MAPMAKER Macintosh V2.0 (Lander et al. 1987) was used to establish the linkage map with the map distance calculated using the Kosambi function. A LOD score of 3.0 was used as the threshold for declaration of linkage. Difference in corresponding intervals between different maps was examined by the Z-test.

Results

Inheritance of the powdery mildew resistance in TA2033 and M80

TA2033 was immune to Bgt 19. Of 53 F_2 plants derived from TA2721 × TA2033, 38 were immune to Bgt19

Table 1 A few STS markers converted from RFLP markers co-segregating with *Pm1a* or developed based on ESTs

STS marker	Source	Forward primer	Reverse primer	Annealing temperature (°C)
MAG1714	PSR148	5′ACGAACTGTATCACAACACCCA3′	5′TAAGCCGAGCATGTAGATGGA3′	53
MAG2166	CDO347	5′ATTCTCGCAGCCATTTCG3′	5′CCAATACCTCCACGGTTACTG3′	53
MAG2185	PSR680	5′GCTCCACTACTTCATCATCC3″	5′TGTTTCCTCTATCACTGACTTG3′	53
MAG1757	BE405531	5′TGCGATTCCAAGCAACATTAGC3′	5′CGACAAGATAGTCACCTGGGAGAG3′	55
MAG1759	CD452874	5′GTACGAGTATGGCAACAGC3′	5′CCAACTCTAGGGGCAGATG3	51

and the remaining were susceptible with a score of 3–5. Among 64 plants of the BC_1 population from M389*2/TA2033, 31 were resistant. The resistance segregation in both populations fits either 3:1 or 1:1 ratio ($\chi^2 = 0.16$ and 0.02, $df = 1$, $P = 0.69$ and 0.89), indicating that a dominant gene controls the powdery mildew resistance in TA2033. Among 25 F_2 progenies from the first combination, five and seven were homozygous resistant and susceptible to Bgt19, indicating that these 25 F_2 plants fit a 1:2:1 ratio ($\chi^2 = 0.36$, $df = 2$, $P = 0.84$) in resistance segregation. The single dominant gene control of the powdery mildew resistance in TA2033 was further confirmed by the resistance segregation of 103 plants from three heterozygous F_2 derived from the cross of TA2033 with TA2716, in which 73 plants were resistant ($\chi^2_{3:1} = 0.73$, $df = 1$, $P = 0.39$). M389, TA2716 and TA2721 in these crosses were all susceptible with the score varied from 4 to 5. The resistance gene in TA2033 is temporarily named as *Mlm2033*.

M80 was resistant to Bgt19 with a score of 0–1; TA2725 and TA2025 were susceptible with a score of 4–5. In the F_2 populations from crosses of M80 with TA2725 and TA2025, 178 and 83 plants were resistant to Bgt19 with a score of 0–2, and 59 and 29 plants were susceptible with a score of 3–5, respectively, fitting the 3:1 ratio ($\chi^2 = 0.001$ and 0.01, $df = 1$, $P = 0.97$ and 0.92), indicating that the powdery mildew resistance in M80 is also controlled by a dominant gene, which was confirmed by progeny tests of 108 F_2 plants from the M80 × TA2025 combination, of which 26 and 25 were homozygous resistant and susceptible to Bgt19, fitting a 1:2:1 segregating ratio ($\chi^2 = 0.35$, $df = 2$, $P = 0.84$). This resistance gene was temporarily designated as *Mlm80*.

Chromosome assignment of *Mlm2033* and *Mlm80*

Initially, 37 SSR markers mapped to the A genome of bread wheat were surveyed for polymorphism between the parents and the bulks pooled from the F_2 plants derived from TA2721 × TA2033 and TA2725 × M80. One of them, GWM344 detected polymorphism between each pair of the parents and between the respective resistant and susceptible pools, with the polymorphic bands different in size in the resistant parents TA2033 and M80 (Fig. 1). Mapping using the F_2 data from TA2721 × TA2033 and M80 × TA2025 demonstrated that *Xgwm344* was closely linked to *Mlm2033* and *Mlm80*. The tight linkage of *Xgwm344* with *Mlm2033* was further confirmed using the populations from M389*2/TA2033 and the three F_3 families from TA2033 × TA2716. Since *Xgwm344* is closely

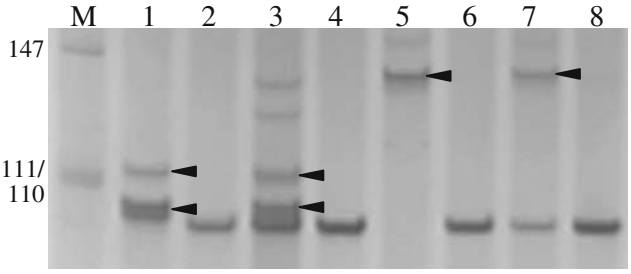

Fig. 1 PCR amplification of DNA from the mapping parents and bulks with GWM344. The *black solid triangles* indicate the polymorphic bands in the resistant parents and R pools that were mapped. M: pUC19/*Msp*I, to the *left* is the marker band size in bp. Lanes *1*, *2*, *3*, *4*, *5*, *6*, *7* and *8* represent TA2033, TA2721, R and S pools from (TA2721 × TA2033) F_2, M80, TA2725, R and S pools from (TA2725 × M80) F_2, respectively

linked to *Pm1e* on the long arm of chromosome 7A (Singrün et al. 2003), 17 more SSR markers mapped to 7AL were then surveyed, five of which, including WMC525, CFA2040, CFA2019, CFA2257 and GWM146, revealed polymorphism in at least one of the four populations. They were all mapped around *Mlm2033* and *Mlm80* (Fig. 2). Loci *Xcfa2040* and *Xgwm344* flanked both genes.

Relationship of *Pm1* with *Mlm2033* and *Mlm80*

Among the five pairs of STS primers converted from RFLP markers linked to *Pm1a*, MAG2166 detected polymorphism between M80 and TA2025 when the PCR products were digested with *Msp*I, MAG1714 detected a polymorphic locus between TA2033 and TA2716, and MAG2185 detected polymorphism between all the six parents examined (Fig. 3). *Xmag2166* co-segregated with *Xgwm344* in the corresponding population (Fig. 2d). *Xmag2185*, derived from RFLP probe PSR680, was the one most closely linked to the resistance genes (Fig. 2), suggesting that either *Mlm2033 and Mlm80* or both are likely allelic or tightly linked to *Pm1a*.

Identification of additional markers linked to *Mlm2033* and *Mlm80*

Twenty-two STS markers were developed from wheat ESTs that are homologous to the CDS in the rice chromosome 6L contig consisting of the seven PACs or BACs shown in Fig. 4. Four of them detected polymorphism between at least one pair of the parents, among which three loci, including *Xmag1759*, *Xmag1810* and *Xmag1986*, were mapped to chromosome 7A (Fig. 2). The mapping positions of these three

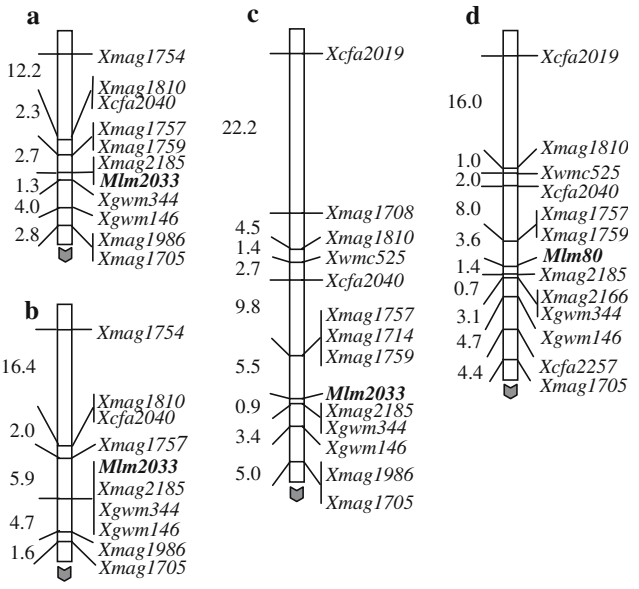

Fig. 2 The marker maps for *Mlm2033* (**a**, **b**, **c**) and *Mlm80* (**d**). **a** TA2721 × TA2033, **b** M389*2/TA2033, **c** TA2033 × TA2716, **d** M80 × TA2025. Genetic distance is shown to the *left* of the maps in cM. *Black arrows* indicate the telomere direction

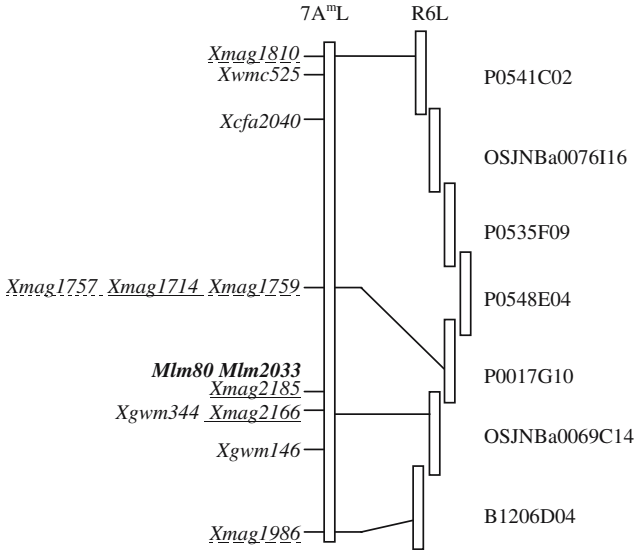

Fig. 4 Comparison of the integrated 7A^mL genetic map with the partial physical map of rice chromosome 6L (R6L). 7A^mL was based on the maps constructed using the populations from TA2033 × TA2716 with the position of *Mlm80* and *Xmag2166* being inferred from M80 × TA2025. To the right of R6L are the names of BACs or PACs ordered in the contig. The length of the *bars* is not scaled on the size. The linker lines between 7A^mL and R6L indicated the homology of the marker with the corresponding BAC or PAC. Marker loci mapped by STS markers developed from RFLP probes, ESTs homologous to rice CDS and other ESTs that were located in the terminal deletion bin of group 7L were underlined with *solid*, *dashed* and *dotted* lines, respectively

loci and the two RFLP-derived loci including *Xmag1714* and *Xmag2166* were in the same order as the corresponding homologous rice CDS in the contig (Fig. 4). Thus, the rice PAC P0017G10 with the sequence homologous to *Xmag1714* and the rice BAC OSJNBa0069C14 with the sequence homologous to *Xmag2166* delimit *Mlm2033* and *Mlm80*.

Fourteen more STS markers developed from wheat ESTs mapped to the terminal deletion bins of the long arms of group 7 chromosomes were surveyed, four of which detected polymorphism between at least one pair of the parents. Their polymorphic loci, including *Xmag1705*, *Xmag1708*, *Xmag1754* and *Xmag1757*, were also mapped to chromosome 7A (Fig. 2), but no markers more closely linked to *Mlm2033 and Mlm80* than *Xmag2185* were found.

Mlm2033 is likely allelic to *Mlm80*

Mlm2033 and *Mlm80* were 0.9 and 1.4 cM from *Xmag2185*. The *Z*-test indicated that the marker interval of *Xmag1759–Xmag2185* in the (TA2033 × TA2716) population (Fig. 2c) was not significantly different from the corresponding one in the (M80 × TA2025) population (Fig. 2d). To examine the allelic relationship of *Mlm2033* with *Mlm80*, the powdery mildew resistance of 12 F_1 plants from the cross of

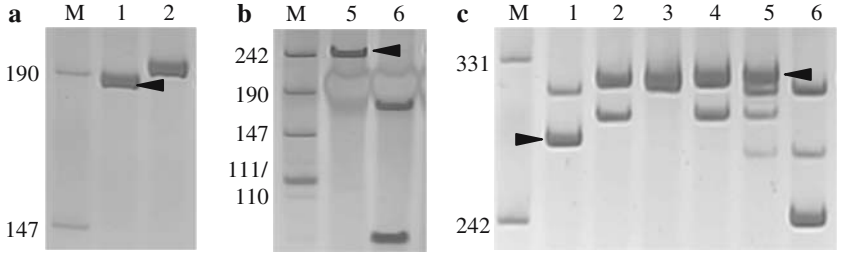

Fig. 3 PCR amplification of DNA from the mapping parents with RFLP-converted STS markers MAG1714 (**a**), MAG2166 (**b**) and MAG2185 (**c**). The PCR products from amplification by MAG2166 were digested with *Msp*I before running the gel. The *black solid triangles* indicate the polymorphic bands in the resistant parents that were mapped. M: pUC19/*Msp*I, to the *left* is the marker band size in bp. Lanes *1*, *2*, *3*, *4*, *5* and *6* represent TA2033, TA2716, M389, TA2721, M80 and TA2025, respectively

TA2033 with M80 and the F_2 populations derived from them were evaluated using the Bgt19 isolate. The F_1 plants were all immune. The F_2 populations were grouped into two categories according to their resistance segregation. One group including 1875 F_2 plants derived from four F_1 had ten plants with a score ≥ 3. The other group had 1657 F_2 plants derived from 8 F_1 that were all resistant with a score of 0–1. Based on the first group data, *Mlm2033* would be linked to *Mlm80* in the repulsion phase with 14.6% recombinant frequency. However, assuming such a recombinant fraction, the probability to have no segregation in a population of 1657 F_2 plants is 1.4×10^{-4}. Therefore, it was highly unlikely to obtain the result of the second group. Since the majority of the F_2 sub-populations did not segregate in resistance and most of the plants in the first group were also resistant, we concluded that the ten susceptible plants were from seed contamination and *Mlm2033* is likely allelic to *Mlm80*.

Discussion

One dominant gene resistant to powdery mildew disease was identified in each of the einkorn accessions TA2033 and M80. Genetic studies and molecular mapping of these two genes, *Mlm2033* and *Mlm80*, indicated that they are located in the *Xmag1759*–*Xmag2185* interval on the long arm of chromosome 7A. Even though *Mlm80* was mapped in the population derived from a cross between *T. boeoticum* and *T. monococcum* and *Mlm2033* was mapped in the population derived from a cross between two *T. monococcum* accessions, the interval distances and orders of common markers closely surrounding *Mlm2033* and *Mlm80* were quite consistent (Fig. 2c, d), suggesting that the recombination between *T. boeoticum* and *T. monococcum* chromosomes was not different from that between *T. monococcum* chromosomes, which is consistent with the results of Dubcovsky and Dvorák (1995).

Quite a few powdery mildew resistance genes have been identified on chromosome 7A. These include *Pm1, Pm9, mlRD30*, and two partially dominant powdery mildew resistances genes originated from *T. monococcum* and *T. timopheevii*, which were located to 2.7 and 1.4 cM proximal to *Xwmc525* in the centromere orientation, respectively (Srnic et al. 2005). *Mlm2033* and *Mlm80* are more than 10 cM away from *Xwmc525* in the opposite direction (Fig. 2). *Pm9* in cultivar 'Normandie' is linked to *Pm1a* with a distance of 8.5 cM (Schneider et al. 1991). The resistance gene *mlRD30* is a recessive powdery mildew resistance gene in a common wheat line and was 1.8 cM from *Xgwm344* (Singrün et al. 2004). *Pm1* has been shown to be a complex locus with multiple alleles conferring dominant powdery mildew resistance including *Pm1a*, *Pm1b*, *Pm1c*, *Pm1d* and *Pm1e*, among which *Pm1b* and likely *Pm1c* were transferred from *T. monococcum* (Hsam et al. 1998), the source for all others except *Pm1d* (transferred from *T. spelta* L.) was *T. aestivum* (Sears and Briggle 1969; Hsam et al. 1998; Singrün et al. 2003). *Mlm2033* and *Mlm80* showed close linkage to or co-segregated with the STS marker derived from PSR680 that co-segregated with *Pm1a* (Neu et al. 2002), implying that that are likely allelic to *Pm1*. But we noticed that, unlike *Mlm2033* and *Mlm80*, *Pm1a* near-isogenic line CI14114 was susceptible to Bgt 19 (data not shown). It would be interesting to investigate how *Mlm2033* and *Mlm80* perform in the hexaploidy genetic background of common wheat.

Neu et al. (2002) demonstrated that the complete linkage of *Xcdo347*, *Xc607*, *Xpsr121*, *Xpsr148* and *Xpsr680* with *Pm1a* is likely caused by suppressed recombination in hexaploid wheat instead of close physical linkage. Our results supported this conclusion. In this study, STS markers derived from RFLP probes C607 and PSR121 did not produce polymorphism between the parents. Among the three loci detected by STS markers derived from the remaining RFLP probes, *Xmag2185* was most tightly linked to both of these two genes, while *Xmag1714* was farthest from *Mlm2033* (Fig. 2c). The genetic distance between *Xmag1714* and *Xmag2185*, which flank *Mlm2033*, was as large as 6.4 cM. Thus, using the diploid wheat populations, we were able to resolve the chromosome region corresponding to *Pm1* with the better resolution, which will certainly help map-based cloning of this gene.

To saturate the region surrounding *Mlm2033* and *Mlm80* with molecular markers, we took advantage of the collinearity of the long arms of wheat chromosome group 7 with rice chromosome 6L (Sorrells et al. 2003). Not all the STS markers derived from wheat ESTs homologous to the rice CDS could be put into the 7AL maps even though polymorphism was detected between the parents, but three of them were mapped around *Mlm2033* and *Mlm80*. We found that *Mlm2033* and *Mlm80* can be further delimited to the region covered by the contig consisting of P0017G10 and OSJNBa0069C14 (Fig. 4). Annotation of this contig sequence did not reveal any known RGAs. Kilian et al. (1997) reported the similar phenomenon in cloning the barley stem rust resistance gene *Rpg1*. A PSR680-derived STS marker MAG2185 was mapped within this region, but PSR680 that might represent a locus

encoding the NBS-LRR disease resistance protein does not has a homolog in the contig. A comparison of the wheat contig carrying *Lr10* locus with the corresponding rice genomic region showed that two RGAs in the wheat contig were absent in rice (Guyot et al. 2004). These results implied that chromosome regions around resistance genes evolve rapidly, resulting in micro-disruption of the collinearity between rice and other grass chromosomes.

With the aim of identifying candidate resistance genes in mind, we have paid special attention to wheat ESTs encoding RGAs. Even though three of the four wheat EST-derived STS marker loci including *Xmag1705*, *Xmag1708* and *Xmag1757* (Fig. 2) could represent RGAs, none of them showed tight linkage to either resistance gene. It should be noted that it was not known in this study if these STS marker loci are genes encoding RGAs. In addition, the fact that less than half of the STS markers derived from RGA-encoding ESTs produced polymorphism could have limited our capacity to identify candidate resistance genes.

Diploid wheat is the A genome donor of hexaploid wheat. Thus, the genome homology makes it possible to transfer the genes in diploid wheat to hexaploid wheat through genetic recombination in sexual crosses. However, as Dubcovsky et al. (1995) have demonstrated that recombination between the A chromosomes of common wheat and the A^m chromosomes of *T. boeoticum* is inhibited by the *Ph1* system of wheat, the utilization of *Ph1* mutants or deletions might be needed to raise the recombination frequency in the transfer process and to diminish potentially deleterious linkage drag. The molecular markers tightly linked to *Mlm2033* and *Mlm80* can facilitate the gene transfer.

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