

Identification of stem rust resistance genes in wheat cultivars in China using molecular markers

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ABSTRACT

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), is a major disease that has been effectively controlled using resistance genes. The appearance and spread of *Pgt* races such as Ug99, TKTTE, and TTTTE, which are virulent to most stem rust-resistant genes currently deployed in wheat breeding programs, renewed the interest in breeding cultivars resistant to wheat stem rust. It is therefore important to investigate the levels of resistance or vulnerability of wheat cultivars to *Pgt* races. Resistance to *Pgt* races 21C3CTHQM, 34MKGQM, and 34C3RTGQM was evaluated in 136 Chinese wheat cultivars at the seedling stage. A total of 124 cultivars (91.2%) were resistant to the three races. Resistance genes *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38* were analyzed using molecular markers closely linked to them, and 63 of the 136 wheat cultivars carried at least one of these genes: 21, 25, and 28 wheat cultivars likely carried *Sr2*, *Sr31*, and *Sr38*, respectively. Cultivars “Kehan 3” and “Jimai 22” likely carried *Sr25*. None of the cultivars carried *Sr24* or *Sr26*. These cultivars with known stem rust resistance genes provide valuable genetic material for breeding resistant wheat cultivars.

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INTRODUCTION

Wheat stem rust caused by *Puccinia graminis* Per. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is a devastating disease that has caused severe yield losses worldwide. Since the deployment of stem rust-resistant wheat cultivars in the second half of the 20th century, stem rust has been successfully controlled in most wheat cultivating areas ([Chen et al., 2015](#)). However, a new race of the stem rust pathogen (Ug99), identified in Uganda in 1999 and highly virulent to resistance gene *Sr31*, was designated as TTKSK under the North American nomenclature system ([Pretorius et al., 2000](#)). Within a few years, virulence of TTKSK to other important stem rust resistance genes (e.g., *Sr24*, *Sr36*, *Sr9h*, *Sr31* + *Sr24*, *Sr31* + *Sr36*, and *Sr31* + *SrTmp*) was detected ([BGRI, 2017](#); [Jin et al., 2008, 2009](#); [Pretorius et al., 2012](#); [Rouse et al., 2014](#)), and 13 variants of Ug99 have now been documented across wheat growing regions in 13 countries ([Food and Agriculture Organization of the United Nations \(FAO\), 2017](#)).

Realizing the disastrous threat on world food security posed by the Ug99 race group, Nobel Peace Prize laureate Norman Borlaug called for a coordinated global campaign to reduce wheat rust epidemics and mitigate the potential impact on food security. The resistance of worldwide wheat accessions (over 200,000) to the Ug99 group was screened in Kenya (He, Xia & Chen, 2008). The results indicated that only 5–15% of the wheat accessions grown globally were resistant to Ug99, and only two of the 118 Chinese wheat cultivars (“Jimai 20” and “Linmai 6”) were resistant to Ug99. The high susceptibility (85–95%) of wheat lines to Ug99 highlighted the potential threat of this group to wheat production worldwide. Furthermore, other broadly virulent *Pgt* races caused wheat stem rust epidemics in recent years. The new race TKTTF (from a genetic lineage distinct from that of Ug99) virulent to the widely grown wheat cultivar “Digalu,” caused yield losses close to 100% in Southern Ethiopia during 2013–2014 (Olivera et al., 2015). In 2016, a new and unusually devastating strain of *Pgt* named TTTTF (virulent to *Sr9e* and *Sr13*) caused the largest outbreak and epidemics of wheat stem rust in Sicily since the 1950s (Bhattacharya, 2017), as tens of thousands of hectares of both durum wheat and bread wheat were infected. Thus, wheat stem rust seems to have returned.

The most effective way to control wheat stem rust is by using resistant genes against this disease to breed and propagate resistant varieties (Pathan & Park, 2007). However, an important issue in the use of resistant varieties is that the simplification of the resistance source may be overcome by variation in the pathogen, resulting in the loss of resistance. Understanding resistance gene content of wheat varieties can effectively avoid this situation, and provide a basis for the reasonable distribution of varieties. Moreover, it is also helpful to discover new genes, enriching the gene pool, and for breeding resistant varieties. Nevertheless, the spread of new *Pgt* races and their variants threatens the safety of wheat production in China (Li et al., 2016). If the conditions are suitable, there is the possibility of wheat stem rust becoming a significant disease. Because the resistance of Chinese wheat varieties to the new races Ug99, TKTTF, and TTTTF is very poor, if these races spread into China they will cause massive losses in wheat production (Cao et al., 2007). We should therefore make full use of wheat cultivar resources to screen for resistant materials. Given the importance of understanding disease resistance genes, those against Ug99, TKTTF, and TTTTF races have been screened and identified worldwide since these races were reported.

In our previous study, the prevalence of *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38* in wheat cultivars from Gansu and Yunnan Province has been finished (Li et al., 2016; Xu et al., 2017), and based on it, this study was carried out. We collected 136 wheat cultivars from two different localities presenting epidemic patterns of wheat stem rust to examine their resistance level to the predominant races of *Pgt* in China. Resistance genes *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38* were detected using molecular markers aiming to screen and identify cultivars that are potentially resistant to emerging races (especially to Ug99, TKTTF, and TTTTF) and map the distribution of those genes in wheat regions based on wheat cultivars’ resistance level to predominant races of *Pgt*. Thus far, our team have identified and characterized these resistant genes in four wheat-producing regions of

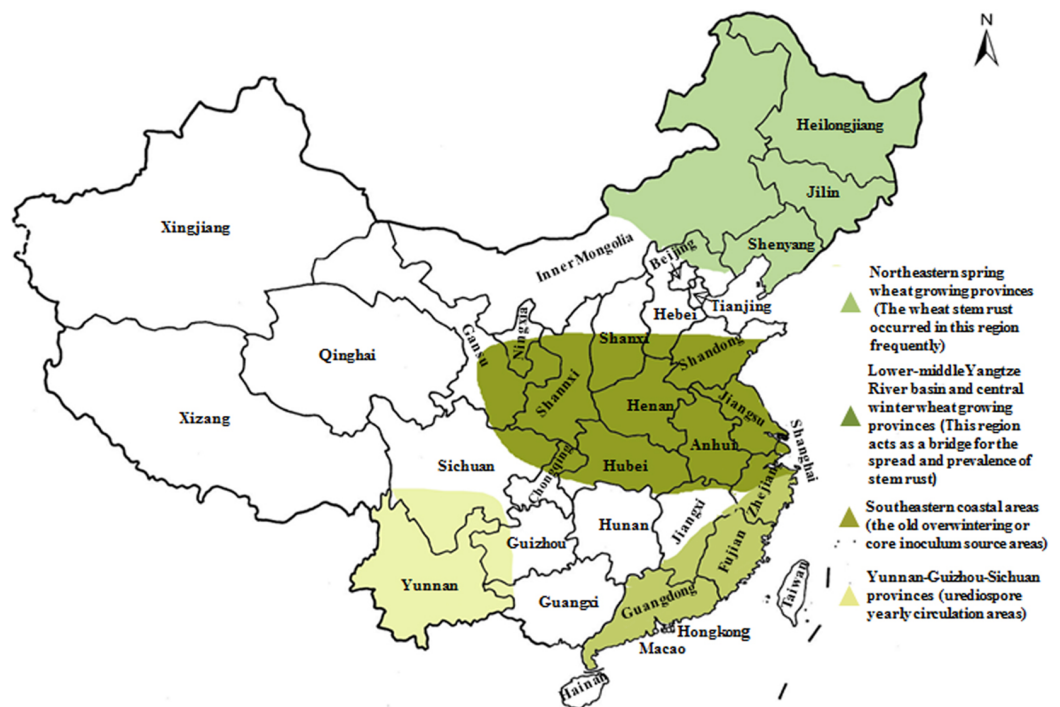


Figure 1 Epidemic patterns of wheat stem rust in four wheat-producing regions in China.

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China (Fig. 1), which will contribute to the deployment of wheat stem rust resistance genes and control of large-scale epidemics of this disease. Additionally, this information will be important for developing potentially durable combinations of stem rust resistance genes in wheat cultivars.

MATERIALS AND METHODS

Wheat cultivars (lines) and *Pgt* races

A total of 136 wheat cultivars (lines) were collected from the largest wheat growing regions in China: the Northeastern spring-wheat growing provinces and the lower-middle Yangtze River basin and central winter-wheat growing provinces. All wheat accessions were provided by researchers from Heilongjiang, Inner Mongolia, Shandong, Shanxi, Anhui, Jiangsu, Beijing, and Ningxia Academies of Agricultural Sciences. A total of six monogenic wheat lines carrying individual *Sr* genes (*Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38*), and 29 differentials for *Pgt*, including the original four Stakman differentials (Little Club, Reliance, Einkorn, and Vernal), five Chinese differentials (Mianzi 52, Huadong 6, Mini 2761, Orofen, and Rulofen), and 20 single *Sr*-gene lines from North America (*Sr5*, *Sr21*, *Sr9e*, *Sr7b*, *Sr11*, *Sr6*, *Sr8a*, *Sr9g*, *Sr36*, *Sr9b*, *Sr30*, *Sr17*, *Sr9a*, *Sr9d*, *Sr10*, *SrTmp*, *Sr24*, *Sr31*, *Sr38*, and *SrMcN*) used worldwide, were provided by the Plant Immunity Institute, Shenyang Agricultural University, China.

Races 21C3CTHQM (*Pgt* isolate Ab3), 34MKGQM (*Pgt* isolate H31), and 34C3RTGQM (*Pgt* isolate XN11) (a new race identified from the alternative host *Berberis*

sp.) were used for evaluating seedling stem rust response in the tested cultivars. These races were isolated and identified by the Plant Immunity Institute, Shenyang Agricultural University, China. The names, virulence/avirulence spectrums, and urediniospores produced method of races were described by *Li et al. (2016)* and *Xu et al. (2017)*.

Seedling infection types

The 136 wheat accessions were planted in 10-cm diameter porcelain pots (each pot contained one cultivar represented by eight to 10 seedlings). A mixture of urediniospores and dried talc (1 g), in a ratio of 1:20 (v:v), was sprayed onto the fully expanded primary leaves of seedling (seven to eight days old) moistened with 0.05% Tween-20. The detail inoculation and cultivation methods followed *Xu et al. (2017)*. Three replicates of the seedling assays were performed for each *Pgt* race. Infection types (ITs) were assessed two weeks after inoculation using the 0–4 IT scale, as described by *Stakman, Stewart & Loegering (1962)*.

DNA extraction

DNA was extracted from the young leaves of seven-day old seedlings grown to the one-leaf stage, using a DNA extraction kit (Sangon Biotech, Shanghai, China). PCR amplifications were followed *Xu et al. (2017)*. Primers were synthesized by Sangon Biotech (China) ([Table 1](#)), and PCR amplification conditions were as described in previous studies ([Table 1](#)). Fragments of the targeted genes were detected by electrophoresis using 2% (W/V) agarose gels and then gels were observed under UV light.

RESULTS

Wheat seedling resistance

The ITs produced by wheat cultivars to races 21C3CTHQ, 34MKGQM, and 34C3RTGQM are listed in [Table 2](#). A total of 124 (91.2%) wheat cultivars were resistant to the three races (ITs 2, 1+, or lower) while the remaining 12 were susceptible (ITs 3-, 3, 3+, and 4) ([Fig. 2](#)). A total of 48 wheat cultivars (35.3%) showed IT 0 to all tested races ([Fig. 2](#)) and 127 showed resistance to the new race 34C3RTGQM.

Detection of stem rust resistance genes using molecular markers

Sr2 screening

The adult plant resistant gene *Sr2*, which provides a durable broad-spectrum to *Pgt* is difficult to screen under field conditions (*Hayden, Kuchel & Chalmers, 2004*). However, the *Sr2*-closely linked microsatellite marker *Xgwm533*, developed by *Hayden, Kuchel & Chalmers (2004)*, typically amplifies a 120-bp fragment from wheat lines known to carry *Sr2*. In the present study, we used this marker to detect *Sr2* and 21 of the 136 wheat varieties showed the *Sr2* fragment ([Fig. 1A](#); [Table 2](#)), suggesting that those wheat varieties carry *Sr2*.

Sr24 screening

Gene *Sr24* is effective against some *Pgt* races in China and it was derived from *Thinopyrum ponticum*. It is widely used in wheat breeding though it has become susceptible to some

Table 1 PCR primers and conditions for the amplification of the tested markers.

Marker	Primers	PCR conditions	
		Temperature (°C)/time	Number of cycles
<i>Xgwm533</i>	5'-GTTGCTTTAGGGGAAAAGCC 5'-AAGGCGAATCAAACGGAATA	92/3 min	One
		92/30 s; 62/30 s; 72/30 s	1 °C Reducing/cycle for seven cycles
		92/30 s; 62/30 s; 72/30 s	47
<i>Sr24#12</i>	5'-CACCCGTGACATGCTCGTA 5'-AACAGGAAATGAGCAACGATGT	94/3 min	One
		94/30 s; 65/30 s; 72/40 s	1 °C Reducing/cycle for seven cycles
		94/30 s; 58/30 s; 72/40 s	30
<i>Gb</i>	5'-CATCCTTGGGGACCTC 5'-CCAGCTCGCATAATCCA	20/1 min	One
		94/3 min	One
		94/30 s; 60/30 s; 72/40 s	30
<i>Sr26#43</i>	5'-AATCGTCCACATTGGCTTCT 5'-CGCAACAAAATCATGCACTA	20/1 min	One
		94/3 min	One
		94/30 s; 56/30 s; 72/40 s	30
<i>SCSS30.2₅₇₆</i>	5'-GTCCGACAATACGAACGATT 5'-CCGACAATACGAACGCCTTG	20/1 min	One
		95/5 min; 60/1 min; 72/30 s	One
		95/1 min; 60/1 min; 72/30 s	35
<i>Iag 95</i>	5'-CTCTGTGGATAGTTACTTGATCGA 5'-CCTAGAACATGCATGGCTGTTACA	72/10 min	One
		94/3 min	One
		94/30 s; 55/60 s; 72/70 s	30
<i>VENTRIUP-LN2</i>	5'-AGGGGCTACTGACCAAGGCT 5'-TGCAGCTACAGCAGTATGTACACAAAA	25/60 s	One
		94/45 s	One
		94/45 s; 65/30 s; 72/7 min	30
		72/1 min	One

Ug99 variants (Jin et al., 2008). Mago et al. (2005) reported that marker *Sr24#12*, linked to *Sr24*, was associated with the 3Ag/1BS Amigo-type translocation, and this marker can amplify a 500-bp fragment in the wheat variety “Westonia/*Sr24*.” Using a diverse collection of wheat germplasm, Yu et al. (2010) showed that this 500-bp PCR fragment was amplified in wheat germplasm carrying *Sr24*. In the present study, 500-bp fragments were amplified in the wheat line “Lc*Sr24Ag*,” suggesting it carries *Sr24* (Fig. 3B) but no fragment was amplified in the other tested varieties (Table 2).

***Sr25* and *Sr26* screening**

Ug99-effective-genes *Sr25* and *Sr26* were transferred into wheat from *T. ponticum*. These two genes were firstly backcrossed into Australian wheat, and some old varieties may carry these genes in China (Cao et al., 1994; Knott, 1961). We used markers *Gb* (amplifies a 130-bp fragment) and *Sr26#43* (amplifies a 207-bp fragment), which are closely linked to genes *Sr25* and *Sr26*, respectively (Liu et al., 2010), to screen these genes in the 136 accessions. The 130-bp fragment was only amplified in Kehan 3 and Jimai 22 (Fig. 3C; Table 2), indicating that only these two wheat varieties carry *Sr25*; the other tested wheat varieties lack *Sr25* and *Sr26* (Fig. 3D; Table 2).

Table 2 Seedling infection types and resistance genes.

Cultivars	Province	Pedigree	Infection types ^a		Sr2	Sr24	Sr25	Sr26	Sr31	Sr38
			21C3CTHQM	34MKGQM						
Xinkehan 9	Heilongjiang	Kefeng 2//Ke74F ₃ -249-3	;1	1	;	-	-	-	-	-
Kehan 2	Heilongjiang	Jiusan 80 jian 119/ Nongda75-65533	;1	1	1	-	-	-	-	+
Kehan 3	Heilongjiang	Ke 61F ₃ -199/ <i>Agropyron glaucum</i>	0	0	0	-	+	-	-	-
Kehan 4	Heilongjiang	Kezhen/Kehong	;1	1	1+	-	-	-	-	+
Kehan 8	Heilongjiang	Ke65F ₂ -196-7/Rulofen	;	0	0	-	-	-	-	-
Kehan 9	Heilongjiang	Kefeng 2//Ke 74F ₃ -249-3	;	0	0	-	-	-	-	-
Kehan 10	Heilongjiang	Kefeng 2//T808/Ke 69-513	;	1	0	-	-	-	-	-
Kehan 11	Heilongjiang	Ke 73-402/Bei 74-205	;	0	0	+	-	-	-	-
Kehan 12	Heilongjiang	Ke 68-88/Ke 68-585-13	;	1	1	-	-	-	-	+
Kehan 13	Heilongjiang	Kefeng 3//Kehan 8	;1	0	0	-	-	-	-	-
Kehan 14	Heilongjiang	Ke 80-10-1/Ke 81 hou 88-0-1	;	1-	1	-	-	-	-	+
Kehan 15	Heilongjiang	Ke 86F ₂ -172/Ke 86F ₅ -325-3	0	0	0	-	-	-	-	-
Kehan 16	Heilongjiang	Jiusan 79F ₅ -541/Ke 80 Yuan 229//Ke 76-750/76F4-779-5//Ke76-413	0	0	0	-	-	-	-	+
Kehan 18	Heilongjiang	Jiusan 1989/Kefeng 5	0	0	0	-	-	-	-	-
Kehan 19	Heilongjiang	Ke 90-99/MY4490	1	0	0	-	-	-	-	+
Kehan 20	Heilongjiang	Ke 89-46/Cundo	;1	0	0	-	-	-	-	+
Kehan 21	Heilongjiang	Ke89F ₆ nan-2/Ke 89F ₁ -1237	1	2	1	-	-	-	-	-
Kefeng 6	Heilongjiang	Ke 85-869/Ke 85-784	;1	;	0	-	-	-	-	-
Kefeng 7	Heilongjiang	Ke 84F ₅ -250-1/84F ₅ -668	;	;	0	-	-	-	-	-
Kefeng 8	Heilongjiang	Kehan 12/Ke 82-371	0	1	;	-	-	-	-	-
Longfu 1	Heilongjiang	Xinshuguang 3/ Liaochun 8	0	1	;	-	-	-	-	-
Longfu 2	Heilongjiang	Longxi 35/Ke 250	;1	1	;	-	-	-	-	-
Longfu 3	Heilongjiang	Longfu 77-4096/S-A-25	0	0	0	-	-	-	-	-
Longfu 4	Heilongjiang	Heiza 266/Ke 79F ₃ -392	;	1	1	-	-	-	-	-
Longfu 5	Heilongjiang	Jiusan B29-/32P	0	0	0	-	-	-	-	-
Longfu 6	Heilongjiang	Longfu 2108/Haishu	0	0	0	-	-	-	-	-
Longfu 7	Heilongjiang	Longfu 3/Gang 98-446	;	0	0	-	-	-	-	-

Cultivars	Province	Pedigree	Infection types ^a		Sr2	Sr24	Sr25	Sr26	Sr31	Sr38
			21C3CTHQ	34MKGQM						
Longfu 8	Heilongjiang	K202 60Coy 1000 Rad	0	0	-	-	-	-	-	-
Longfu 9	Heilongjiang	Kejian 23 60Coy/180Gy	0	0	-	-	-	-	-	-
Longfu 10	Heilongjiang	Ke 87-183 γ1.1 kRad	0	0	-	-	-	-	-	-
Longfu 11	Heilongjiang	Longfu 81-8106 60 Coy 1.1 kRad	0	0	-	-	-	-	-	+
Longfu 12	Heilongjiang	Jia 5 60 Coy	;	0	-	-	-	-	-	-
Longfu 13	Heilongjiang	Unknown	2	1	-	-	-	-	-	-
Longfu 14	Heilongjiang	F ₀ (Ke 86F6-545/Hei 85-1584) γ1.0 Rad	1	0	-	-	-	-	-	-
Longfu 16	Heilongjiang	Unknown	0	0	;	1	-	-	-	-
Longfu 18	Heilongjiang	Long 94-4083 mutagenesis	;	0	+	-	-	-	-	-
Longfu 19	Heilongjiang	SP4/Longmai 26	0	0	-	-	-	-	-	-
Longfu 20	Heilongjiang	Xiaoyan 6/Long 94-4083	1-	0	-	-	-	-	-	-
Longmai 10	Heilongjiang	Dongnong 101/Yuanzhong 3908	0	0	-	-	-	-	-	-
Longmai 15	Heilongjiang	Ke 76-686/Tieling 3	1	1+	-	-	-	-	-	-
Longmai 20	Heilongjiang	Unknown	0	0	;	-	-	-	-	-
Longmai 23	Heilongjiang	Unknown	0	0	-	-	-	-	-	-
Longmai 24	Heilongjiang	Unknown	0	0	-	-	-	-	-	-
Longmai 26	Heilongjiang	Long 87-7129/Ke 88F22060	;	0	+	-	-	-	-	+
Longmai 27	Heilongjiang	Unknown	;	1	-	-	-	+	-	-
Longmai 30	Heilongjiang	Long 90?05098/Long 90?06351	1	0	-	-	-	-	-	+
Longmai 31	Heilongjiang	Longmai 20/PSN/BOW//Longmai 206	0	0	-	-	-	-	-	-
Longmai 32	Heilongjiang	Long 94-4018/Ke 88F ₂ 165-3	0	0	-	-	-	-	-	-
Longmai 33	Heilongjiang	Longmai 26/Jiusan 3u92	;	0	+	-	-	-	-	-
Longmai 34	Heilongjiang	F ₁ (Zhong B054-3/2 ⁺ Longmai 15//97 Chanjian489(3)/Longmai 26	;	;	+	-	-	-	-	-
Longmai 35	Heilongjiang	Ke 90-513/Longmai 26	1-	0	-	-	-	-	-	+
Longmai 36	Heilongjiang	Ke 92-387/Long 99F ₃ -6725-1	0	0	-	-	-	-	-	-

(Continued)

Cultivars	Province	Pedigree	Infection types ^a												
			21C3CTHQ	34MKGQM	34C3RTGQM	Xgwm533	Sr24#12	Gb	Sr25	Sr26	Sr31	Sr38			
Longmai 37	Heilongjiang	Long 2003M8059-3/ Long 01D1572-2	;	1	0	0	+	-	-	-	-	-	-	-	-
Longmai 39	Heilongjiang	Long 03F3-6519/Longfu 20-378	2	2	0	0	+	-	-	-	-	-	-	-	-
Kefeng 2	Heilongjiang	Kehan 7/Ke 68F ₄ -585-13	0	1-	1	+	-	-	-	-	-	-	-	-	-
Kefeng 3	Heilongjiang	Kehan 8/Kehong/ Kezheng/Nadadoles	;	1-	;	-	-	-	-	-	-	-	-	-	-
Kefeng 4	Heilongjiang	Ke 71F4-370-7/Moyi 66	0	0	;	-	-	-	-	-	-	-	-	-	-
Kefeng 5	Heilongjiang	Ke 76-250/Ke 76F ₄ - 799-5	1	0	0	-	-	-	-	-	-	-	-	-	-
Kefeng 6	Heilongjiang	Ke 85-869/Ke 85-784	;	2	1-	-	-	-	-	-	-	-	-	-	+
Kefeng 10	Heilongjiang	Kehan 12/Ke 89RF ₆ 287	0	0	;	-	-	-	-	-	-	-	-	-	-
Kenda 4	Heilongjiang	82-5621/Ke 79-369	0	0	0	-	-	-	-	-	-	-	-	-	-
Kenda 5	Heilongjiang	Longfu 5009/Nongda 84-838	0	0	0	-	-	-	-	-	-	-	-	-	-
Kenda 6	Heilongjiang	Nongda 89-2729/Bei 89- 22	0	1-	0	-	-	-	-	-	-	-	-	-	-
Kenda 7	Heilongjiang	Nongda 89-2729/Bei 89- 22	0	0	0	-	-	-	-	-	-	-	-	-	-
Kenda 8	Heilongjiang	Nongda 89-2729/Bei 86- 1701	0	0	0	-	-	-	-	-	-	-	-	-	-
Kenda 9	Heilongjiang	Nongda 88-1116-8/ Bei88-26	2	1	1-	-	-	-	-	-	-	-	-	-	-
Kenda 10	Heilongjiang	Nongda 94-3537/Bei 90- 1201	1	1	1	-	-	-	-	-	-	-	-	-	-
Kenda 11	Heilongjiang	Jiusan 93u92/Ke 90-514	0	0	0	-	-	-	-	-	-	-	-	-	-
Kenda 12	Heilongjiang	Jiadongmai 19/Nongda 96-2543	0	1	1-	-	-	-	-	-	-	-	-	-	-
Kenda 13	Heilongjiang	Unknown	0	0	0	+	-	-	-	-	-	-	-	-	-
Kenjiu 9	Heilongjiang	Xiyin 1/Jiusan 80- 41123-7-3	0	0	0	-	-	-	-	-	-	-	-	+	-
Kenjiu 10	Heilongjiang	Jiusan 84-7251/Jiusan 87148/Ke	0	0	1-	-	-	-	-	-	-	-	-	-	+
Kechun 2	Heilongjiang	Ke 90-514/Ke 93RF ₆ - 128/Ke 90-514	;	1	1-	+	-	-	-	-	-	-	-	-	+
Kechun 5	Heilongjiang	Ke 99F2-33-3/Jiusan 94- 9178	0	0	0	-	-	-	-	-	-	-	-	+	-
Kechun 8	Heilongjiang	Ke 99F ₂ -33-3/Jiusan 94- 9178	0	0	0	-	-	-	-	-	-	-	-	+	+

Cultivars	Province	Pedigree	Infection types ^a		Sr2	Sr24	Sr25	Sr26	Sr31	Sr38
			21C3CTHQ	34MKGQM						
Kechun 9	Heilongjiang	Ke 99F ₂ -33-3/Jiusan 94-9178	0	1+	2	-	-	-	+	+
Xiaobing 33	Heilongjiang	<i>A. glaucum/Triticum aestivum</i>	0	0	0	-	-	-	-	-
Beimai 6	Heilongjiang	Jiusan 93-3U92/Ke 90-514	0	2	0	-	-	-	-	+
Beimai 9	Heilongjiang	Jiusan 97F ₄ -1057/Jiusan 97F ₄ -255F ₁ /119-54-4-3	2	1	1	-	-	-	-	+
Longken 402	Heilongjiang	Unknown	1	1	0	-	-	-	-	-
2010j159	Heilongjiang	Unknown	0	0	2	+	-	-	+	-
Norstar	Heilongjiang	Unknown	1	2	1+	+	-	-	-	+
Dongnong 125	Heilongjiang	Unknown	;1	0	2	-	-	-	-	+
Nongmai 850	Beijing	Unknown	1	0	0	+	-	-	+	-
Zhongmai 8	Beijing	Hehua 971-3/Ji Z76	1+	1	0	-	-	-	-	+
Jingdong 8	Beijing	Afuler 5238-016/Hongliang 4//Jingnong 79-106	2	0	1	-	-	-	+	-
Zhongmai 895	Beijing	Zhoumai 16/Liken 4	1	0	1	-	-	-	+	-
Chimai 2	Inner Mongolia	Wenge 7/Kehan 6	1	0	1	-	-	-	-	-
Chimai 5	Inner Mongolia	Wenge 1/Ke 76 tiao 295	;1	1	1-	-	-	-	-	-
Chimai 7	Inner Mongolia	Ke 76 tiao 295/Wenge 1	2	1	1	-	-	-	-	-
Ba 13p51	Inner Mongolia	Unknown	;1-	0	1	+	-	-	+	-
Shannong 22	Shandong	PH82-2-2/954072	2	1+	2	-	-	-	-	-
Shannong 23	Shandong	Tal (Ms2) recurrent selection	2	2	2	-	-	-	+	-
Shannong 24	Shandong	Tal (Ms2) recurrent selection	1-	0	2	-	-	-	+	-
Jimai 19	Shandong	Lunai 13/Linfen 5064	4	4	4	-	-	-	-	-
Jimai 20	Shandong	Lunai 14/Lu 884187	; 3	0	0	+	-	-	-	+
Jimai 21	Shandong	865186/Chuanongda 84-1109/Ji 84-5418	3	4	4	-	-	-	-	-
Jimai 22	Shandong	935024/935106	0	0	1	-	+	-	-	+
Jimai 44	Shandong	Jinan 17/954027	2	; 2	2	+	-	-	-	+
Yannong 19	Shandong	Yan 1933/Shan 82-29	;1	; 3	;	-	-	-	-	+
Yannong 21	Shandong	Heyan 1933/Shan 8229	3-	2	1	-	-	-	-	-
Yannong 23	Shandong	Yan 1061/Lunai 14	3	4	3	-	-	-	-	-
Tainong 18	Shandong	Laizhou 137/Yan 369-7	2	0	1	-	-	-	-	-

(Continued)

Cultivars	Province	Pedigree	Infection types ^a					Sr2	Sr24	Sr25	Sr26	Sr31	Sr38
			21C3CTHQ	34MKGQM	34C3RTGQM	Xgwm533	Sr24#12						
Taishan 23	Shandong	876161/881414	1+	0	1-	-	-	-	-	-	+	-	
Taishan 24	Shandong	904017/Zhenzhou 8329	3	4	3-	-	-	-	-	-	-	-	
Luyuan 502	Shandong	9940168/Jimai 19	;	0	0	-	-	-	-	-	+	-	
Tanmai 98	Shandong	Jining 13/942	4	3	2	-	-	-	-	-	-	-	
Lumai 21	Shandong	Yanzhong 144/Baofeng 7228	3+	4	4	-	-	-	-	-	-	-	
Jinan 17	Shandong	Linfen 5064/Lumai 13	4	4	4	-	-	-	-	-	-	-	
Liangxing 66	Shandong	Ji91102/Ji 935031	2	3	1	-	-	-	-	-	-	-	
Liangxing 99	Shandong	Ji 91102/Lumai14//PH85-16	3-	4	0	-	-	-	-	-	-	-	
Zhoumai 28	Henan	Zhoumai 18/Zhoumai 22//Zhou 2168	1	;	;	-	-	-	-	-	+	-	
Zhoumai 762	Henan	Unknown	2	;	;	-	-	-	-	-	+	+	
Luomai 6010	Henan	Yuanyang /Luo152/82C6/M	2	;	2	-	-	-	-	-	-	-	
Guomai 301	Henan	G883/Pumai 9	2	0	2	-	-	-	-	-	-	+	
Zhoumai 27	Henan	Zhoumai 16/Aikang 58	1	0	;	-	-	-	-	-	+	-	
Xumai 33	Henan	Neixiang 991/Zhoumai 16	0	1	0	-	-	-	-	-	+	-	
Xinmai 29	Henan	Yanzhan 4110/Zhoumai 16	4	4	4	-	-	-	-	-	-	-	
Annong 0711	Henan	Yannong 19/Aanong 0016	2	1	2	-	-	-	-	-	-	-	
Anke 157	Henan	Taishan 241/Xinong 1718	2	3	4	-	-	-	-	-	-	-	
Pumai 053	Henan	Bainong AK58/Zhoumai 18	1	1	0	-	-	-	-	-	+	-	
Kaimai 22	Henan	Zhoumai 18/ Bainong AK58	1-	1	0	-	-	-	-	-	+	-	
Zhenmai 1860	Henan	Unknown	1	;	;	-	-	-	-	-	+	-	
Wornai 9	Henan	Laizhou 953/Bainong AK58	1	1	1	-	-	-	-	-	+	-	
Ning 52	Ningxia	Yong 403/Yongliang 15// Yong 1147/230	0	0	0	+	-	-	-	-	+	-	
Ning 39	Ningxia	Yong 833/Ningchun 4	;	0	0	+	-	-	-	-	-	-	
Ningchun 4	Ningxia	Suonuola 64/Hongtu	0	0	0	-	-	-	-	-	-	-	
Ning 51	Ningxia	Yong 3002/Ningchun 4	0	0	0	+	-	-	-	-	-	-	

Table 2 (continued).

Cultivars	Province	Pedigree	Infection types ^a		Sr2	Sr24	Sr25	Sr26	Sr31	Sr38
			2IC3CTHQ	34MKGQM						
Ningchun 53	Ningxia	Ningchun 39/Moxige M7021	0	0	+	-	-	-	-	-
Ningdong 11	Ningxia	RENAN//Beinong 2/Beijing 841	1+	0	-	-	-	+	+	+
Linfeng 3	Shanxi	Linyuan 86-7065/Linyuan 81-5011	2	0	-	-	-	-	-	-
Jinmai 90	Shanxi	Jinmai 47/02L013	4	4	-	-	-	-	-	-
Wanmai 38	Jiangsu	Yanzhong 114/85-15-9	1+	0	-	-	-	-	-	+
Wansu 0217	Jiangsu	Unknown	2	2	-	-	-	-	-	-
Huaimai 4064	Jiangsu	Unknown	1	1	-	-	-	+	+	-
Wanmai 1643	Jiangsu	Unknown	0	0	+	-	-	-	-	-

Notes:

Seedling infection types produced by three races of *P. tritici* f. sp. *tritici* and molecular detection of resistance genes *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38* in the 136 wheat cultivars (lines).

^a Infection types (ITs) are based on a 0–4 scale where ITs of 0, 1, and 2 are indicative of a resistant (low) response and ITs of 3 or 4 of a susceptible (high) response; Symbols + and – indicate slightly larger and smaller pustule sizes, respectively (Stakman, Stewart & Loegering, 1962).

^b Symbol “+” indicates the cultivar (line) carry the tested genes; “–” indicates the cultivar (line) don’t carry the tested genes.

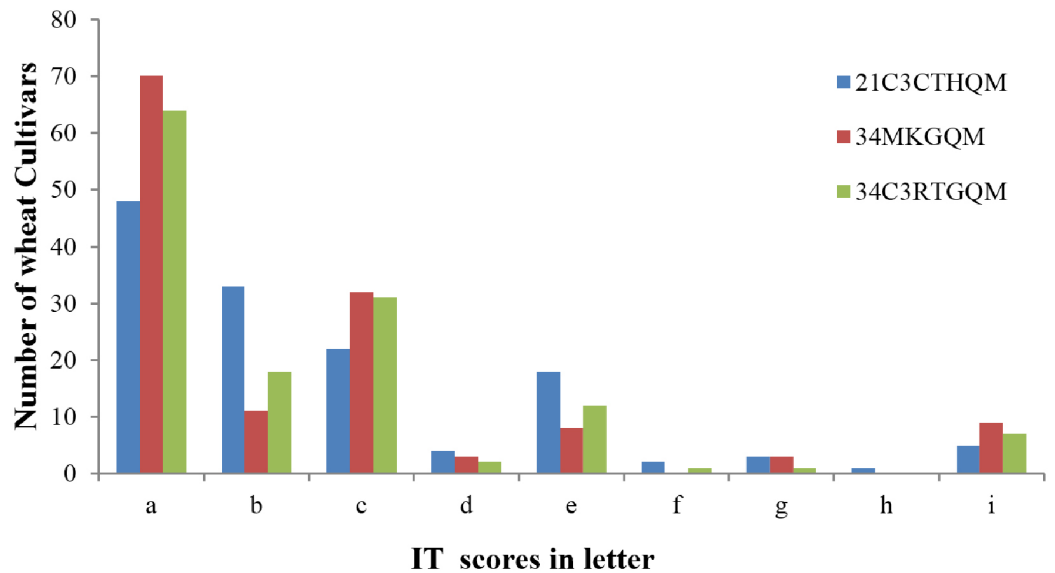


Figure 2 Screening for resistance genes against three *Pgt* races in wheat seedlings. Seedling infection type (IT) scores have been converted to letters to facilitate reading: a, 0; b, -1; c, 1; d, 1+; e, 2; f, 3-; g, 3; h, 3+; i, 4.

Full-size [DOI: 10.7717/peerj.4882/fig-2](https://doi.org/10.7717/peerj.4882/fig-2)

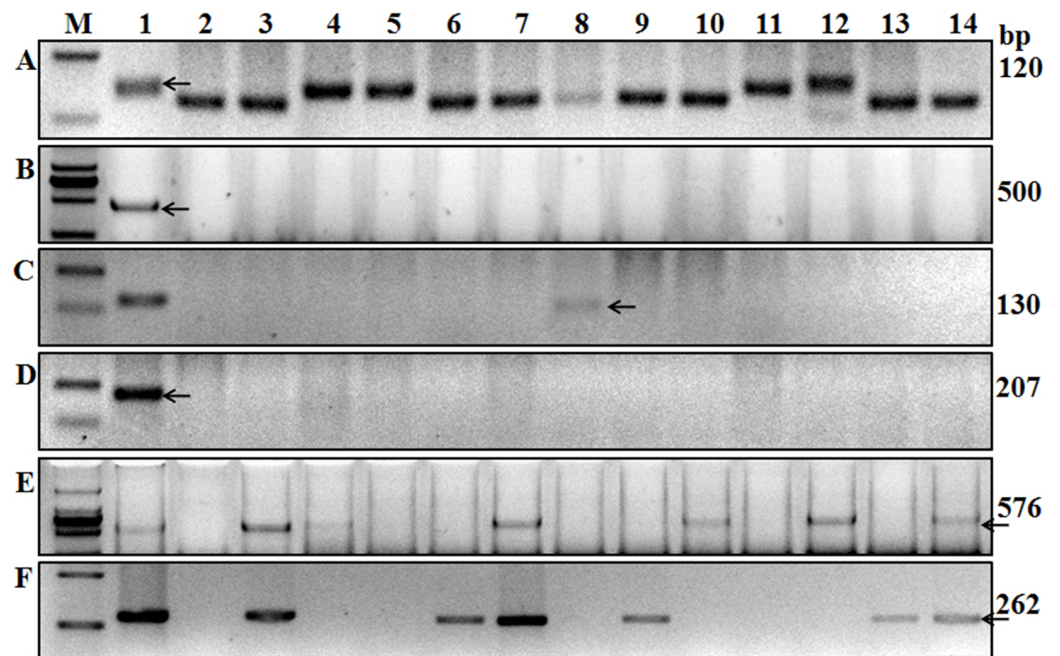


Figure 3 Amplification results for some of the wheat cultivars tested using six markers. A, Xgwm533; B, Sr24#12; C, Gb; D, Sr26#43; E, SCSS30.2576; F, VENTRIUP-LN2. Lanes A1 to F1 are results of Hope, LcSr24Ag, Agatha/9*LMPG, Eagle, Sr31/6*LMPG, and Trident cultivars. Lanes 2–14 are Kenda 9, Kechun 8, Nongmai 850, Longfu 18, Kenjiu 9, Ningdong 11, Jimai 22, Yannong 19, Taishan 23, Ning 39, Ning 52, Wanmai 38, and Zhumai 762 cultivars. M is the DNA ladder used to identify the specific sequences of each molecular marker.

Full-size [DOI: 10.7717/peerj.4882/fig-3](https://doi.org/10.7717/peerj.4882/fig-3)

Sr31 and Sr38 screening

The effective resistance of *Sr31* and *Sr38* to *Pgt* was overcome by Ug99, as no race with virulence to these genes had been found in China (*Li et al., 2016*). Genes *Sr31* and *Sr38* were widely used in wheat programs. Markers SCSS30.2₅₇₆ (amplifies a 576-bp fragment) and *Iag95* (amplifies a 1,100-bp fragment) linked to *Sr31*, and the 2NS-specific primer *VENTRIUP-LN2* (amplifies a 262-bp fragment), linked to the rust resistance gene cluster *Lr37-Sr38-Yr17*, were used in the present study to screen *Sr31* and *Sr38*. Fragment sizes consistent with the presence of both resistant genes were amplified in 25 wheat cultivars and in the positive control *Sr31/6*LMPG* using markers SCSS30.2₅₇₆ and *Iag95*, and in 28 wheat cultivars using marker *VENTRIUP-LN2*.

DISCUSSION

It is reported that the resistance of wheat cultivars to *Pgt* is higher in the northern rather than in the southern wheat region, especially in varieties from North China where stem rust is prone to occur. Results obtained in the present study are similar to that previously reported. In total, 124 (91.2%) wheat cultivars were resistant to the three *Pgt* races (ITs 2, 1+, or lower), and the resistance level of the accessions from Heilongjiang was higher than that of accessions from other provinces. All wheat cultivars from Heilongjiang Province were resistant to races 21C3CTHQ, 34MKGQM, and 34C3RTGQM, as wheat lines must be resistant to *Pgt* for being registered in Heilongjiang. In addition, the resistance level of wheat lines from Heilongjiang is tested by the Plant Immunity Institute, Shenyang Agricultural University, every year using the 21C3 and 34 *Pgt* race groups. Therefore, all registered cultivars registered in Heilongjiang should present ITs below 3, which was confirmed in the present study (0, 1, 1-, and 2 ITs were found; [Table 2](#)). Wheat cultivars from the lower-middle Yangtze River basin and central winter-wheat growing provinces were also highly resistant (73.9%) to the tested *Pgt* races.

Gene *Sr2*, originated from *Triticum dicoccum* Schronk, was transferred into North American and International Maize and Wheat Improvement Center (CIMMYT) wheat breeding programs in 1925, and since then it has been extensively used in many regions worldwide (*Borlaug, 1968*). In the present study, marker *Xgwm533*, which was used to detect *Sr2*, revealed that only 21 of the 136 wheat varieties were likely to carry this gene. Such cultivars might be resistant to Ug99, as the high resistance of Jimai 20 to Ug99 tested in Kenya in 2006 has been attributed to the *Sr2* gene carried by this cultivar (*He, Xia & Chen, 2008*). But it is difficult to conclude that these 21 wheat varieties carry *Sr2*, because many North American, Australian and CIMMYT lines which predicted not carry this gene can amplified a 120-bp fragment (*Jemanesh et al., 2013; Mago et al., 2011*).

The wheat stem rust gene *Sr24*, derived from *T. ponticum*, is effective against most *Pgt* races, including race TTKSK (i.e., Ug99). Races virulent to *Sr24* are rare in the *Pgt* population in North America (*Jin et al., 2008*). This gene has been used as a differential in North America and worldwide race surveys, but a new variant of race TTKSK with *Sr24* virulence has arisen in Kenya, South Africa, Tanzania, Ethiopia, Mozambique, and Uganda (*BGRI, 2017*). Leaf rust gene *Lr24* in association with *Sr24* provides resistance to all *Pgt* isolates. Thus, we used marker *Sr24#12*, completely linked to *Sr24* (*Mago et al.,*

2005), to screen for *Sr24/Lr24* genes in the 136 wheat accessions. None of the tested cultivars carried *Sr24*, although previous research using postulated gene presence based on marker data showed that some Chinese wheat cultivars might be carrying this gene (Cao et al., 2007). On the other hand, the study conducted by Zhang et al. (2008) supports our result as none of the 23 wheat cultivars they screened using molecular markers linked to *Lr24* carried this gene. Thus, more races and molecular markers should be used to confirm whether Chinese wheat cultivars carry *Sr24* or not.

Genes *Sr25* and *Sr26*, derived from *T. ponticum*, are effective against Ug99 and all *Pgt* races in China, and their use is increasing based on their resistance to Ug99 (Bariana et al., 2007). Novel genetic tools based on molecular marker technologies were developed to tag the presence of those genes (Mago et al., 2005; Liu et al., 2010). In the present study, we used molecular markers *Gb*, linked with *Sr25*, and *Sr26#43*, linked with *Sr26*, to identify these genes. Two wheat cultivars, “Kehan 3” (Ke61F₃-199/*Agropyronglaucum*) and “Jimai 22” (935024/935106) are likely to carry *Sr25*. Pedigree tracking indicated that “Kehan 3” parents contained *A. glaucum*, but *Sr25* is derived from *T. ponticum*, so the result obtained for this cultivar might not be accurate. Expectedly, none of the wheat varieties carried *Sr26*, as this gene is not widely used in breeding programs in China (Li et al., 2016). Results obtained here are similar to those of previous studies; for example, using marker *Sr26#43*, Li et al. (2016) detected that none of the 119 wheat materials examined carried *Sr26*.

Gene *Sr31*, derived from “Petkus” rye, is located on 1BL/1RS. It is distributed in wheat cultivars worldwide, but was transferred into Chinese wheat backgrounds from the Soviet Union and Romania in the 1960s (Jiang et al., 2007). Since then, the wheat cultivars “Alondra S,” “Aftab LeEr,” “Kavkaz,” and “Luofulin” lines carrying *Sr31* have been released in wheat growing regions in China. Although, this gene is susceptible to Ug99, it is effective against TKTF and TTTTF and all *Pgt* races in China (Pretorius et al., 2000; Olivera et al., 2015; Bhattacharya, 2017; Li et al., 2016). Markers *Iag95* and *SCSS30.2₅₇₆*, which were used to screen the gene *Sr31* in the present study, revealed that 25 wheat cultivars contained *Sr31*, and pedigree information and low ITs supported these results. Thus, *Sr31* should be used in breeding programs in China in combination with other genes resistant to Ug99 to ensure that Chinese wheat cultivars are resistant to Chinese *Pgt* races and to Ug99.

Gene *Sr38*, originated from *T. ventricosum*, is widely used due to its association with the stripe rust gene *Yr17* and the leaf rust gene *Lr37* that confer resistance to the three species of wheat rust pathogens (Delibes et al., 1993; Dyck & Lukow, 1988). Genes *Yr17* and *Lr37* were reported from wheat cultivars in China using molecular markers linked to them (Peng et al., 2013; Xue et al., 2014). In the present study, the marker *VENTRIUP-LN2*, which is linked with the *Sr38-Yr17-Lr37* cluster of rust resistance genes, was used and the specific PCR fragment for this marker was detected in 28 of the 136 wheat cultivars examined. These 28 cultivars presented low ITs indicating they carry *Sr38*. Gene *Sr38* is susceptible to Ug99, similar to gene *Sr31*, but resistant to all *Pgt* races in China (Cao et al., 2007). Therefore, in China, it should be used in combination with genes resistant to Ug99 through gene pyramiding.

Molecular markers linked to resistance genes are an alternative to gene postulation and may allow breeders to identify resistance genes rapidly and accurately (*Goutam et al., 2013*). Combining molecular markers with pedigree information of the tested varieties can greatly increase the success of gene postulation (*Yu et al., 2010*). Due to the rapid development of molecular markers and to the great importance of the new *Pgt* races, molecular markers closely linked to resistance genes against such races have been frequently reported, and many have been converted to simple sequence repeat (*Mago et al., 2013; Tsilo, Jin & Anderson, 2007*), sequence tagged site/cleaved amplified polymorphic sequence (*Helguera et al., 2003; Mago et al., 2011*), sequence tagged site (*Mago et al., 2005; Bansal et al., 2014*), and simple sequence repeats/amplified fragment length polymorphism markers (*Periyannan et al., 2014*). This approach overcomes gene interactions and plant stage-dependent gene expression problems associated with traditional gene postulation.

CONCLUSION

In the present study, we used molecular markers to determine if *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38* were present in the 136 wheat cultivars examined. Overall, genes *Sr31*, and *Sr38* were differently distributed across wheat regions in China and none of the wheat cultivars contained *Sr24* and *Sr26*. Additional studies will be needed to verify the gene postulations for *Sr2* and *Sr25*. These cultivars comprising stem rust resistance genes are valuable genetic materials for future wheat-breeding plans.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Xiaofeng Xu performed the experiments, authored or reviewed drafts of the paper.
- Depeng Yuan prepared figures and/or tables.
- Dandan Li performed the experiments.
- Yue Gao performed the experiments.
- Ziyuan Wang performed the experiments.
- Yang Liu analyzed the data, prepared figures and/or tables.
- Siting Wang analyzed the data, prepared figures and/or tables.
- Yuanhu Xuan conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Hui Zhao prepared figures and/or tables.
- Tianya Li conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Yuanhua Wu conceived and designed the experiments.

Data Availability

The following information was supplied regarding data availability:

The raw data are provided in a [Supplemental File](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.4882#supplemental-information>.

REFERENCES

- Bansal U, Bariana H, Wong D, Randhawa M, Wicker T, Hayden M, Keller B. 2014.** Molecular mapping of an adult plant stem rust resistance gene *Sr56* in winter wheat cultivar Arina. *Theoretical and Applied Genetics* **127**(6):1441–1448 DOI [10.1007/s00122-014-2311-1](https://doi.org/10.1007/s00122-014-2311-1).
- Bariana HS, Brown GN, Bansal UK, Miah H, Standen GE, Lu M. 2007.** Breeding triple rust resistant wheat cultivars for Australia using conventional and marker-assisted selection technologies. *Australian Journal of Agricultural Research* **58**(6):576–587 DOI [10.1071/ar07124](https://doi.org/10.1071/ar07124).
- BGRI. 2017.** A global wheat rust monitoring system. Available at http://rusttracker.cimmyt.org/?page_id=22.
- Bhattacharya S. 2017.** Deadly new wheat disease threatens Europe's crops. *Nature* **542**(7640):145–146 DOI [10.1038/nature.2017.21424](https://doi.org/10.1038/nature.2017.21424).
- Borlaug NE. 1968.** Wheat breeding and its impact on world food supply. In: Finlay KW, Shephard KW, eds. *Proceedings of the 3rd International Wheat Genetics Symposium*. Canberra: Australian Academy of Sciences, 1–36.
- Cao YY, Han JD, Zhu GQ, Zhang L. 2007.** Ug99, a new virulent race of *Puccinia graminis* f. sp. *tritici*, and its effect on China. *Plant Protection* **6**:86–89.
- Cao YY, Yao P, Zhu GQ, Wu YS. 1994.** A preliminary analysis of probable genes for stem rust resistance and resistance stability of 41 wheat cultivars in China. *Journal of Shenyang Agricultural University* **25**:392–397.
- Chen SS, Rouse MN, Zhang WJ, Jin Y, Akhunov E, Wei YM, Dubcovsky J. 2015.** Fine mapping and characterization of *Sr21*, a temperature- sensitive diploid wheat resistance gene effective

- against the *Puccinia graminis* f. sp. *tritici* Ug99 race group. *Theoretical and Applied Genetics* **128**(4):645–656 DOI [10.1007/s00122-015-2460-x](https://doi.org/10.1007/s00122-015-2460-x).
- Delibes A, Romero D, Aguaded S, Duce A, Mena M, Lopez-Brana I, Andrés MF, Martin-Sanchez JA, García-Olmedo F. 1993.** Resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a ‘stepping–stone’ procedure. *Theoretical and Applied Genetics* **87**(3):402–408 DOI [10.1007/bf01184930](https://doi.org/10.1007/bf01184930).
- Dyck PL, Lukow OM. 1988.** The genetic analysis of two inter specific sources of leaf rust resistance and their effect on the quality of common wheat. *Canadian Journal of Plant Science* **68**(3):633–639 DOI [10.4141/cjps88-076](https://doi.org/10.4141/cjps88-076).
- Food and Agriculture Organization of the United Nations (FAO). 2017.** Spread of damaging wheat rust continues: new races found in Europe, Africa, Central Asia. Available at <http://www.fao.org/news/story/en/item/469467/icode/> (accessed 3 February 2017).
- Goutam U, Kukreja S, Tiwari R, Chaudhary A, Gupta RK, Dholakia BB, Yadav R. 2013.** Biotechnological approaches for grain quality improvement in wheat: present status and future possibilities. *Australian Journal of Cereal Science* **7**:469–483.
- Hayden MJ, Kuchel H, Chalmers KJ. 2004.** Sequence tagged microsatellites for the Xgwm533 locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*T. aestivum* L.). *Theoretical and Applied Genetics* **109**(8):1641–1647 DOI [10.1007/s00122-004-1787-5](https://doi.org/10.1007/s00122-004-1787-5).
- He ZH, Xia XC, Chen WQ. 2008.** Breeding for resistance to new race Ug99 of stem rust pathogen. *Journal of Triticeae Crops* **28**:170–173.
- Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L. 2003.** PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Science* **43**(5):1839–1847 DOI [10.2135/cropsci2003.1839](https://doi.org/10.2135/cropsci2003.1839).
- Jemanesh KH, Karl H, Ayele B, Ravi PS, Marion SR. 2013.** Haplotype analysis of molecular markers linked to stem rust resistance genes in Ethiopian improved durum wheat varieties and tetraploid wheat landraces. *Genetic Resources and Crop Evolution* **60**(3):853–864 DOI [10.1007/s10722-012-9880-0](https://doi.org/10.1007/s10722-012-9880-0).
- Jiang YY, Chen WQ, Zhao ZH, Zeng J. 2007.** Threat of new wheat stem rust race Ug99 to wheat production in China and counter measure. *Plant Protection* **27**:14–16.
- Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch T Jr. 2008.** Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* **92**:923–926.
- Jin Y, Szabo LJ, Rouse MN, Fetch T Jr, Pretorius ZA, Wanyera R, Njau P. 2009.** Detection of virulence to resistance gene *Sr36* within the TTKS race lineage of *Puccinia graminis* f. sp. *tritici*. *Plant Disease* **93**(4):367–370 DOI [10.1094/pdis-93-4-0367](https://doi.org/10.1094/pdis-93-4-0367).
- Knott DR. 1961.** The inheritance of rust resistance VI. The transfer of stem rust resistance from *Agropyron elongatum* to common wheat. *Canadian Journal of Plant Science* **41**(1):109–123 DOI [10.4141/cjps61-014](https://doi.org/10.4141/cjps61-014).
- Li TY, Cao YY, Wu XX, Xu XF, Wang WL. 2016.** Seedling resistance to stem rust and molecular marker analysis of resistance genes in wheat cultivars of Yunnan, China. *PLOS ONE* **11**(10): e0165640 DOI [10.1371/journal.pone.0165640](https://doi.org/10.1371/journal.pone.0165640).
- Liu S, Yu LX, Singh RP, Jin Y, Sorrells ME, Anderson JA. 2010.** Diagnostic and co-dominant PCR markers for wheat stem rust resistance genes *Sr25* and *Sr26*. *Theoretical and Applied Genetics* **120**(4):691–697 DOI [10.1007/s00122-009-1186-z](https://doi.org/10.1007/s00122-009-1186-z).
- Mago R, Bariana HS, Dundas IS, Spielmeier W, Lawrence GL. 2005.** Development of PCR markers for the selection of wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. *Theoretical and Applied Genetics* **111**(3):496–504 DOI [10.1007/s00122-005-2039-z](https://doi.org/10.1007/s00122-005-2039-z).

- Mago R, Brown-Guedira G, Dreisigacker S, Breen J, Jin Y, Singh R, Appels R, Lagudah ES, Ellis J, Spielmeyer W. 2011. An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. *Theoretical and Applied Genetics* 122(4):735–744 DOI 10.1007/s00122-010-1482-7.
- Mago R, Verlin D, Zhang P, Bansal U, Bariana H, Jin Y, Ellis J, Hoxha S, Dundas I. 2013. Development of wheat-*Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2s#1 chromosome. *Theoretical and Applied Genetics* 126(12):2943–2955 DOI 10.1007/s00122-013-2184-8.
- Olivera P, Newcomb M, Szabo LJ, Rouse M, Johnson J, Gale S, Luster DG, Hodson D, Cox JA, Burgin L, Hort M, Gilligan CA, Patpour M, Justesen AF, Hovmöller MS, Woldeab G, Hailu E, Hundie B, Tadesse K, Pumphrey M, Singh RP, Jin Y. 2015. Phenotypic and genotypic characterization of race TKTTF of *Puccinia graminis* f. sp. *tritici* that caused a wheat stem rust Epidemic in Southern Ethiopia in 2013–14. *Phytopathology* 105(7):917–928 DOI 10.1094/phyto-11-14-0302-fi.
- Pathan AK, Park RF. 2007. Evaluation of seedling and adult plant resistance to stem rust in European wheat cultivars. *Euphytica* 155(1–2):87–105 DOI 10.1007/s10681-006-9308-z.
- Peng X, Ren MJ, Zhang SS, Xu RH. 2013. Molecular detection of wheat leaf rust resistance gene *Lr34* and *Lr37*. *Guizhou Agricultural Science* 41:13–16.
- Periyannan S, Bansal U, Bariana H, Deal K, Luo MC, Dvorak J, Lagudah E. 2014. Identification of a robust molecular marker for the detection of the stem rust resistance gene *Sr45* in common wheat. *Theoretical and Applied Genetics* 127(4):947–955 DOI 10.1007/s00122-014-2270-6.
- Pretorius ZA, Singh RP, Wagoire WW, Payne TS. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. *Plant Disease* 84(2):203 DOI 10.1094/pdis.2000.84.2.203b.
- Pretorius ZA, Szabo G, Boshoff WHP, Herselman L, Visser B. 2012. First report of a new TTKSF race of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in South Africa and Zimbabwe. *Plant Disease* 96(4):590 DOI 10.1094/pdis-12-11-1027-pdn.
- Rouse MN, Nirmala J, Jin Y, Chao SM, Fetch T Jr, Pretorius ZA, Hiebert CW. 2014. Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99. *Theoretical and Applied Genetics* 127(8):1681–1688 DOI 10.1007/s00122-014-2330-y.
- Stakman EC, Stewart DM, Loegering WQ. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. Washington, D.C.: United States Department of Agriculture, Agricultural Research Service, 1–53.
- Tsilo JT, Jin Y, Anderson JA. 2007. Microsatellite markers linked to stem rust resistance allele *Sr9a* in wheat. *Crop Science* 47(5):2013–2020 DOI 10.2135/cropsci2007.02.0087.
- Xu XF, Li DD, Liu Y, Gao Y, Wang ZY, Ma YC, Yang S, Cao YY, Xuan YH, Li TY. 2017. Evaluation and identification of stem rust resistance genes *Sr2*, *Sr24*, *Sr25*, *Sr26*, *Sr31* and *Sr38* in wheat lines from Gansu Province in China. *PeerJ* 5:e4146 DOI 10.7717/peerj.4146.
- Xue WB, Xu X, Mu JM, Wang QL, Wu JH, Huang LL, Kang ZS. 2014. Evaluation of stripe rust resistance and genes in Chinese elite wheat varieties. *Journal of Triticeae Crops* 34:1054–1060.
- Yu L, Liu S, Anderson JA, Singh RP, Jin Y, Dubcovsky J, Gina BJ, Bhavani S, Morgounov A, He Z, Huerta-Espino J, Sorrells ME. 2010. Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. *Molecular Breeding* 26(4):667–680 DOI 10.1007/s11032-010-9403-7.
- Zhang N, Yang WX, Wang HY, Ke YM, Jia JZ, Liu DQ. 2008. Molecular identification of leaf rust resistance in 23 diploid wild relatives of wheat. *Journal of Triticeae Crops* 28:691–696.