***PmLX66* and *PmW14*: New Alleles of *Pm2* for Resistance to Powdery Mildew in the Chinese Winter Wheat Cultivars Liangxing 66 and Wennong 14**

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Abstract

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Wheat powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*) can be effectively managed by growing resistant cultivars. ‘Liangxing 66’ and ‘Wennong 14’ are the current winter wheat cultivars grown in northern China where powdery mildew is epidemic. Both cultivars have been demonstrated to carry single dominant genes for resistance to powdery mildew, tentatively designated *PmLX66* and *PmW14*, on chromosome 5DS and share common linked markers with *Pm2*. Allelism tests were performed using a total of 15,657 plants of F₂ segregating populations to determine the relationship between *PmLX66*, *PmW14*, and *Pm2*. All progeny from the crosses Liangxing 66 × ‘Ulka/8*Chancellor’ (Ulka/8*Cc),

Wennong 14 × Ulka/8*Cc, and Liangxing 66 × Wennong 14 were resistant when tested with *B. graminis* f. sp. *tritici* isolate E20, indicating that *PmLX66* and *PmW14* are allelic to *Pm2* and to each other. Liangxing 66 was resistant to 76.7% of the 60 *B. graminis* f. sp. *tritici* isolates from northern China, a slightly smaller proportion than Ulka/8*Cc (78.3%). However, Wennong 14 (85.0%) was more resistant against this set of *B. graminis* f. sp. *tritici* isolates than Ulka/8*Cc and Liangxing 66. Liangxing 66 and Wennong 14 differed from Ulka/8*Cc in respect to a number of *B. graminis* f. sp. *tritici* isolates. Based on these findings, *PmLX66* and *PmW14* are new alleles at the *Pm2* locus.

Powdery mildew, caused by the biotrophic fungus *Blumeria graminis* f. sp. *tritici*, is a major constraint to common wheat (*Triticum aestivum* L.) production throughout the world, especially in the wheat-producing regions with maritime or semicontinental climates (Cowger et al. 2012). Wheat production in China has been suffering from this destructive disease since the 1970s. Currently, the area affected by powdery mildew in China ranges from 6 to 8 million ha, which encompasses nearly all the major wheat-producing regions of the country, with more severe damage in the winter- than spring-wheat regions (<http://cb.natasc.gov.cn>).

Losses in yield and end-use quality caused by powdery mildew can be limited by fungicides but the use of resistant cultivars is the preferred way to reduce the effects of epidemics and minimize yield losses (Hulbert et al. 2001). Reduced input cost for farmers is another benefit of growing powdery mildew-resistant cultivars.

Availability of powdery mildew resistance genes (*Pm*) is a prerequisite for developing resistant cultivars. Over 60 genes conferring qualitative resistance to wheat powdery mildew have been mapped (<http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolListPageAction.do?page=-1>). Most loci contain a single resistance allele but some (e.g., *Pm1*, *Pm3*, *Pm4*, *Pm5*, *Pm8*, and *Pm24*) have multiple alleles (Hsam and Zeller 1997; Huang et al. 2003; Schmolke et al. 2012; Singrün et al. 2003; Xue et al. 2012; Yahiaoui et al. 2009). These genes are race specific, short lived, and frequently overcome by virulent pathotypes. Genes *Pm6* and *Pm8*, for example, were widely deployed in commercial wheat cultivars or in combinations with other powdery mildew resistance genes in China (Li et al. 2011;

Sang et al. 2006; Zhan et al. 2010) and other countries (Purnhauser et al. 2011; Rabinovich 1998). The results of a recent study using 1,082 *B. graminis* f. sp. *tritici* isolates collected from different wheat-production regions of China demonstrated that the majority of isolates (>70%) were virulent on *Pm6* and *Pm8* (Zeng et al. 2014). In contrast, the number of *B. graminis* f. sp. *tritici* isolates that are virulent on *Pm2* remained low in some regions of China and other parts of the world (Cao et al. 2010; Lutz et al. 1992, 1995; Zhou et al. 2002), although *Pm2* was identified several decades ago (Pugsley and Carter 1953).

The use of a specific powdery mildew resistance gene depends on its effectiveness and the agronomic performance of wheat cultivars containing it. Therefore, not all identified genes are easily used in developing cultivars due to linkage drag (Summers and Brown 2013). ‘Liangxing 66’ and ‘Wennong 14’ are commercial winter wheat cultivars that have broad-spectrum resistance to powdery mildew in northern China. Resistance in these cultivars was reportedly controlled by single dominant genes tentatively designated *PmLX66* and *PmW14* on chromosome 5DS, on which *Pm2* is located (Huang et al. 2012; Song et al. 2014).

As a follow-up study, the present allelism test was conducted using large segregating populations derived from the crosses between Liangxing 66, Wennong 14, and ‘Ulka/8*Chancellor’ (Ulka/8*Cc), a near-isogenic line with known *Pm2* produced by repeatedly backcrossing ‘Ulka’ with the recurrent parent ‘Chancellor’ (Briggle 1969). The reactions of Liangxing 66 and Wennong 14 to additional *B. graminis* f. sp. *tritici* isolates collected from northern China were examined. The objectives of this study were to elucidate the relationship between *PmLX66*, *PmW14*, and *Pm2* and determine the effectiveness of these newly identified genes.

Materials and Methods

Plant materials. Winter wheat Liangxing 66, developed from the cross ‘Ji 91102’ × ‘Ji 935031’ by the Shandong Liangxing Seed Co. Ltd., Ningjin, Shandong, has been commercialized in a wide region of Shandong, Hebei, Shanxi, Henan, Anhui, Jiangsu, and Shaanxi provinces and Tianjin across the Yellow and Huai River Valleys winter wheat zone and the northern winter wheat zone (<http://www.cnvpv.com/>). Wennong 14, a winter wheat cultivar produced by

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Table 1. Infection types produced on seedlings of Liangxing 66 (*PmLX66*), Wennong 14, Ulka/8**Cc* (*Pm2*), and Tabasco (*Pm48*) and virulence frequencies on different known powdery mildew resistance genes of a set of *Blumeria graminis* f. sp. *tritici* isolates originating from different wheat-producing regions of China

Isolates	Origin	Avirulent/virulent on <i>Pm</i> genes ^c	Liangxing 66 (<i>PmLX66</i>)	Wennong 14 (<i>PmW14</i>)	Ulka/8* <i>Cc</i> (<i>Pm2</i>)	Tabasco (<i>Pm48</i>)	Chancellor	Zhongzuo 9504	Virulence frequency (%)
Bg44-4	Zhanhua, Shandong	1c,2,3,3b,3g,4a,4b,5e,6,13,21,24, 33/1a,3c,5a,8,19,46	3	3	0	3	3	4	31.6
Bg44-5	Zhanhua, Shandong	1a,1c,4b,5e,13,17,21,24,33/2, 3,3b,3c,3g,4a,5a,6,7,8,19,46	3	3	3	3	3	3	57.1
Bg44-6	Zhanhua, Shandong	1a,1c,4a,4b,5e,13,19,21,24,33, 46/2,3,3b,3c,3g,5a,6,8	3	3	3	1	3	3	42.1
Bg79-1	Jining, Shandong	1c,5e,13,17,21,24,33/1a,2,3,3b, 3c,3g,4a,4b,5a,7,8,19,46	3	3	3	3	3	4	65.0
Bg79-2	Jining, Shandong	1c,2,5e,8,13,21,24,33,46/1a,3,3b, 3c,3g,4a,4b,5a,6,19	3	3	0	0	3	4	52.6
Bg79-3	Jining, Shandong	1a,1c,2,3b,4a,4b,5e, 6,13,19,21,24,46/3,3c, 3g,5a,7,8,17,33	3	3	0	0	3	3	38.1
Bg80-3	Juxian, Shandong	1a,1c,2,3b,5a,5e, 13,17,21,24,46/3,3c,3g, 4a,4b,7,8,19,33	0	0	0	1	3	3	45.0
Bg81-2	Pingyi, Shandong	1a,1c,2,5e,13,21,24, 33,46/3,3b,3c,3g,4a, 4b,5a,6,7,8,17,19	0	0	0	0	3	3	57.1
Bg81-3	Pingyi, Shandong	1c,2,5e,13,17,21,24,46/1a, 3,3b,3c,3g,4a,4b, 5a,7,8,19,33	0	0	0	0	3	4	60.0
Bg82-1	Shoushan, Shandong	1c,5e,8,13,21,24,46/ 1a,2,3,3b,3c,3g, 4a,4b,5a,6,7,17,19,33	3	0	4	0	3	4	66.7
Bg82-2	Shoushan, Shandong	1c,2,5e,6,8,13,17,21, 24,33,46/1a,3,3b,3c,3g,4a, 4b,5a,7,19	0	0	0	0	3	3	47.6
Bg82-3	Shoushan, Shandong	1c,2,3,3b,3c,3g,4a,4b,5a, 5e,6,13,17,21,24, 33,46/1a,8,19	0	0	0	0	3	3	14.3
Bg83-1	Wendeng, Shandong	1c,3b,3g,4a,4b,5e,6, 13,17,21,24,33,46/ 1a,2,3,3c,5a,7,8,19	3	0	3	0	3	3	38.1
Bg83-2	Wendeng, Shandong	1c,3b,3c,4a,4b,5a,5e, 8,13,17,21,24,33/ 1a,2,3,3g,7,19,46	3	0	3	3	3	3	35.0
Bg84-1	Yuncheng, Shandong	1c,3,3b,4a,4b,8,13, 17,21,24,33,46/ 1a,2,3c,3g,5a,5e,6,7,19	1	3	3	0	3	4	42.9
Bg84-2	Yuncheng, Shandong	1a,1c,2,3b,5e,13, 21,24,46/3,3c,3g,4a, 4b,5a,6,7,8,17,19,33	0	0	0	0	4	4	57.1
Bg84-3	Yuncheng, Shandong	1a,1c,3b,5e,13,17,21, 24,33,46/2,3,3c,3g,4a, 4b,5a,7,8,19	3	0	3	0	3	3	50.0
Bg85-1	Zhaoyuan, Shandong	1a,1c,2,3,4a,4b,21,24, 33,46/3b,3c,3g,5a,5e,6, 7,8,13,17,19	1	0	2	0	4	3	52.4
Bg85-2	Zhaoyuan, Shandong	1a,1c,2,4b,13,17,21, 24,33,46/3,3b,3c,3g, 4a,5a,5e,7,8,19	0	0	0	0	3	4	50.0
Bg85-3	Zhaoyuan, Shandong	1c,2,3,4a,4b,13,21,24, 33,46/1a,3b,3c,3g,5a,5e, 6,7,8,17,19	0	0	0	0	4	4	52.4
Bg88-2	Haiyang, Shandong	1c,3b,5e,21,24,46/1a,2,3,3c, 3g,4a,4b,5a, 6,7,8,13,17,19,33	0	0	3	0	3	4	71.4
Bg88-3	Haiyang, Shandong	1a,1c,2,3b,5e,13,21, 24,46/3,3c,3g,4a,4b, 5a,6,7,8,17,19,33	1	0	0	0	3	4	57.1
Bg57-3	Xingtai, Hebei	1a,1c,2,3,5e,21,24, 46/3b,3c,3g,4a,4b,5a,6,7, 8,13,17,19,33	0	0	0	0	4	4	61.9
Bg57-4	Xingtai, Hebei	1c,2,3,5e,6,13,17,21, 24,46/1a,3b,3c,3g,4a,4b, 5a,7,8,19,33	0	0	0	0	4	4	52.4

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^c 1 = Festival, *Pm1a*; 2 = *Pm1c*; 3 = Ulka/8**Cc*, *Pm2*; 4 = + *Pm3a*; 5 = CI 14121, *Pm3b*; 6 = Sonora/8**Cc*, *Pm3c*; 7 = Courtot, *Pm3g*; 8 = Khaphi/8**Cc*, *Pm4a*; 9 = VPM1, *Pm4b*; 10 = CI 14125, *Pm5a*; 11 = Fuzhuang 30, *Pm5e*; 12 = Coker 747, *Pm6*; 13 = CI 141879, *Pm7*; 14 = PI 361879, *Pm8*; 15 = 96-282, *Pm13*; 16 = Amigo, *Pm17*; 17 = 96-286, *Pm19*; 18 = R77/6*Bainong 3217, *Pm21*; 19 = Chiyacao, *Pm24*; 20 = 2636-32R, *Pm33*; 21 = Tabasco, *Pm48*.

Table 1. (continued from preceding page)

Isolates	Origin	Avirulent/virulent on <i>Pm</i> genes ^z	Liangxing 66 (<i>PmLX66</i>)	Wenlong 14 (<i>PmW14</i>)	Ulka/8*Cc (<i>Pm2</i>)	Tabasco (<i>Pm48</i>)	Chancellor	Zhongzuo 9504	Virulence frequency (%)
Bg57-5	Xingtai, Hebei	1a,1c,2,4a,4b,5e,8,13, 17,21,24,33/ 3,3b,3c,3g,5a,7,19,46	0	0	0	3	3	4	40.0
Bg69-1	Cixian, Hebei	1a,1c,2,3b,3c,4b,5e,13,21,24,33, 46/3,3g,4a,5a,6,7,8,19	0	0	0	0	3	4	40.0
Bg69-2	Cixian, Hebei	1a,1c,2,3b,3c,5e,13, 17,21,24,46/3,3g,4a, 4b,5a,7,8,19,33	0	0	0	0	3	4	50.0
Bg69-3	Cixian, Hebei	1a,1c,2,3b,3g,5a, 5e,8,13,17,21,24, 33,46/3,3c,4a,7,19	0	0	0	0	3	4	31.6
Bg70-1	Gucheng, Hebei	1a,1c13,21,24, 46/2,3,3b,3c,3g, 4a,4b,5a,5e,6, 7,8,17,19,33	0	0	4	0	4	4	71.4
Bg70-2	Gucheng, Hebei	1a,1c,2,13,21,24,33, 46/3,3b,3c,3g,4a,4b,5a, 5e,6,7,8,17,19	0	0	0	0	4	4	66.7
Bg70-3	Gucheng, Hebei	1a,1c,2,5e,21,24, 46/3,3b,3c,3g,4a,4b,5a, 6,7,8,13,17,19,33	0	0	0	0	4	4	71.4
Bg70-?	Gucheng, Hebei	1a,1c,2,5e,13,21, 24/3,3b,3c,3g,4a,4b, 5a,7,8,17,19,33,46	0	0	0	3	3	4	70.0
Bg71-2	Shahe, Hebei	1a,1c,2,5e,6,13, 21,24,46/3,3b,3c,3g, 4a,4b,5a,8,19,33	0	0	0	0	3	3	52.6
Bg71-3	Shahe, Hebei	1a,1c,2,6,13,21,24, 46/3,3b,3c,3g,4a,4b,5a, 5e,7,8,17,19,33	0	0	0	0	4	4	61.9
Bg72	Shijiazhuang, Hebei	1a,1c,2,3b,4a,4b,5e, 6,21,24,33,46/3,3c, 3g,5a,8,19	0	0	0	0	3	3	33.3
Bg73-2	Yuanshi, Hebei	1a,1c,2,3,5e,21,24,46/ 3b,3c,3g,4a,4b,5a,6,7,8, 13,17,19,33	0	0	0	0	4	4	61.9
Bg73-3	Yuanshi, Hebei	1a,1c,2,4b,5e,6,13, 19,21,24,46/3,3b,3c,3g, 4a,5a,8,33	0	0	0	0	3	3	47.4
Bg74-1	Zhuozhou, Hebei	1a,1c,2,3g,4a,4b,5e,13, 21,24,33,46/3,3b,3c,5a, 6,7,8,19	0	0	0	0	3	3	40.0
Bg74-2	Zhuozhou, Hebei	1a,1c,2,4b,5e,13,17,21, 24,33,46/3,3b,3c,3g,4a, 5a,7,8,19	0	0	0	0	3	4	45.0
Bg74-3	Zhuozhou, Hebei	1a,1c,2,3,4a,4b,5e, 13,21,24,33,46/3b,3c,3g, 5a,6,7,8,19	1	0	0	0	3	4	42.9
Bg75-1	Xunxian, Henan	1a,1c,2,3b,5e,13,17,21, 24,46/3,3c,3g,4a,4b,5a, 6,8,19,33	0	0	0	0	3	4	47.6
Bg75-2	Xunxian, Henan	1a,1c,2,5e,8,13,21,24, 46/3,3b,3c,3g,4a,4b,5a, 6,7,19,33	0	0	0	0	3	3	60.0
Bg75-3	Xunxian, Henan	2,3b,5e,13,21,24, 46/1a,1c,3,3c,3g,4a,4b, 5a,6,8,19,33	3	0	0	0	3	3	57.9
Bg76-1	Lankao, Henan	5e,6,21,24,46/1a,1c,2, 3,3b,3c,3g,4a,4b, 5a,7,8,13,19,33	3	0	4	0	4	4	71.4
Bg76-3	Lankao, Henan	1a,1c,2,3,3b,3g,4a, 4b,5e,19,21,24,33, 46/3c,5a,6,8,13	0	0	0	0	3	3	26.3
Bg77-1	Xihua, Henan	3b,3c,5a,5e,13,17, 21,24,46/1a,1c,2,3,3g,4a, 4b,6,7,8,19,33	3	1	3	1	3	4	47.6
Bg77-3	Xihua, Henan	1a,1c,2,5e,13,17, 21,24/3,3b,3c,3g,4a, 4b,5a,6,7,8,19,33	0	0	0	ND	3	3	60.0

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Table 1. (continued from preceding page)

Isolates	Origin	Avirulent/virulent on <i>Pm</i> genes ^a	Liangxing 66 (<i>PmLX66</i>)	Wenong 14 (<i>PmW14</i>)	Ulka/8* <i>Cc</i> (<i>Pm2</i>)	Tabasco (<i>Pm48</i>)	Chancellor	Zhongzuo 9504	Virulence frequency (%)
Bg78-1	Xinxiang, Henan	1a,1c,2,3,3b,5e,6, 21,24,46/3c,3g,4a,4b,5a, 7,8,13,17,19,33	0	0	0	0	4	4	52.4
Bg78-2	Xinxiang, Henan	1a,1c,2,5e,8,13,21, 24/3,3b,3c,3g,4a,4b,5a, 6,7,17,19,33	0	0	1	0	3	3	61.9
Bg78-3	Xinxiang, Henan	1a,1c,2,3,3b,3c,3g,4a,4b, 5a,5e,6,13,17,19,21, 24,33,46/8	0	0	0	0	3	3	9.5
Bg68-1	Beijing	1a,1c,2,3b,3c,4a,4b, 5e,6,13,17,21,24,33, 46/3,3g,5a,7,8,19	0	0	0	0	3	3	28.6
Bg68-2	Beijing	1a,1c,2,4a,4b,5e,6,13,21, 24,33,46/3,3b,3c,3g,5a, 7,8,19	0	0	0	0	3	4	40.0
Bg68-3	Beijing	1a,1c,2,3b,4a,5a,5e,6, 13,21,24,33,46/3,3c,3g, 4b,7,8,17,19	0	0	0	0	3	3	47.6
Bg87	Beijing	1a,1c,2,3b,4a,4b,5e,13, 21,24,33,46/3,3c,3g,5a, 6,7,8,17,19	0	0	0	0	3	4	47.6
Bg91-2	Beijing	1a,1c,5e/2,3,3b,3c,3g, 4a,4b,5a,6,7,8, 17,19,21,24,33	0	3	4	ND	4	4	84.2
Bg86-1	Yangzhou, Jiangsu	1a,1c,2,5e,8,13,21,24, 33,46/3,3b,3c,3g,4a,4b, 5a,7,17,19	0	0	0	0	3	4	50.0
Bg86-2	Yangzhou, Jiangsu	1c,2,3b,5e,13,21, 24,33,46/1a,3,3c,3g,4a, 4b,5a,7,8,17,19	3	0	0	2	3	4	55.0
Bg86-3	Yangzhou, Jiangsu	1a,1c,2,3,4a,4b,6, 17,21,24,33,46/3b,3c,3g, 5a,5e,7,8,13,19	0	0	0	0	3	4	42.9
Bg89-1	Wenjiang, Sichuan	1a,1c,2,3,4b,5e, 21/3b,3c,3g,4a,5a,6, 7,8,17,19,24,33	2	3	0	ND	3	4	68.4
Bg90-1	Wenjiang, Sichuan	1a,1c,2,5e,7,17, 21/3,3b,3c,3g,4a,4b,5a, 6,8,19,24,33	0	0	0	ND	3	4	63.2

the Wennong Seed Co. Ltd., Tai'an, Shandong, has been commercialized in the northern part of the Yellow and Huai River Valleys winter wheat zone (Chen and Sun 2012) and the provincial level in Shandong (Sun et al. 2011). Wheat line Ulka/8**Cc*, with *Pm2* (Briggle 1969), and the German wheat 'Tabasco', carrying gene *Pm48* (which was redesignated from *Pm46*) (Gao et al. 2012), were used for comparison purposes.

A set of 21 wheat genotypes that carry single *Pm* genes was used to determine the avirulence or virulence arrays of *B. graminis* f. sp. *tritici* isolates. Chancellor and 'Zhongzuo 9504' wheat were used as susceptible controls and the latter was also used to maintain and increase the *B. graminis* f. sp. *tritici* isolates. To determine allelic relationships, Liangxing 66, Wennong 14, and Ulka/8**Cc* were intercrossed to produce segregating F₂ populations.

***B. graminis* f. sp. *tritici* isolates.** Sixty *B. graminis* f. sp. *tritici* isolates were collected from fields across different parts of northern China and used to test lines with *PmLX66*, *PmW14*, *Pm2*, and *Pm48* (Table 1). Single-pustule progeny of each isolate were made three times prior to disease assessments. Isolates were maintained on seedlings of Zhongzuo 9504. In a previous study, the resistance to *B. graminis* f. sp. *tritici* isolate E20 (avirulence/virulence formula: *Pm1c*, *Pm2*, *Pm4a*, *Pm4b*, *Pm4c*, *Pm5e*, *Pm12*, *Pm13*, *Pm16*, *Pm20*, *Pm21*, *Pm24*, *Pm33*, and *Pm43/Pm1a*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3g*, *Pm5a*, *Pm6*, *Pm7*, *Pm8*, *Pm17*, *Pm19*, and *Pm40*) (Zhao et al. 2013) was controlled by single dominant genes in Liangxing 66 and Wennong 14 as demonstrated with the F₂ segregating populations derived from the crosses between resistant Liangxing 66 and Wennong 14 and

susceptible 'Jingshuang 16' (Song et al. 2012). Thus, isolate E20 was used to inoculate the F₂ populations in the allelism tests.

Molecular marker analysis. Total genomic DNA was extracted from young seedling leaves using the cetyltrimethylammonium bromide protocol (Saghai-Marooof et al. 1984). Sequence-characterized amplified region (SCAR) marker *SCAR203* (forward: 5'-TAAACCCTGAGAATGCAG-3', reverse: 5'-GAGACCATGAGACCCACC-3') and simple-sequence repeat (SSR) marker *Xcfd81* (forward: 5'-TATCCCCAATCCCCCTCTTTC-3', reverse: 5'-GTCAATTGTGGCTTGTCCT-3'), closely linked to *PmLX66*, *PmW14*, and *Pm2* (Li et al. 2009; Huang et al. 2012; Song et al. 2014), were used to examine Liangxing 66, Wennong 14, and Ulka/8**Cc*. Each reaction mixture (10 µl) consisted of 20 ng of template DNA, 0.1 µM each primer, 0.2 mM dNTPs, 1× polymerase chain reaction (PCR) buffer, 1.5 mM MgCl₂, and 1 U of *Taq* DNA polymerase. Amplification of DNA was carried out in a Biometra T3000 Thermocycler (ABL, New York) with the following conditions: 94°C for 4 min, followed by 38 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min. The reaction was terminated after a final extension at 72°C for 10 min and then maintained at 4°C. The PCR products were separated on 8% nondenatured polyacrylamide gels with a 29:1 ratio of acrylamide and bisacrylamide, and visualized by silver staining.

Allelism tests. Seedlings of the F₂ populations were inoculated with the *B. graminis* f. sp. *tritici* isolate E20 when the first leaves were fully unfolded by brushing the conidiospores from susceptible seedlings of Zhongzuo 9504. Approximately 15 day after inoculation, infection types (IT) on the primary leaf of each plant was scored on a scale of 0 to 4.

Fig. 1. Amplification profile by markers *SCAR203* and *Xcfd81* from Liangxing 66, Wennong 14, Ulka/8*Cc, and Zhongzuo 9504. Arrow indicates the target bands specific for *Pm2*.

Table 2. Analysis of allelism relationships between powdery mildew resistance genes *PmLX66*, *PmW14*, and *Pm2* when tested against *Blumeria graminis* f. sp. *tritici* isolate E20 in the F_2 populations derived from Liangxing 66 (*PmLX66*), Wennong 14 (*PmW14*), and Ulka/8*Cc (*Pm2*)

Cultivar or cross	N ^z	Infection type		
		0	1	3 or 4
Liangxing 66 (<i>PmLX66</i>)	20	20	0	...
Wennong 14 (<i>PmW14</i>)	20	20	0	...
Ulka/8*Cc (<i>Pm2</i>)	20	0	20	...
Zhongzuo 9504 (control)	20	0	0	20
Liangxing 66 × Ulka/8*Cc	1,344	1,124	220	0
	2,275	1,955	320	0
Wennong14 × Ulka/8*Cc	2,072	1,968	104	0
	4,113	3,128	985	0
Liangxing 66 × Wennong 14	1,571	1,507	64	0
	4,282	3,770	512	0

^z Total number of plants.

Reaction to *B. graminis* f. sp. *tritici* isolates. A detached-leaf method was used to compare reactions to different *B. graminis* f. sp. *tritici* isolates in Liangxing 66 (*PmLX66*), Wennong 14 (*PmW14*), Ulka/8*Cc (*Pm2*), and Tabasco (*Pm48*) (Limpert et al. 1988). Three leaf segments from different seedlings of each genotype were embedded in media on plates containing agar at 6 g liter⁻¹ supplemented with benzimidazole at 50 mg liter⁻¹ and inoculated separately with *B. graminis* f. sp. *tritici* isolates by dusting conidiospores on susceptible seedlings of Zhongzuo 9504 prior to incubation at 18 to 21°C in a growth cabinet.

IT on each leaf segment was scored on a 0-to-4 scale, where 0 = no visible symptom, 0; = necrotic flecks, 1 = necrosis with low sporulation, 2 = necrosis with moderate sporulation, 3 = no necrosis with moderate to high sporulation, and 4 = no necrosis with full sporulation, and where 0 is immune and 4 represents heavy sporulation (Xiao et al. 2013). The phenotype of each wheat line to each isolate was categorized as either resistant (IT 0 to 2) or susceptible (IT 3 to 4) and the experiment was conducted twice.

Results

Molecular marker analysis of the segregating populations. SCAR marker *SCAR203* and SSR marker *Xcfd81*, which were tightly linked to *PmLX66*, *PmW14*, and *Pm2*, amplified the same band from Liangxing 66, Wennong 14, and Ulka/8*Cc (Fig. 1). These bands were slightly smaller than that from susceptible Zhongzuo 9504. *SCAR203* was used to confirm the genotypes of selected F_2 segregating populations used in allelism tests. The amplified fragment specific to *Pm2* was present in 64, 70, and 82 random F_2 plants derived from the crosses Liangxing 66 × Ulka/8*Cc, Wennong 14 × Ulka/8*Cc, and Liangxing 66 × Wennong 14, respectively (data not shown).

Allelism test of *PmLX66*, *PmW14*, and *Pm2*. When inoculated with *B. graminis* f. sp. *tritici* isolate E20, Liangxing 66 and Wennong 14 showed a resistant reaction with IT 0, whereas Ulka/8*Cc produced IT 1. To determine the allelic relationship between *PmLX66*, *PmW14*, and *Pm2*, phenotypes of the F_2 plants derived from the crosses between these wheat genotypes were determined in two separate tests using *B. graminis* f. sp. *tritici* isolate E20 (Table 2). In the first test, 1,344 F_2 plants from the cross Liangxing 66 × Ulka/8*Cc produced 1,124 plants with IT 0 and 220 plants with IT 1. In the second test, all of the 2,275 F_2 plants from this cross had a resistant phenotype (IT 0 or 1) and none was susceptible. Among the 2,072 plants from the cross Wennong 14 × Ulka/8*Cc in the first test, 1,968 plants produced IT 0 and 104 plants had IT 1. None of them was susceptible. In the second test, all of the 4,113 F_2 plants from this cross had IT 0 or 1. In total, 5,853 F_2 plants originating from the cross Liangxing 66 × Wennong 14 were tested against *B. graminis* f. sp. *tritici* isolate E20 in two separate tests. Most plants produced IT 0 and a small proportion of plants had IT 1. No plants were found to have a susceptible

Fig. 2. Frequency of isolates from Shandong and other provinces in China that were avirulent on Liangxing 66 (*PmLX66*), Wennong 14 (*PmW14*), Ulka/8*Cc (*Pm2*), and Tabasco (*Pm48*).

phenotype. In total, none of the 15,657 F₂ plants from the three crosses examined was susceptible to isolate E20. These results indicate that genes *PmLX66* and *PmW14* in Liangxing 66 and Wennong 14, respectively, were allelic to *Pm2* in Ulka/8**Cc*, and *PLX66* and *PmW14* are also allelic with each other.

Reactions of Liangxing 66, Wennong 14, Ulka/8Cc*, and Tabasco to *B. graminis* f. sp. *tritici*.** Sixty isolates were inoculated separately to wheat lines carrying 21 different *Pm* genes and the susceptible controls Chancellor and Zhongzuo 9504 (Table 1). A small percentage of isolates (approximately 1.7 to 5.0%) were virulent on cultivars possessing *Pm1c*, *Pm21*, and *Pm24*. The percentage of isolates that were virulent on cultivars carrying *Pm5e* and *Pm13* were 13.3 and 16.1%, respectively. Over 80% of isolates were virulent on cultivars carrying *Pm3c*, *Pm3g*, *Pm5*, *Pm7*, *Pm8*, and *Pm19*. A range of 46.7 to 76.7% of isolates were virulent on cultivars with *Pm3*, *Pm3b*, *Pm4a*, *Pm4b*, *Pm6*, *Pm17*, and *Pm33*. Isolates Bg91-2 (Beijing) was virulent on all genes except *Pm1c* and *Pm5e*, isolate Bg78-3 (Xinjiang, Henan province) was avirulent on all genes except *Pm7* and *Pm8*, and isolate Bg82-3 (Shoushan, Shandong province) was avirulent on all the genes except *Pm1*, *Pm8*, and *Pm19* (Table 1). All the *B. graminis* f. sp. *tritici* isolates were virulent on the susceptible controls Chancellor and Zhongzuo 9504, with IT 3 or 4.

The avirulence frequencies of isolates on Liangxing 66 (*PmLX66*), Wennong 14 (*PmW14*), Ulka/8**Cc* (*Pm2*), and Tabasco (*Pm48*) were 76.7% (46/60 isolates), 85.0% (51/60 isolates), 78.3% (47/60 isolates), and 89.3% (50/56 isolates), respectively (Fig. 2). Both Liangxing 66 and Wennong 14 were developed in Shandong province; therefore, 22 *B. graminis* f. sp. *tritici* isolates collected from that province were tested. Among them, the frequencies of *B. graminis* f. sp. *tritici* isolates from Shandong province that were avirulent on Liangxing 66, Wennong 14, Ulka/8**Cc*, and Tabasco were 54.5, 68.2, 59.1, and 81.8%, respectively. These wheat genotypes were even more effective against the *B. graminis* f. sp. *tritici* isolates from other provinces than those from Shandong province.

Among the 60 *B. graminis* f. sp. *tritici* isolates examined, Liangxing 66 produced the same infection types as Ulka/8**Cc* in reaction to 51 isolates but differed from Ulka/8**Cc* in reaction to 9 isolates (Table 1). Four isolates were avirulent on Liangxing 66 (IT 0 or 1) but virulent on Ulka/8**Cc* (IT 3 or 4), and five isolates were virulent on Liangxing 66 (IT 3 or 4) but avirulent on Ulka/8**Cc* (IT 0). Liangxing 66 displayed the same phenotypes as Tabasco in reaction to 44 *B. graminis* f. sp. *tritici* isolates out of 56 isolates tested. These two cultivars exhibited different IT in response to 12 isolates, and Tabasco was resistant to all except 2 isolates.

Wennong 14 differed from Ulka/8**Cc* in reaction to 12 *B. graminis* f. sp. *tritici* isolates. Seven isolates were avirulent on Wennong 14 but virulent on Ulka/8**Cc*. Isolate Bg77-1 resulted in IT 1 on Wennong 14 but IT 3 on Ulka/8**Cc*. In contrast, Wennong 14 and Ulka/8**Cc* had IT 3 and 0, respectively, in their reactions to four isolates. Among the eight *B. graminis* f. sp. *tritici* isolates, which had different infection types between Wennong 14 and Tabasco, three isolates exhibited IT 0 and 3, while five isolates produced IT 3 and 0 on Wennong 14 and Tabasco, respectively.

Liangxing 66 and Wennong 14 differed in their reactions to 11 *B. graminis* f. sp. *tritici* isolates. Wennong 14 was resistant to 8 of 11 isolates (IT 0 or 1) but susceptible to 3 isolates (IT 3 or 4). However, Liangxing 66 was susceptible to all of these *B. graminis* f. sp. *tritici* isolates except for isolates Bg84-1, Bg89-1, and Bg91-2. Of 56 isolates examined, Liangxing 66 and Wennong 14 were different from Tabasco in the reactions to 13 and 8 isolates, respectively. In addition, isolate Bg44-5 originating from Zhanhua, Shandong province, was virulent, whereas 37 isolates were avirulent on the four genotypes.

Discussion

Previous studies have demonstrated that the dominant genes *PmLX66* and *PmW14* were present in Liangxing 66 and Wennong 14 and molecular marker analyses indicated that *PmLX66* and *PmW14* shared the same locus as *Pm2* on chromosome 5DS on the basis of linked markers *Xcfd81* and *SCAR203*. Allelism tests carried out in the present work indicated that the three genes were allelic.

Given the different responses to various *B. graminis* f. sp. *tritici* isolates found in the present work as well as in earlier studies (Huang et al. 2012; Song et al. 2014), *PmLX66* and *PmW14* must be new alleles of *Pm2* locus.

In addition to Liangxing 66 and Wennong 14, *Pm* genes on chromosome 5DS have been identified in several Chinese wheat lines; for example, 'D57' (Ma et al. 2011), 'Nongda 399' (Li et al. 2013), and 'X3986-2' (Ma et al. 2014), as well British 'Brock' wheat (Li et al. 2009; Lillemo et al. 2010). The close linkages between these powdery mildew resistance genes and *Pm2* was established based on common linked molecular markers such as *Xcfd81*. Study of a cross between Tabasco and Ulka/8**Cc* showed that the gene *Pm48* in Tabasco was different from *Pm2* despite their common molecular marker *Xcfd81* (Gao et al. 2012).

Pm2 has been widely utilized in breeding programs in China and Europe (Bennett 1984; Liu et al. 2000). High virulence frequencies for *Pm2* in *B. graminis* f. sp. *tritici* isolates were reported in some areas of the United States (Niewoehner and Leath 1998; Parks et al. 2008; Persaud and Lipps 1995) and China (Shi et al. 2009). In a recent study, the virulence frequency in *B. graminis* f. sp. *tritici* isolates from different regions of China on Ulka/8**Cc* varied from 31.8% (north China) to 58.3% (Xinjiang) (Zeng et al. 2014). In previous studies, Liangxing 66 and Wennong 14 were resistant to approximately 60% (25 of 42) and 82.7% (43 of 52) of isolates, respectively, from northern China (Huang et al. 2012; Song et al. 2014). In this study, Liangxing 66 and Wennong 14 conferred comparable or slightly higher frequencies and different arrays of resistance compared with Ulka/8**Cc* to a set of 60 *B. graminis* f. sp. *tritici* isolates from different parts of China. Additionally, Liangxing 66 and Wennong 14 are commercial cultivars and more adaptive to the local environments than Ulka/8**Cc*.

In summary, genes *PmLX66* and *PmW14* in Liangxing 66 and Wennong 14 winter wheat, previously mapped on chromosome 5DS, were effective against over 70% of the *B. graminis* f. sp. *tritici* isolates originating from wheat-producing regions in China where they are commercialized. These genes confer different reaction patterns in response to *B. graminis* f. sp. *tritici* isolates compared with the *Pm2* gene derived from Ulka, with which they are allelic. Because Liangxing 66 and Wennong 14 are widely grown commercial winter wheat cultivars and free of linkage drag, the identification of genes for resistance to powdery mildew will facilitate their application in developing disease-resistant cultivars or in combination with other powdery mildew resistance genes for effectively controlling the disease in wheat production.

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