Theor Appl Genet (2013) 126:3081–3089
DOI 10.1007/s00122-013-2194-6

ORIGINAL PAPER

Genetic analysis and detection of the gene *MlLX99* on chromosome 2BL conferring resistance to powdery mildew in the wheat cultivar Liangxing 99

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Received: 14 April 2013 / Accepted: 6 September 2013 / Published online: 24 September 2013

Abstract
Key message **The effectiveness of wheat cultivar Liangxing 99 against powdery mildew was shown to be controlled by a single dominant gene located on a new locus of chromosome 2BL in the bin 2BL2-0.35-0.50.**
Abstract Liangxing 99, one of the most widely grown commercial cultivars in the winter wheat (*Triticum aestivum*) producing regions in northern China, was shown to provide a broad spectrum of resistance to *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates originating from that region. Using an F_2 population and $F_{2:3}$ lines derived from a cross of Liangxing 99 × Zhongzuo 9504, genetic analysis demonstrated that a single dominant gene, designated *MlLX99*, was responsible for the resistance of Liangxing 99 to *Bgt* isolate E09. The results of molecular analysis indicated that this gene is located on chromosome 2BL and flanked by the SSR marker *Xgwm120* and EST-STS marker *BE604758* at genetic distances of 2.9 and 5.5 cM, respectively. Since the flanking markers of *MlLX99* were previously mapped to the bin 2BL2-0.36-0.50, *MlLX99* must be located in this chromosomal region. *MlLX99* showed a different resistance reaction pattern to 60 *Bgt* isolates from *Pm6*, *Pm33*, and *PmJM22*, which were all previously mapped on chromosome 2BL, but differed in their positions from *MlLX99*. Due to its unique position on chromosome 2BL, *MlLX99* appears to be a new locus for resistance to powdery mildew. Liangxing 99 has shown superior yield performance and wide adaptation to different agricultural conditions, which has resulted in its extensive use as a wheat cultivar in China. The identification of resistance gene *MlLX99* facilitates the use of this cultivar in the protection of wheat from damage caused by powdery mildew.

Communicated by A. E. Melchinger.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2194-6) contains supplementary material, which is available to authorized users.

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Introduction

Wheat (*Triticum aestivum* L.) is a popular staple food crop in China. From 1950 to 2011, wheat production and the average yield increased from <20 million tons and 0.66 t/ha to 114.5 million tons and 4.84 t/ha, respectively; while the total acreage of wheat production remained approximately the same (22 million ha in 1950 vs. 23.4 million ha in 2011) (He et al. 2001, http://www/stats.gov.cn). The development of high yielding cultivars has made a significant contribution to the yield increase in addition to improvements in crop production. As wheat suffers from yield losses caused by diseases, the development of disease resistant cultivars is a priority in many wheat breeding programs.

Epidemics of powdery mildew, which are caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), result in significant economic losses in many temperate wheat producing regions of the world (Cowger et al. 2012). This fungal

disease has become widespread throughout most winter wheat regions in China since the 1970s. In the last decade, powdery mildew has occurred on about 6 ~ 8 million ha of wheat annually, making it one of the most serious diseases in the country (http://cb.natesc.gov.cn/sites/cb/). Although widespread occurrence of powdery mildew in China is relatively recent compared to other diseases, such as stripe rust (caused by *Puccinia striiformis* Westend.), outbreaks of powdery mildew have resulted in significant yield reductions. In 1990, powdery mildew covered an area of some 12 million ha, leading to an estimated loss of 1.438 billion kg of grain (Huo et al. 2002). Wheat infected by powdery mildew also produces poor quality grain, which influences the milling and baking quality of wheat (Everts et al. 2001).

In China, early efforts to improve resistance to powdery mildew have largely relied on the powdery mildew (*Pm*) resistance gene *Pm8* originating from the wheat-rye (*Secale cereale* L.) chromosome translocation T1BL·1RS in the wheat cultivars Lovrin 13, Pregornaia 2, and Kavkaz, which were introduced from Romania in the 1970s (Zhuang and Li 1993). The overwhelming majority of cultivars released during the 1980s and 1990s carried this gene (Zhou et al. 2004; Li et al. 2011). However, overreliance on *Pm8* has increased the frequency of isolates that are virulent to this gene and soon resulted in its being overcome in almost all the wheat producing regions. In addition to *Pm8*, the resistance genes *Pm2*, *Pm4a*, *Pm6*, *Pm2+6*, and *Pm21* were frequently used in Chinese wheat cultivars (Zhuang 2003; Sang et al. 2006; Li et al. 2007; Zhan et al. 2010). At present, over 60 genes or alleles for resistance to powdery mildew have been documented (McIntosh et al. 2008, 2012). In a recent study, a few genes, such as *Pm1c*, *Pm16*, *Pm20*, and *Pm21*, were highly effective against *Bgt* isolates collected from Shandong and Hebei provinces, which are the major winter wheat producing regions in China. *Pm1a*, the *Pm3* alleles, *Pm5*, *Pm7*, and *Pm8* are no longer effective (Zhao et al. 2013).

Tests of a large number of wheat cultivars identified some resistant commercial cultivars, however, their frequency was low (Li et al. 2011). A number of widely grown commercial cultivars carry single dominant genes for resistance to powdery mildew (Song et al. 2012). The availability of molecular markers has facilitated the identification of powdery mildew resistance genes in several Chinese wheat cultivars. The powdery mildew resistance gene in the wheat cultivar Yumai 66 was located on chromosome 2AL with the linked marker *Xksum193* (Hu et al. 2008a). A single gene on chromosome 7BL is associated with the powdery mildew resistance in Tangmai 4 (Hu et al. 2008b). A dominant gene on chromosome 2BL of Jimai 22 is responsible for its resistance to powdery mildew (Yin et al. 2009). Zhoumai 22 carries gene *PmHNK* on chromosome 3BL, which is linked to the SSR marker *Xwmc291* (Xu et al. 2010). *PmHNK54* on chromosome 2AL is linked to the SSR markers *Xbarc5* and *Xgwm312*, which confers resistance to powdery mildew in Zheng 9754 (Xu et al. 2011). The powdery mildew resistance in Liangxing 66 is conferred by a single dominant gene of the locus *Pm2* on chromosome 5DS (Huang et al. 2012). Liangxing 99 is an outstanding winter wheat cultivar that has being commercialized and grown over a wide range of wheat producing regions in northern China, such as Shandong, Hebei, Shanxi, Shaanxi, Jiangsu, and Anhui provinces (http://www.sdny.gov.cn/art/). Due to its superior yield performance and wide adaptation, Liangxing 99 has served as a control cultivar in the national and Shandong provincial wheat yield trials. This cultivar was highly resistant to powdery mildew at both seedling and adult stages (Li et al. 2011).

The objectives of this study were (1) to test the seedling reactions of Liangxing 99 to a collection of *Bgt* isolates from the regions where it is grown commercially, (2) to understand the inheritance of its resistance to powdery mildew, and (3) to determine the chromosome location of the resistance gene in Liangxing 99 by means of molecular marker analysis.

Materials and methods

Plant materials

Liangxing 99 (pedigree: Ji 91102/Lumai 14//PH85-16) was developed by the Shandong Liangxing Seed Co. Ltd., Ningjin, Shandong province, China. Liangxing 99 was crossed to the susceptible wheat cultivar Zhongzuo 9504 to produce F_1, F_2, and $F_{2:3}$ populations for genetic analysis of its powdery mildew resistance. The F_2 population was used to map the powdery mildew resistance gene in Liangxing 99, and the F_2-derived lines in F_3 generation of Liangxing 99 × Zhongzuo 9504 each consisting of 15 F_3 seedlings were tested against *Bgt* isolate E09 to establish the genotypes of F_2 plants. The winter wheat cultivars Jingshuang 16 and Zhongzuo 9504 were used as the susceptible controls in the powdery mildew assessments. Jimai 22 (pedigree: 935024/935106) and a set of 34 differential wheat cultivars or lines with known powdery mildew resistance genes or gene combinations were used to compare their responses to different isolates of *Bgt* with those of Liangxing 99 (Zhao et al. 2013). Ten seedlings of each genotype were separately inoculated with each isolate and this experiment was conducted twice.

Disease assessments at the seedling stage

Sixty single-spore *Bgt* isolates, collected from Shandong, Hebei, Henan, and Shanxi provinces, where Liangxing 99

is grown, as well as Beijing, Jiangsu, Yunnan, and Guizhou, were used to determine the reactions of wheat entries to powdery mildew (Supplementary Table 1). Isolate E09 was used to test the two parents, DNA bulks, and genetic populations for mapping the gene conferring resistance to powdery mildew in Liangxing 99.

Evaluation of the seedling reactions to different *Bgt* isolates was carried out in a greenhouse at the Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China. The parents and the differential wheat entries, as well as the mapping populations, were grown in plastic trays with 5 × 10 holes (5 × 5 cM). Seedlings at the one-leaf-stage were dusted with fresh conidiospores from the susceptible cultivar Zhongzuo 9504. The plants were separated into either a resistant group when the Infection types (ITs) were 0–2 or a susceptible group when the ITs were 3–4. After incubation for 24 h in a dew chamber, the inoculated plants were grown at 18–20 °C with a 60 % relative humidity and a 12 h light/12 h dark photoperiod. Infection types (ITs) on the first leaf of each plant were rated using a 0–4 scale about 15 days after inoculation when severe symptoms were present on the susceptible controls Jingshuang 16 and Zhongzuo 9504.

Marker analysis

Following the cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al. 1984), genomic DNA was extracted from the leaf tissues of young seedlings. Equal amounts of DNA from 10 highly resistant or susceptible F_2 plants were pooled to perform bulked segregant analysis (BSA) (Michelmore et al. 1991). Simple sequence repeat (SSR) and expressed sequence tag (EST) markers that were previously used to map the wheat genome (http://wheat.pw.usda.gov/) were tested for polymorphisms between the two parents and the contrasting DNA bulks. Using a Biometra T3000 Thermocycler (ABI, New York, USA), DNA amplification was performed with the reaction mixture (10 μL) containing 50 ng of template DNA, 0.2 μM of the forward and reverse primers, 1 U of *Taq* polymerase, 0.4 mM dNTPs, and 2 μL 10× buffer with 20 mM Mg^{2+}. The amplification was programmed at 94 °C for 4 min; 35 cycles of 94 °C for 40 s, 52–60 °C for 40 s, and 72 °C for 1 min. The reaction was terminated after an extension at 72 °C for 10 min. The resulting PCR products were mixed with 2 μL loading buffer (98 % formamide, 10 mM EDTA, 0.25 % bromophenol blue, and 0.25 % xylene cyanol) prior to separation on 1 % agarose gel or 8 % nondenaturing polyacrylamide gel (Acr:Bis = 19:1 or 39:1). The banding patterns were visualized by silver staining (Santos et al. 1993). The polymorphic markers obtained were then used to map the gene for resistance to powdery mildew in Liangxing 99.

Statistical analysis and linkage map construction

A comparison of the pattern of infection types on the race differentials and Liangxing 99 was made to identify lines with similar reactions to the *Bgt* isolates. Chi squared (χ^2) test for the goodness of fit was performed to determine the deviations of observed data from expected F_2 and $F_{2:3}$ segregation ratios. Linkage between markers and the resistance gene in Liangxing 99 was established with the software Mapmaker/Exp Version 3.0b (Lincoln et al. 1993). Genetic distances were determined using the Kosambi function. The logarithm of the odds ratio (LOD) threshold score was set at 3.0 and the maximum distance allowed between markers was set at 50.0 cM (Kosambi 1944).

Results

Comparison of resistance reaction patterns in response to *Bgt* isolates between Liangxing 99 and different wheat accessions with known resistance genes

Wheat seedlings at the one-leaf-stage were tested against 60 *Bgt* isolates. These isolates differed in virulence on 34 powdery mildew resistance genes or gene combinations (Supplementary Table 1). All the isolates were avirulent on *Pm24*, and a low virulence frequency (1.7–6.7 %) was observed on cultivars carrying genes *Pm1c*, *Pm16*, *Pm20*, *Pm21*, *PmH*, and *Mlxbd*. The virulence frequencies on cultivars carrying *Pm2*, *Pm5e*, *Pm12*, *Pm13*, *Pm17*, *Pm40*, *Pm2+6*, and *Pm5+6* ranged from 19.0 to 45.0 %. The range of virulence frequencies on cultivars carrying *Pm1a*, *Pm3b*, *Pm4a*, *Pm4b*, *Pm4c*, *Pm6*, *Pm33*, *Pm44*, *PmDR147*, and *PmPS5A* was 56.7–76.7 %. Over 80 % of the isolates tested were virulent on cultivars carrying *Pm3a*, *Pm3c*, *Pm3 g*, *Pm5a*, *Pm7*, *Pm8*, *Pm19*, *PmY39*, and *Pm1+2+9*.

Liangxing 99 was resistant to 52 of the 60 isolates tested (86.7 %), except for Bg41-2, Bg46-1, Bg50-1, and Bg52-1 from Shandong province, Bg56-3, Bg62-1, and Bg62-2 from Hebei province, and B13 from Jiangsu province. This indicates that Liangxing 99 is effective against powdery mildew over a wide range of wheat producing regions in northern China. Comparisons of Liangxing 99 with the differential wheat entries revealed similar patterns between Liangxing 99 and *Pm2+6* in their resistance reactions to 51 isolates out of the 60 isolates tested, showing the greatest similarity over other known powdery mildew resistance genes or gene combinations. Liangxing 99 differed from *Pm2* in its reactions to 13 isolates, *Pm6* for 26 isolates, *Pm33* for 32 isolates, and *Pm5+6* for 13 isolates (Supplementary Table 1). Both of the cultivars Jingshuang 16 and

Zhongzuo 9504 were susceptible to all the isolates tested as expected.

To compare the powdery mildew reaction patterns of Liangxing 99 and Jimai 22, these two cultivars, together with Coker 747 (*Pm6*), 2632-32R (*Pm33*), Ulka/8*Cc (*Pm2*), and Zhongzuo 9504, were tested using 11 *Bgt* isolates from the 60 isolate collection. Liangxing 99 differed from Jimai 22 in its reactions to isolates E11, E16, and B13 (Fig. 1). Among the 11 *Bgt* isolates, Liangxing 99 differed in its reaction from Coker 747 (*Pm6*), 2632-32R (*Pm33*), and Ulka/8*Cc (*Pm2*) to 7, 5, and 3 isolates, respectively.

Genetic analysis of resistance to powdery mildew in Liangxing 99

Liangxing 99 was resistant to the *Bgt* isolate E09 with an IT of 0, while Zhongzuo 9504 was highly susceptible with an IT of 4. Twenty F_1 plants derived from Liangxing 99 × Zhongzuo 9504 cross produced similar reactions to the isolate E09 as the resistant parent Liangxing 99 (Table 1). Among the 286 F_2 plants tested, 216 resistant plants and 70 susceptible plants were observed. A Chi squared test indicated that these plants segregated in a ratio of 3:1 ($P = 0.84$). The F_2 plants were grown in a

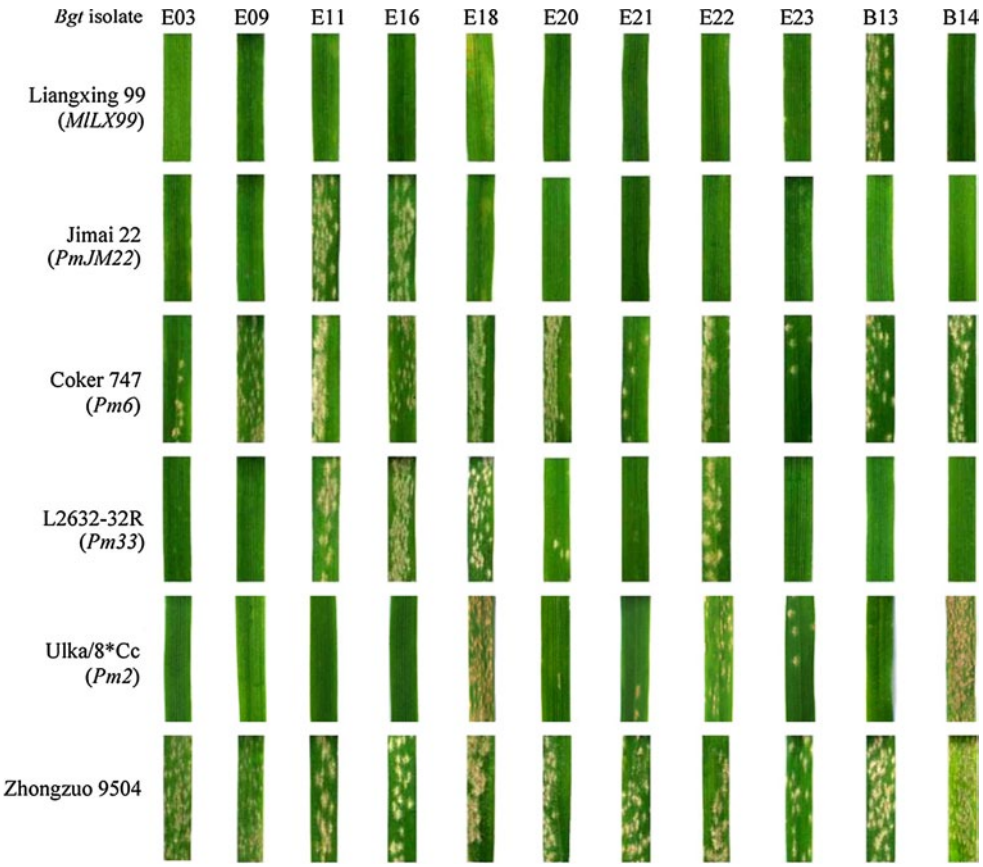

Fig. 1 Reactions of Liangxing 99, Jimai 22, Coker 747 (*Pm6*), 2632-32R (*Pm33*), Ulka/8*Cc (*Pm2*) and Zhongzuo 9504 to different isolates of *Blumeria graminis* f. sp. *tritici* from northern China (see Supplementary Table 1)

Table 1 Genetic analysis of resistance to isolate E09 of *Blumeria graminis* f. sp. *tritici* in F_1, F_2, and $F_{2:3}$ progenies derived from Liangxing 99 × Zhongzuo 9504 cross

Parents and cross	Generation	Total number of plants/families	Observed ratio			Expected ratio	χ^2	*P* value
			HR	Seg	HS			
Liangxing 99	P_R	20	20		0			
Zhongzuo 9504	P_S	20	0		20			
Liangxing 99 × Zhongzuo 9504	F_1	20	20		0			
Liangxing 99 × Zhongzuo 9504	F_2	286	216		70	3:1	0.0420	0.84
Liangxing 99 × Zhongzuo 9504	$F_{2:3}$	232	65	110	57	1:2:1	1.1724	0.56

P_R resistant parent, *P_S* susceptible parent, *HR* homozygous resistant, *Seg* segregating (heterozygous resistant), *HS* homozygous susceptible

greenhouse and produced 232 $F_{2:3}$ lines. The $F_{2:3}$ lines were categorized as 66 homozygous resistant: 113 segregating: 59 homozygous susceptible lines, which fits to the expected ratio of 1:2:1 ($P = 0.56$) for the monogenic mode of inheritance with regard to resistance to the isolate E09. These results demonstrate that a single dominant gene confers resistance to powdery mildew in Liangxing 99, which was tentatively designated *MlLX99*.

Molecular mapping of the powdery mildew resistance gene in Liangxing 99

To identify the position of *MlLX99*, 283 SSR markers distributed on all the different wheat chromosomes were used to perform bulked segregant analysis using the mapping population of Liangxing 99 × Zhongzuo 9504. Marker *Xgwm47*, which is located on chromosome arm 2BL, was polymorphic between the two parents and the contrasting DNA bulks, showing potential linkage with *MlLX99*. Therefore, the polymorphisms of an additional 77 markers on chromosome 2BL were examined, which resulted in the identification of seven polymorphic markers, i.e., *Xgwm191*, *Xcfd73*, *Xwmc441*, *Xgwm120*, *XAG24*, *Xwmc175*, and *Xwmc500*. These polymorphic markers were thus used to genotype the F_2 segregating population of Liangxing 99 × Zhongzuo 9504, and a linkage map spanning chromosome arm 2BL (62.5 cM in length) was constructed (Fig. 2a). *MlLX99* was flanked by markers *Xgwm120* and *Xwmc441*. Since the closest marker *Xgwm120* from *MlLX99* was mapped to the deletion bin 2BL2-0.36-0.50, 66 EST-STS markers that were previously mapped in this chromosome region (http://wheat.pw.usda.gov/cgi-bin/westsql/bin_candidates.cgi?bin=2BL2-0.36-0.50) were further tested for discovering the polymorphic markers. Two EST-STS markers *BE604758* and *BF292219* were associated with *MlLX99*. Based on the linkage analysis; *MlLX99* is located on chromosome 2BL in region 2BL2-0.36-0.50 and is flanked by markers *Xgwm120* and *BE604758* at genetic distances of 2.9 and 5.5 cM, respectively (Figs. 2b and 3).

Comparison of the positions between *MlLX99* and other powdery mildew resistance genes on chromosome 2BL

The polymorphisms for markers linked to the genes previously mapped on the long arm of chromosome 2B also were determined using the parental cultivars Liangxing 99 and Zhongzuo 9504, as well as the DNA bulks. Although the markers CINAU123, CINAU124, and CINAU127, which are tightly linked to gene *Pm6*, were polymorphic between the two parents and the DNA bulks, they were not associated with *MlLX99* based on testing of the F_2 mapping population.

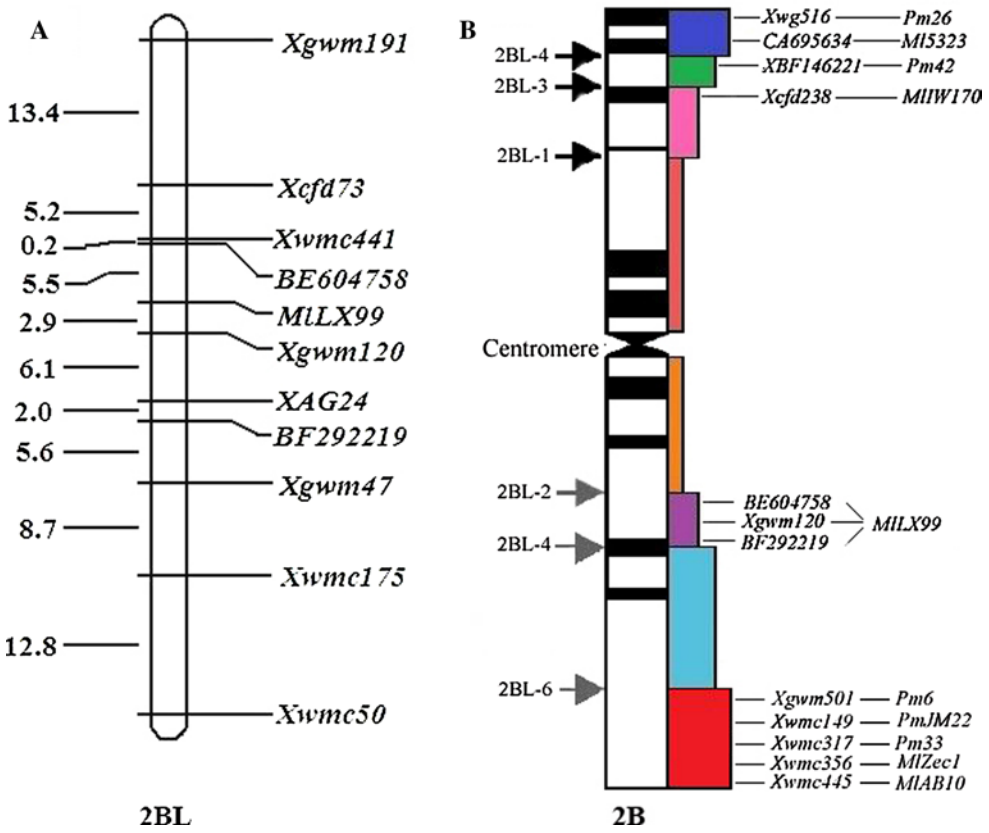

Fig. 2 Linkage map (**a**) and chromosome bin physical map (**b**) of *MlLX99* on wheat chromosome 2B. Genetic distances are shown to the *left* in cM. The different bins of chromosome 2B are indicated by *different colors*

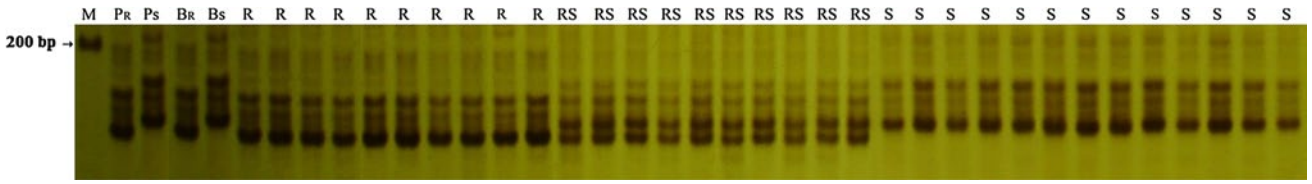

Fig. 3 A profile of amplification with SSR marker *Xgwm120* from the parents, DNA bulks, and selected F_2 plants from the Liangxing 99 × Zhongzuo 9504 cross. *M* 100 bp DNA ladder, P_R resistant parent Liangxing 99, P_S susceptible parent Zhongzuo 9504, B_R resistant bulk, B_S susceptible bulk, *R* homozygous resistant F_2 plants, *RS* heterozygous resistant F_2 plants, *S* homozygous susceptible F_2 plants

Table 2 Detection of polymorphism in the *MlLX99* mapping population derived from cross Liangxing 99 × Zhongzuo 9504 with the markers linked to the known powdery mildew resistance genes on chromosome 2BL

Marker	Resistance gene	Polymorphism[a]		Linkage to *MlLX99*	Reference
		Parents	Bulks		
Xgwm47	*Pm6*, *MlZec1*	+	+	+	Wang et al. (2007) Mohler et al. (2005)
Xgwm501	*Pm6*	−	−	−	Wang et al. (2007)
NAU/STS$_{BCD135\text{-}1}$	*Pm6*	−	−	−	Ji et al. (2008)
NAU/STS$_{BCD135\text{-}2}$	*Pm6*	−	−	−	Ji et al. (2008)
CINAU123	*Pm6*	+	+	−	Qin et al. (2011)
CINAU124	*Pm6*	+	+	−	Qin et al. (2011)
CINAU127	*Pm6*	+	+	−	Qin et al. (2011)
Xwmc149	*PmJM22*	−	−	−	Yin et al. (2009)
Xwmc317	*Pm33*	−	−	−	Zhu et al. (2005)
Xwmc356	*MlZec1*	−	−	−	Mohler et al. (2005)
Xgwm526	*MlZec1*	−	−	−	Mohler et al. (2005)
Xwmc445	*MlAB10*	−	−	−	Maxwell et al. (2010)

[a] +, polymorphic or linked
−, non-polymorphic or unlinked

The SSR marker *Xgwm47* associated with *Pm6* was 16.6 cM away from *MlLX99* (Fig. 2a). The *Pm6*-linked STS markers NAU/STS$_{BCD135\text{-}1}$ and NAU/STS$_{BCD135\text{-}2}$ and SSR marker *Xgwm501* did not show any polymorphisms between the parents or the DNA bulks. Similarly, the *Pm33*-linked marker *Xwmc317*, the *MlZec1*-linked marker *Xwmc356*, the *PmJM22*-linked marker *Xwmc149*, and the *MlAB10*-linked markers *Xwmc445*, *Xwmc447*, and *Xwmc498* also were not polymorphic (Table 2). These findings indicate that the above marker alleles were not associated with the resistance of Liangxing 99 to powdery mildew. Moreover, the SSR markers *Xgwm120* and *Xwmc441* that flanked *MlLX99* did not produce the diagnostic bands in Coker 747 (*Pm6*), L2632-32R (*Pm33*), or Jimai 22 (*PmJM22*) (data not shown). These results demonstrate that *MlLX99* does not share the same loci with the other powdery mildew resistance genes that have been mapped on chromosome 2BL.

Discussion

Resistance to powdery mildew in the wheat cultivar Liangxing 99 was confirmed at the seedling stage in a controlled environment. The resistance against the *Bgt* isolate E09 in Liangxing 99 was inherited as a monogenic trait. A single dominant gene, tentatively designated *MlLX99*, was assigned to chromosome 2BL using a linkage map constructed with the polymorphic SSR and EST markers. Based on the differences in their positions, reactions to *Bgt* isolates, and origins, *MlLX99* represents a new locus that differs from other powdery mildew resistance genes previously located on chromosome 2BL.

The wheat chromosome 2B carries a number of genes conferring resistance to powdery mildew. *Pm6* was the first gene localized on chromosome 2BL, which is inherited from *T. timopheevii* (Zhuk.) Zhuk. via CI 12633 (Jørgensen and Jensen 1972, 1973). Because *Pm6* alone or in combination with other genes such as *Pm2* and *Pm5* was still effective against powdery mildew in a wide range of wheat production regions throughout the world (Věchet 2006; Purnhauser et al. 2011; Zhao et al. 2013), many studies have been carried out to find markers linked to *Pm6*. Tao et al. (2000) established the linkage between the RFLP marker *Xbcd135* and *Pm6* at genetic distance of 1.6 cM, and this marker was assigned to the deletion bin 2BL6-0.89-1.00. Based on this linked RFLP marker, two PCR-based STS markers *NAU/STS*$_{BCD135\text{-}1}$ and *NAU/STSB*$_{CD135\text{-}1}$ were shown to be linked to *Pm6* (Ji et al. 2008). Using SSR and STS markers, analysis of a set of *T. aestivum–T. timopheevii* introgression lines containing different sizes

of *T. timopheevii* chromosome segments confirmed the localization of *Pm6* on the distal end of chromosome 2BL (Ji et al. 2007). According to its physical position, the SSR marker *Xgwm501* associated with *Pm6* was also localized in the same chromosome region (Sourdille et al. 2004; Wang et al. 2007). All these findings provide strong evidence that *Pm6* resides on the distal region of chromosome 2BL. Qin et al. (2011) finely mapped *Pm6* with the aid of comparative genomics analysis resulting in the identification of a set of STS markers that were tightly linked to or co-segregated with *Pm6*.

Pm33 is another gene that has been mapped on chromosome 2BL. This gene was introgressed into the wheat cultivar Laizhou 953 via line Am9, which was derived from the cross between *T. carthlicum* Nevski acc. PS5 and *Aegilops umbellulata* Zhuk. acc. Y39, as a bridge. The results of molecular marker analysis demonstrated that *Pm33* originated from *T. carthlicum* acc. PS5 (Zhu et al. 2005). The physical position of the SSR marker *Xwmc317*, which is tightly linked to *Pm33*, resides on the distal region of chromosome 2BL based on the consensus map of wheat (http://wheat.pw.usda.gov/cgi-bin/cmap/viewer). The temporarily designated gene *MlZec1* derived from wild emmer [*T. turgidum* L. ssp. *dicoccoides* (Koern. *ex* Asch. & Graebner) Thell.] was located on chromosome 2BL in the deletion bin 2BL6-0.89-1.00 and was linked to SSR marker *Xwmc356* (Mohler et al. 2005). Another gene, *MlAB10* of wild emmer origin, was located on the same part of chromosome 2BL as *MlZec1* (Maxwell et al. 2010). Finally, the powdery mildew resistance gene in the Chinese wheat cultivar Jimai 22 was also localized in the same chromosome region as *Pm6*, *Pm33*, and *MlZec1* (Yin et al. 2009). The localization of these powdery mildew resistance genes, together with *Sr9*, *Sr16*, and *Sr28* for resistance to stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks.), *Lr50* for resistance to leaf rust (*P. triticina* Eriks), and *Yr7* for resistance to stripe rust (*P. striiformis*) (McIntosh et al. 2008), provides evidence that there might be a resistance gene cluster in the distal region of chromosome 2BL.

Results of molecular marker analysis assigned *MlLX99* to the deletion bin 2BL2-0.36-0.50 (Fig. 2b). These results indicated that *MlLX99* was not likely located at the same position as other powdery mildew resistance genes previously mapped on chromosome 2BL. *MlLX99* was not associated with the wild emmer-derived genes *Pm26* (Rong et al. 2000), *pm42* (Hua et al. 2009), *MlIW170* (Liu et al. 2012), and *Ml5323* derived from *T. turgidum* ssp. *dicoccum* Ischrank ex Shübler) Thell. (Piarulli et al. 2012), which are located on 2BS.

In the present study, *MlLX99* showed different reaction patterns against the *Bgt* isolates tested from those of the powdery mildew race differentials (Supplementary Table 1), confirming the uniqueness of this gene from other powdery mildew resistance genes. Most identified genes for resistance to powdery mildew on chromosome 2B, such as *Pm6*, *Pm33*, *MlZec1*, and *MlAB10* on 2BL, as well as *pm26*, *Pm42*, *MlIW170*, and *Ml5323* on 2BS, are derived from the wild related species of wheat. Undesirable traits that are transferred into wheat along with the desirable genes may limit their usefulness in breeding programs, despite this *Pm6* has been used in developing resistant wheat cultivars (Bennett 1984; Purnhauser et al. 2011). The increase in the frequency of virulent isolates on *Pm6* prevents further reliance on this gene for controlling powdery mildew unless it is used in combination with other genes such as *Pm2* and *Pm5* (Věchet 2006; Purnhauser et al. 2011; Zhao et al. 2013). *Pm33*, *MlZec1*, *MlAB10*, *MlIW170*, and *Ml5323* have not been used in wheat breeding in China, possibly because of their alien origin, vulnerability to the current *Bgt* isolates or unavailability to breeders.

Unlike genes from wild related species, *MlLX99* is preferable to breeders because it originates from common wheat and is associated with superior agronomic performance. It can be easily incorporated into other wheat backgrounds to develop new cultivars without any limitation of chromosome recombination. Liangxing 99 has been grown over a wide range of the wheat producing region of northern China. The identification of a powdery mildew resistance gene in Liangxing 99 and closely linked molecular markers will facilitate the use of this cultivar in wheat improvement against powdery mildew. In addition, Liangxing 99 was also resistant to powdery mildew at the adult stage (Li et al. 2011). A recombinant inbred line population has been constructed (HJ Li, unpublished data), which allows mapping of the gene(s) or QTL(s) that confer resistance in Liangxing 99 to powdery mildew at adult stage. The results of the current study indicated that genes *Pm1c*, *Pm16*, *Pm20*, *Pm21*, *PmH*, and *Mlxbd* had lower frequencies of isolate virulence than *MlLX99* (Supplementary Table 1). However, these genes have not been used in China, except for *Pm21* (Li et al. 2007). The combination of *MlLX99* with certain of these powdery mildew resistance genes will increase the effectiveness of the resistance in wheat cultivar Liangxing 99 against powdery mildew.

In conclusion, the wheat cultivar Liangxing 99 displayed a broad spectrum of resistance to the *Bgt* isolates collected from the winter wheat producing regions in northern China. Genetic analysis demonstrated that resistance to powdery mildew in Liangxing 99 was controlled by a single dominant gene, tentatively designated *MlLX99*. This gene was localized on a unique position in the interstitial region (2BL2-0.35-0.50) of chromosome 2BL. *MlLX99* differed from genes *Pm6*, *Pm33*, *MlZec1*, *PmJM22*, and *MlAB10* that were previously mapped on the distal part of chromosome 2BL (2BL6-0.89-1.00) in chromosome location, reactions to different *Bgt* isolates, and

origins. Based on these findings, *MlLX99* is most likely a new locus on chromosome 2BL for resistance to powdery mildew. Due to its superior agronomic performance, the powdery mildew resistance of Liangxing 99 can be preferably used in breeding programs. The identification of molecular markers associated with *MlLX99* will facilitate its quick and efficient incorporation into other wheat backgrounds.

Acknowledgments We are grateful to Dr. RL Conner of the Morden Research Station, Agriculture and Agri-Food Canada, for critical review of this manuscript. The authors thank Dr. Yilin Zhou of Institute of Plant Protection, Chinese Academy of Agricultural Sciences, and Dr. Hongxing Xu of Center for Agro-Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences for their providing of some *Bgt* isolates. This study was financially supported by the National Program on Key Basic Research Project (973 Program, 2009CB118304) and Modern Agro-industry Technology Research System (CARS-3-1).

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