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Identification of Stem Rust Resistance Genes in *Triticum* Wheat Cultivars and Evaluation of Their Resistance to *Puccinia graminis* f. sp. *tritici*

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Abstract: Wheat stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*), poses a substantial threat to global wheat production. Utilizing stem rust resistance (*Sr*) genes represents an economically viable, effective, and environmentally friendly approach to disease control. In this study, gene postulation, molecular testing, and pedigree analysis were used to identify the presence of *Sr* genes in 45 wheat cultivars. In addition, the resistance of these cultivars was evaluated against two predominant *Pgt* races, 34MRGQM and 21C3CTHTM, at the adult-plant stage during 2021–2022. The results identify seven *Sr* genes (*Sr31*, *Sr38*, *Sr30*, *SrTmp*, *Sr22*, *Sr19*, and *Sr5*) within 35 wheat cultivars. Among these, 23 cultivars contained *Sr31*, whereas *Sr5* and *SrTmp* were present in four cultivars each. Han 5316, Shimai 15, Shiyou 20, and Kenong 1006 exhibited the presence of *Sr19*, *Sr22*, *Sr30*, and *Sr38*, respectively. Molecular studies confirmed the absence of *Sr25* and *Sr26* in any of the wheat cultivars. During field evaluation, 37 (82.2%) and 39 (86.7%) wheat cultivars demonstrated resistance to races 34MRGQM and 21C3CTHTM, respectively. Moreover, 33 wheat cultivars (73.3%) exhibited resistance to all the tested races. These study findings will significantly contribute to future research in wheat pre-breeding and abiotic stress tolerance.

Keywords: wheat stem rust; *Puccinia graminis* f. sp. *tritici*; gene postulation; *Sr* gene

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1. Introduction

Wheat, as the third major staple crop, plays a crucial role in the global food supply and food security. However, its production is persistently affected by various biological and abiotic diseases [1,2]. Wheat stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), has a severe impact on wheat production worldwide [3]. Since the 1970s, the use of wheat cultivars carrying *Sr31* has effectively controlled the disease globally [4]. However, *Pgt* exhibits the ability to constantly change its virulence, overcome cultivar resistance, and lead to epidemics. For example, Ug99, a new race of *Pgt* overcoming *Sr31* resistance, was first documented in Uganda in 1999. This race, named as TTKS according to the international nomenclature for *Pgt*, was later renamed as TTKSK due to the addition of a fifth differential set to the nomenclature [5,6]. TTKSK exhibits characteristics of rapid mutation and virulence. Currently, 15 mutated races belonging to the TTKSK lineage have been identified in 14 countries over the past two decades, successfully evading the resistance of globally utilized *Sr* genes (*Sr31*, *Sr24*, *Sr36*, *Sr31* + *Sr36* + *Sr38*, and *Sr24* + *Sr31* + *Sr38*) [7–11].

More seriously, this race and its derivative races have demonstrated highly pathogenic characteristics in the majority of wheat cultivars worldwide. Between 2005 and 2010, over 200,000 wheat cultivars, advanced breeding materials, and germplasm collections originating from 22 wheat-producing countries in Asia and Africa were evaluated for disease resistance in Kenya and Ethiopia. The results indicate that less than 10% of the materials exhibited a certain degree of resistance to Ug99 [12].

In 2013, the emergence of the new race TKTTF triggered an outbreak of wheat stem rust in Ethiopia, resulting in nearly 100% yield loss for the wheat cultivar "Digalu" [13]. Since the mid-20th century, wheat stem rust prevalence has become increasingly uncommon in Western Europe, resulting in the neglect of resistance breeding. Consequently, European wheat cultivars are vulnerable to wheat stem rust due to a lack of resistance. However, during the 21st century, a resurgence of wheat stem rust occurred in several European countries, where it had been absent for decades. In 2013 and 2014, TKTTF was detected in Germany [14], Denmark, and the United Kingdom in Europe [15], leading to severe losses in wheat yield. In 2016, the stem rust race TTTTF caused the breakdown of resistance in numerous durum and bread wheat varieties in Sicily, representing a significant threat that has been identified in other European countries [16]. In addition, an outbreak of wheat stem rust in Switzerland in 2017 resulted in a significant reduction in wheat productivity [14]. In 2020, an unprecedented occurrence of wheat stem rust was detected for the first time across multiple locations in Ireland. Transcriptome sequencing revealed that the isolates exhibited a remarkably close genetic relationship with race TKTTF [17].

Historically, the incidence of wheat stem rust in China has occurred mainly in the northeast region of Inner Mongolia, northwest spring wheat areas, and southern Yunnan [18]. Incomplete statistical records indicate that, from the 1920s to the 1970s, nine significant epidemics ravaged the wheat-planted regions of the northeast, resulting in staggering losses. For instance, in 1923 and 1948, losses amounted to 7.4×10^9 kg and 5.6×10^9 kg, respectively. Moreover, the devastating outbreaks of 1956 and 1958 in the Jianghuai wheat region led to wheat losses of up to 1×10^{10} kg [19]. However, since the 1970s, this disease has been effectively contained, primarily occurring sporadically in localized areas.

The *Sr31* gene is present in approximately 60% of wheat cultivars in China, making the Ug99 race group a substantial threat to the safe production of wheat in China [20]. Hence, it is imperative to conduct analyses on wheat cultivars to assess their resistance to wheat stem rust and identify the specific resistance genes present, thereby aiding local wheat production risk assessment. To date, more than 70 *Sr* genes have been identified and named, originating from both cultivated wheat and its wild relatives [21]. Although most of these confer race-specific resistance, certain resistance genes, including *Sr2*, *Sr55*, *Sr57*, and *Sr58*, do not exhibit race specificity. In recent years, gene postulation and molecular marker technology, combined with cultivar pedigree analysis, have been extensively used to identify wheat stem rust resistance genes. For instance, Mourad et al. utilized gene-specific markers to analyze the presence of *Sr* genes in 330 genotypes from two nurseries in Nebraska [22]. The results indicate the existence of nine *Sr* genes (*Sr24*, *Sr31*, *Sr38*, *Sr6*, *Sr7b*, *Sr9b*, *Sr36*, *Sr1RSamigo*, and *SrTmp*) within these genotypes. Similarly, Haile et al. employed STS and SSR markers linked to major *Sr* genes to screen these genes in 58 tetraploid wheat materials in Ethiopia [23]. Li et al. conducted molecular marker assays to screen Yunnan wheat cultivars for resistance genes, revealing that 12 of the 119 wheat cultivars tested contained *Sr28*, 43 contained *Sr31*, 1 contained *Sr32*, and 10 contained *Sr38* [24]. Moreover, Wu et al. applied molecular markers linked with *Sr22*, *Sr25*, *Sr26*, and *Sr28* to detect the presence of these genes in 119 Yunnan wheat cultivars (lines) and 20 CIMMYT Ug99 resistance materials. Their analysis identified two CIMMYT materials containing *Sr25*, one containing *Sr26*, and one containing *Sr28*, while among the 119 wheat cultivars, only 12 were discovered to contain *Sr28* [25].

The Shandong, Shanxi, Hebei, and Henan provinces are prominent wheat production areas in China. The latest statistical yearbook illustrates that the combined wheat cultivation

area in these provinces amounts to 1.25×10^7 square hectares, accounting for 52.9% of the national cultivation area. The wheat yield in these regions reaches 8.15×10^4 million kg, accounting for 59.53% of the national wheat yield (http://www.stats.gov.cn/, accessed on 1 January 2024). Historically, these regions have been plagued by recurrent wheat stem rust outbreaks, which gravely affect local wheat production [26,27]. With the persistent emergence of *Pgt* on a global scale, it is of great significance to clarify the resistance of wheat cultivars to wheat stem rust and to identify the major resistance genes contained in wheat cultivars. Therefore, this study employed molecular testing, gene postulation, and lineage analysis to identify the presence of resistance genes in 45 prominent cultivars from Shandong, Shanxi, and Hebei provinces. In addition, we investigated their field responses to two predominant races (34MRGQM and 21C3CTHTM) in China.

2. Materials and Methods

2.1. Plant Materials and Pgt Races

Dr. Yan Hongfei from the Hebei Agricultural University of Hebei Province (latitude 38°82′ E, longitude 115°44′ N), China, contributed to the collection of 45 distinct wheat cultivars for this study (Supplemental Table S1). Forty-three monogenic lines used in this study were sourced from the Institute of Plant Immunity, Shenyang Agricultural University. These lines were instrumental in evaluating the virulence spectrum of *Pgt* and confirming the reliability of the molecular markers employed. The Little Club cultivar was used as a universally susceptible control. To assess the resistance capacity of the wheat cultivars to diverse races, 10 distinct races (34C3MKGSM, 34C6MTGSM, 34MRGQM, 21C3CTTTM, 21C3CTHTM, 34MKGQM, 34MTGSM, 21C3CTTSC, 34C3MTGQM, and MTSRR) of *Pgt* with different virulence spectra were identified by applying an international nomenclature for *Pgt* at the Institute of Plant Immunity, Shenyang Agricultural University [5,20].

2.2. Determination of Infection Types

The 10 *Pgt* races were propagated in a greenhouse and stored at 4 °C. In a controlled glass greenhouse environment, seeds of 45 wheat lines and 43 known *Sr* monogenic lines were individually planted in pots (10 cm in diameter and 12 cm in height) filled with a vermiculite and sand mixture (3:2, *v*/*v*), leveled to pH 7.2, with 10 seeds per pot for each line. The susceptible wheat cultivar LC, lacking any resistance genes to stem rust, was included as a control. Infection types (ITs) were performed at the one-leaf stage (approximately 8–10 d old) of the plants. The first leaves were sprayed with a 0.05% Tween 20 aqueous solution, followed by dust inoculation with a mixture of talcum and urediniospores (20:1, *v*/*v*) [3]. Subsequently, another round of spraying with a 0.05% Tween 20 aqueous solution was performed to create a moisturizing film. The inoculated wheat plants were then maintained at a temperature range from 18 °C to 20 °C for 16 h and cultivated in the greenhouse at a temperature of 20 °C ± 1 °C. Each isolate was subjected to three tests. When the wheat cultivar LC was fully sporulated, ITs were recorded approximately two weeks post-inoculation using a 0–4 scale. Low ITs of *Pgt* in wheat (0, 1−, 1, 1+, 2, and 2+) were classified as resistant, whereas high ITs (3−, 3, 3+, and 4) were categorized as susceptible [28]. According to the infection phenotype of the cultivar to be tested and the phenotype of the monogenic lines of the known *Sr* gene, the presence of the respective gene was deduced adhering to the method described by Dubin et al. [29].

2.3. Field Evaluation at the Adult-Plant Stage (APS)

In the third week of March, in both 2021 and 2022 (70–72 d after sowing), all wheat cultivars designated for testing purposes were meticulously planted within the experimental grounds of Shenyang Agricultural University (latitude 41°49′ N, longitude 123°33′ E, and altitude 67 m). The cultivation method involved planting a single row of each cultivar (1 m in length with a row spacing of 25 cm). In addition, for every 10 rows, a row of the susceptibility control cultivar LC was included. The field test utilized two prevalent races, 34MRGQM and 21C3CTHTM, which have dominated in China over the past three

decades. An inoculation test was conducted at the jointing stage, with the soil maintained completely humid using water on the day of inoculation. Inoculation commenced at sunset, involving the application of a 20% Tween 20 solution to create a layer of dew on the leaves, followed by dust inoculation with a 1:30 volume ratio of urediniospores and talcum powder. The inoculated wheat plants were covered with plastic to maintain moisture for 14 h [30]. Maximum severity and infection response (IR) were assessed during the heading and flowering stages using a modified Cobb scale, as described by Roelfs et al. [31]. The climatic conditions are listed in Supplemental Table S2.

2.4. Molecular Markers for Stem Rust Resistance Gene

Genomic DNA was extracted from 45 wheat lines using CTAB [32]. All cultivars were detected using molecular markers that were closely linked to *Sr24*, *Sr25*, *Sr26*, *Sr31*, and *Sr38*. Polymerase chain reaction (PCR) systems were adjusted following the provided instructions. Table 1 outlines the PCR annealing temperature, array size, and primer sequences.

Table 1. Genome location, sequence of primer, and conditions for PCR amplification of molecular marker.

Gene	Chromosome	Marker	Sequence of Primer (5′→3′)	PCR Amplification Conditions		Size of Markers (bp)
				Temperature (°C)/Time	N. of Cycles	
Sr24	3DL	Sr24#50	CACCCGTGACATGCTCGTA AACAGGAAATGAGCAACGATGT	94/3 min 94/30 s; 57/30 s; 72/40 s 20/1 min	1 30 1	500
Sr25	7DL	Gb	CATCCTTGGGGACCTC CCAGCTCGCATACATCCA	94/3 min 94/30 s; 60/30 s; 72/40 s 20/1 min	1 30 1	191
Sr26	6AL	Sr26#43	AATCGTCCACATTGGCTTCT CGCAACAAAATCATGCACTA	94/3 min 94/30 s; 56/30 s; 72/40 s 20/1 min	1 30 1	207
Sr31	1BL	SCSS30.2$_{576}$	GTCCGACAATACGAACGATT CCGACAATACGAACGCCTTG	95/5 min 95/1 min; 60/1 min; 72/30 s 72/10 min	1 35 1	576
		Iag95	CTCTGTGGATAGTTACTTGATCGA CCTAGAACATGCATGGCTGTTACA	94/3 min 94/30 s; 55/60 s; 72/70 s 25/60 s	1 30 1	1100
Sr32	2AS	csSr32#2	CAAATGAATAGAAAAACCCGTGCT CACACACTGTTTTCCGTTGC	94/3 min 94/30 s; 60/60 s; 72/70 s 25/60 s	1 30 1	152
Sr38	2AS	VENTRIUP-LN2	GGGGCTACTGACCAAGGCT TGCAGCTACAGCAGTATGTACACAAAA	94/45 s 94/45 s; 65/30 s; 72/1 min 72/7 min	1 30 1	259

3. Results

3.1. Sr genes in the Wheat Cultivars Based on Gene Postulation and Molecular Marker Analysis

The seedling ITs of 43 monogenic lines containing known *Sr* genes and 45 wheat test lines when tested with 10 Chinese *Pgt* races are shown in Tables 2 and 3, respectively. Nine lines with *Sr31*, *Sr38*, *Sr44*, *Sr40*, *Sr33*, *Sr26*, *Sr21*, *SrTt3*, and *Sr9e* genes were resistant to all races, exhibiting ITs ranging from 0 to 2. Conversely, 10 lines with *Sr39*, *Sr16*, *Sr9g*, *Sr9f*, *Sr9d*, *Sr9b*, *Sr9a*, *Sr8a*, *Sr7b*, and *Sr6* were susceptible to all races, demonstrating ITs ranging from 3 to 4. These observations indicate that these 19 *Sr* genes could not be accurately identified by gene postulation. However, the remaining lines, containing *Sr38*, *Sr37*, *Sr36*, *Sr35*, *Sr34*, *Sr32*, *Sr30*, *Sr28*, *Sr27*, *Sr25*, *Sr24*, *Sr23*, *Sr22*, *Sr19*, *Sr18*, *Sr17*, *Sr15*, *Sr13*, *Sr12*, *Sr11*, *Sr10*, and *Sr5*, exhibited high and low ITs to the tested races. Therefore, these 22 genes could be identified through gene postulation. Through a combination of molecular detection and gene postulation methods, seven *Sr* genes (*Sr31*, *Sr38*, *Sr5*, *Sr19*, *Sr22*, *Sr30*, and *SrTmp*) were identified in the 36 wheat cultivars.

Table 2. Seedling test of 43 wheat lines inoculated with 10 races of *P. graminis* f. sp. *tritici.*

No.	Lines	Infection Types [a]									
		Pgt 1 [b]	*Pgt 2*	*Pgt 3*	*Pgt 4*	*Pgt 5*	*Pgt 6*	*Pgt 7*	*Pgt 8*	*Pgt 9*	*Pgt 10*
1	ISr5-Ra (*Sr5*)	4	4	3	4	4	3	3	1	1	1
2	ISr6-Ra (*Sr6*)	3	3	3	3	3+	3	3+	3	3+	3
3	ISr7b-Ra (*Sr7b)*	4	3	3	3+	3	4	4	4	3	4
4	ISr8a-Ra (*Sr8a*)	4	3	3	4	1	4	3	3+	3+	4
5	ISr9a-Ra (*Sr9a*)	4	4	4	4	4	3+	4	3	3	4
6	W2691Sr9b (*Sr9b*)	4	4	4	4	4	3	3	3+	4	3
7	ISr9d-Ra (*Sr9d*)	4	3+	3	3	4	4	3+	4	4	3
8	Vernstine (*Sr9e)*	0	;1	1+	;	;	1	1	1−	;	1−
9	CnsSr9f (*Sr9f)*	4	4	3	4	3	4	4	3+	4	3
10	CnsSr9g (*Sr9g*)	4	3+	3	3+	4	3	4	3	4	4
11	W2691Sr10 (*Sr10*)	2	3−	3−	;	1	1 + N	3	3	3	3
12	Lee (*Sr11*)	4	3+	1	4	4	0	3	4	4	3
13	Bt/TcSr12 (*Sr12*)	4	2	3	1+	3+	3	3−	4	3−	3
14	W2691Sr13 (*Sr13*)	0	3−	3−	1+	1	1+	3	3	3−	3
15	W2691Sr15 (*Sr15*)	0	4	3−	;	3	3	3+	3+	4	3
16	ISr16-Ra (*Sr16*)	4	3+	3	3−	3+	3	3	3	3	4
17	Prelude/8*Mq/2*/Esp 5/8/9 (*Sr17*)	1−	;	1	;	;	0	;	3	3	4
18	LcSr18R1 (*Sr18*)	0	3−	3	3+	3	1−	3+	4	1+	4
19	LcSr19Mq (*Sr19*)	0	2	1	0	1	1+	3	1+	0	1
20	CnS_T_mono_deri (*Sr21*)	1	1	2	1	1−	1+	1−	1	1−	2
21	SwSr22T.B. (*Sr22*)	2	2	0	3+	0	1+	0	1	3−	0
22	Exchange selection (*Sr23*)	1	1+	4	0	1	;	;	1	3	3
23	LcSr24Ag (*Sr24*)	3	3+	3	4	3−	3	3	3	;1−	3C
24	LcSr25Ars (*Sr25*)	0	3−	0	3	0	1	4	3+	0	0
25	Eagle (*Sr26*)	0	1+	1	2	1	1	1+	1	0	1
26	73,214,3-1/9*LMPG (*Sr27*)	3	1	4	0	1+	4	3−	3	3+	1−
27	W2691*Sr28* (*Sr28*)	1	3	3	4	3−	3	3+	3+	3+	4
28	BtS30Wst (*Sr30*)	4	1+	;	1	1−	1+	;	3+	3	1
29	Sr31/6*LMPG (*Sr31*)	2	1−	1	;	;	1	;	1	;	1−
30	CnsSr32 (*Sr32*)	1+	1	3	4	;	3−	3+	3	1	3−
31	RL5450 (*Sr33*)	0	2	2	0	1+	1+	2	2	2	1+
32	Compair (*Sr34*)	3	2	;	4	1	1+	3+	3+	3−	3+
33	Mq(2)5XG2919 (*Sr35*)	3	1−	;	1+	;	0	1−	;	3−	4
34	CI12632/8*LMPG (*Sr36*)	4	0	0	1	0	0	1	4	4	0
35	W2691Sr37 (*Sr37*)	0	3−	0	1	;	1	1+	1	4	1
36	Trident (*Sr38*)	1	;1−	;	;	;	1	;	;	;	;
37	RL6082 (*Sr39*)	3	3+	3	3−	3	3−	3−	3	3−	3
38	RL6088 (*Sr40*)	1	1+	2	0	0	1+	1	2	1	1+
39	TAF 2 (*Sr44*)	1	0	2	1	0	1+	0	2	2	1+
40	Media (*Srdp-2*)	4	1+	1+	3	0	−	1	1+	3	2
41	CnsSrTmp (*SrTmp*)	3	0	1	;	1N	0	;	3N	1−	3−
42	Fed/SrTt3 (*SrTt-3*)	2	1+	2	1+	;	2	2	1+	1−	1
43	BT*Wld* (*SrWld*)	4	2	1+	;	1	−	1+	3	3−	1+
44	Little club	4	4	4	4	4	3+	4	4	3+	4

[a] Infection types were assessed on a 0–4 scale, where high ITs of 3 or 4 were considered resistant, and low ITs of; 0, 1, or 2 were considered susceptible. The symbols + and − indicate slightly larger and smaller pustule sizes, respectively. [b] Races 1–10 represent the tested races: MTSRR, 34C6MTGSM, 34C3MTGQM, 34C3MKGSM, 34MRGQM, 34MKGQM, 34MTGSM, 21C3CTTTM, 21C3CTTSC, and 21C3CTHTM.

Table 3. Seedling infection types and absence or presence of *Sr* genes in 45 wheat cultivars based on molecular markers and gene postulation using 10 races of *P. graminis* f. sp. *tritici*.

No.	Cultivar	*Sr* Gene	Infection Types [a]									
			Pgt 1 [b]	*Pgt 2*	*Pgt 3*	*Pgt 4*	*Pgt 5*	*Pgt 6*	*Pgt 7*	*Pgt 8*	*Pgt 9*	*Pgt 10*
1	Shannong 22	*Sr31* [d]	1	1	0	1+	1	2−	2	1+	0	1+
2	Lunxuan 061	*Sr31* [d]	1+	1	1	1+	1	1+	0	1+	1+	1+
3	Nongda 5181	*Sr31* [d]	2+	0	0	1	1	2	0	1+	0	1+
4	Chang 6154	*Sr31* [d]	1	0	0	1	;	0	;	;	0	−
5	Chang 4738	−	1	;	;	1	1	1	0	2	0	−
6	Nongda 212	−	2	1	0	3	;	1+	;	2	;	1+
7	Liaochun 18	*Sr31* [d]	1	;	0	2	1	1+	0	2	0	0
8	Jinuo 200	*Sr31* [d]	1	0	2	2−	1	1+	0	1+	0	0
9	Henong 7106	*Sr31* [d]	1−	0	1+	1+	1	0	0	2	0	0

Table 3. *Cont.*

No.	Cultivar	*Sr* Gene	Infection Types [a]									
			Pgt 1 [b]	*Pgt 2*	*Pgt 3*	*Pgt 4*	*Pgt 5*	*Pgt 6*	*Pgt 7*	*Pgt 8*	*Pgt 9*	*Pgt 10*
10	Jimai 518	*Sr31* [d]	1	1	2	1	1+	1+	0	1+	0	0
11	Jimai 325	*Sr31* [d]	2+	1	2	1	2−	1+	0	1+	0	1+
12	Heng 136	*Sr5* [c]	3	3	3	3−	3	4	3	1+	0	1+
13	Heng 6632	*Sr31* [d]	1+	;	1	1	1	1	0	1+	0	1
14	Cangmai 119	–	1	;	1+	2	1++	1+	0	;	0	1+
15	Shannong 19	*Sr31* [d]	0	1	0	2	1	0	0	0	0	–
16	Jimai 21	–	;	2	0	3	3−	3	0	1−	;	1+
17	Jimai 22	*SrTmp* [c]	3	1	1	0	;	1	0	3	0	3
18	Tanmai 98	*Sr31* [d]	1	2	0	2	1	2	;	1	0	1+
19	Shimai 15	*Sr22* [c]	2	2+	1	3	1+	1+	0	2	3−	1+
20	Henong 826	*Sr5* [c]	3	4	4	3+	4	3+	3	1+	0	1+
21	H6756	*Sr31* [d]	1	1−	–	1+	1+	0	1−	0	0	0
22	Aikang 58	*Sr31* [d]	2+	;	1	;	;	0	;	0	0	0
23	Jinan 17	*SrTmp* [c]	3	1+	1+	1	1	2	;	3−	0	3
24	Han 617	–	2N	1	;	2+	1+	0	0	1	0	1−
25	Gaoyou 9618	*Sr31* [d]	1	2	1	2	1+	2	0	1−	0	;
26	Kunpeng 1	*Sr31* [d]	1	2	0	2	1	0	0	1	;	1
27	Lin 4	*Sr31* [d]	2+	1	0	0	2	1	1	0	0	0
28	Linmai 2	*Sr31* [d]	1	1	1−	2	1	1−	0	1−	0	1+
29	Nongda 399	*Sr31* [d]	2	2	2−	2	+	;	0	1	0	;
30	Lunxuan 103	–	1	1	0	1+	1	;	0	;	0	1
31	Kenong 1006	*Sr38* [d]	;	1+	0	2	;	1	0	;	0	1
32	Jimai 19	*SrTmp* [c]	3	2	1	;	1	1	1	3N	0	3
33	Han 5316	*Sr19* [c]	1	1	;	2	1	;	3	;	0	1+
34	Shunmai 1718	*Sr5* [c]	4	3	3	3+	3+	3−	3−	1+	;	1+
35	Yunhan 618	*Sr5* [c]	3+	3	3	4	3+	3	3	1+	2	1
36	Jimai 738 strong gluten	–	1+	2	0	2	3−	3	;	2	0	1+
37	Jishi 02-1 strong gluten	*Sr5* [c]	3	4	4	3	4	3	3−	2	;	0
38	Yingzao 2018 strong gluten	–	;	2−	;	2	;	;	;	2	0	1+
39	Ke 2009 strong gluten	–	2	2	0	3+	1+	1−	0	3	0	1+
40	Shiyou 20 strong gluten	*Sr30* [d]	4	2	0	2	1+	1+	0	3	3−	1+
41	Heng S29	*Sr31* [d]	2+	;	0	0	;	;	0	1	0	1+
42	Kenong 2011	*Sr31* [d]	2+	;	2−	1	1+	1	1+	1+	0	1
43	Xingmai 27	*Sr31* [d]	1	2	1	1−	1+	1	0	2	0	0
44	Shannong 28	*SrTmp* [c]	3	1	2	1	;	1	0	3	0	3
45	Jimai 120	*Sr31* [d]	1	2	0	1−	1+	1	0	1+	;	1
46	Little club	–	4	4	4	4	4	3+	4	4	3+	4

[a] Infection types were assessed on a 0–4 scale, where high ITs of 3 or 4 were considered resistant, and low ITs of ;, 0, 1, or 2 were considered susceptible. The symbols +, −, and ++ indicate slightly larger and smaller pustule sizes, respectively. [b] *Pgt 1–10* represent the tested races: MTSRR, 34C6MTGSM, 34C3MTGQM, 34C3MKGSM, 34MRGQM, 34MKGQM, 34MTGSM, 21C3CTTTM, 21C3CTTSC, and 21C3CTHTM. [c] *Sr* genes derived through gene postulation. [d] *Sr* genes confirmed through molecular marker detection.

The results of the molecular markers confirmed the presence of *Sr31* in the 23 wheat cultivars (Figure 1 and Table 3). All wheat cultivars containing *Sr31* were further confirmed by gene postulation because these lines were also resistant to all tested races. Five cultivars (Heng 136, Henong 826, Shunmai 1718, Yunhan 618, and Jishi 02-1 strong gluten) demonstrated low ITs to three *Sr5*-avirulent races (21C3CTTTM, 21C3CTTSC, and 21C3CTHTM), and high ITs to all other races, suggesting the presence of *Sr5*. Han 5316 displayed high ITs for 34MTGSM and low ITs for all other races, which was consistent with the resistance spectrum of the monogenic line containing *Sr19*. Therefore, it was postulated that Han 5316 contains *Sr19*. The susceptibility of Shimai 15 to 34MRGQM, but resistance to other races, suggested the presence of *Sr22.* Based on the susceptibility of the Shiyou 20 cultivar to races MTSSR, 21C3CTTTM, and 21C3CTTSC, coupled with its resistance to all other races, it was likely that Shiyou 20 harbored the *Sr30* resistance gene. Four wheat cultivars (Jimai 22, Jinan 17, Jimai 19, and Shannong 28) were resistant to seven *SrTmp* avirulent races (34C6MTGSM, 34C3MTGQM, 34C3MKGSM, 34MRGQM, 34MKGQM, 34MTGSM, and 21C3CTTSC), and susceptible to three *SrTmp* virulent races (MTSRR, 21C3CTTTM, and 21C3CTHTM), suggesting the potential presence of *SrTmp*. Furthermore, molecular marker analysis revealed that Kenong 1006 contained *Sr38*, whereas no wheat cultivars harbored the genes *Sr25* and *Sr26*.

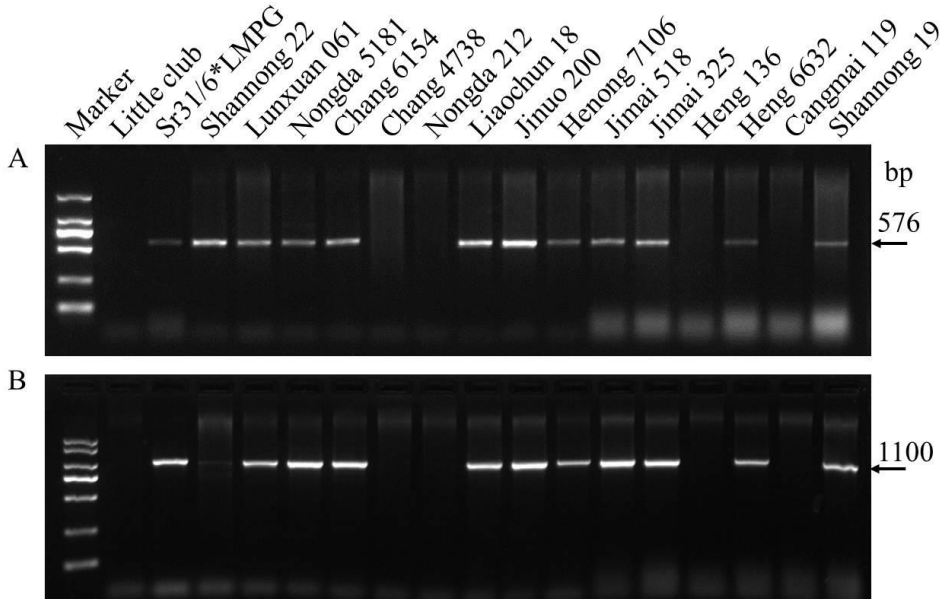

Figure 1. Detection results for parts of the wheat cultivar with markers SCSS30.2$_{576}$ and Iag95. (**A**) SCSS30.2$_{576}$; (**B**) Iag95.

3.2. Field Evaluation

From 2021 to 2022, the IRs of 45 wheat cultivars were evaluated against two predominant races: 34MRGQM and 21C3CTHTM (Table 4). The IRs of all the wheat cultivars were divided into three categories: immunity, resistance to moderate resistance, and susceptibility to moderate susceptibility (Table 5). In field trials conducted in 2021 and 2022, 37 wheat cultivars (82.2%) and 39 wheat cultivars (86.7%) showed resistance to races 34MRGQM and 21C3CTHTM, respectively. Overall, 33 wheat cultivars (73.3%) were resistant to all tested races.

Table 4. Pedigree of tested cultivars, and stem rust infection response (IRs) [a] at the adult-plant stage during 2021 and 2022.

No.	Cultivar	Pedigree	34MRGQM		21C3CTHTM	
			2021	2022	2021	2022
1	Shannong 22	Ta1 (Ms2) wheat recurrent selection population	5 R	5 R	0 R	5 R
2	Lunxuan 061	Recurrent quality populations of dwarf male-sterile wheat	0 R	5 R	0 R	0 R
3	Nongda 5181	Nongda 3097/Lunxuan 987	0 R	5 R	0 R	0 R
4	Chang 6154	Jinmai 63/Yun 8337-17-5-2-1	5 R	5 R	0 R	10 R
5	Chang 4738	82230-6/94-5383	0 R	0 R	0 R	0 R
6	Nongda 212	Nongda 3338/S180	0 R	5 R	10 R	10 R
7	Liaochun 18	Liaochun 10 mutants	5 R	5 R	0 R	0 R
8	Jinuo 200	03 Nuo F3-1186/Lankao 906	30 R	5 R	0 R	0 R
9	Henong 7106	Henong 9923/Henong 4631	5 R	5 R	0 R	0 R
10	Jimai 518	Taigu Male-sterile populations	20 R	10 R	5 R	0 R
11	Jimai 325	Ji 5157/Shi 02-7221	20 R	5 R	20 R	5 R
12	Heng 136	Heng 4119/Shijiazhuang 1	40 MS	50 S	5 R	0 R
13	Heng 6632	Black wheat/Heng 8116	5 R	10 R	0 R	0 R
14	Cangmai 119	8341699/CA8694	90 S	80 S	90 S	90 S
15	Shannong 19	(83(3)-113/1604) F3//886059	30 R	5 R	30 R	5 R
16	Jimai 21	865186/Chuannongda 84-1109//Ji 84-5481	30 MS	40 MS	10 R	30 R
17	Jimai 22	935024/935106	5 R	5 R	20 MS	60 S
18	Tanmai 98	Jining 13/942	5 R	5 R	0 R	0 R
19	Shimai 15	(GS Jimai 38/92R137)/GS Jimai 38	30 R	5 R	30 R	20 R
20	Henong 826	Shi 6365/95 Guan 26	60 S	50 MS	10 R	5 R
21	H6756	Laizhou 953/9024-85/Ji 87-5108	0 R	5 R	0 R	5 R
22	Aikang 58	Zhoumai 11//Wenmai 6/Zhengzhou 8960	40 R	0 R	0 R	10 MR
23	Jinan 17	Linfen 5064/Lumai 13	20 R	5 R	80 S	60 S
24	Han 6172	Han 4032/Zhongyin 1	10 R	20 R	5 R	10 R
25	Gaoyou 9618	8515-4/8901-11-14	50 R	40 R	10 R	20 MR
26	Kunpeng 1	9411 (8901/Annong 8455)/9502 (8903/Xiaobing 33)	60 R	50 R	50 R	60 R
27	Lin 4	Lumai 23/Lin 9015	5 R	5 R	0 R	0 R

Table 4. *Cont.*

No.	Cultivar	Pedigree	34MRGQM		21C3CTHTM	
			2021	2022	2021	2022
28	Linmai 2	Lumai 23/Lin 90-15	0 R	0 R	0 R	0 R
29	Nongda 399	Torino/Henong 2552/Nongda 9516//Shi 4185	30 MR	20 MR	0 R	5 R
30	Lunxuan 103	Shimai 12/Shijiazhuang 8	5 R	5 R	10 R	5 R
31	Kenong 1006	Kn 9204//Kn 9204/Gaomai 5///9204R	0 R	0 R	0 R	0 R
32	Jimai 19	Lumai 13/Linfen5064	0 R	0 R	20 MS	40 MS
33	Han 5316	(Han 7808/CA8059) F4//85 Zhong47	20 R	5 R	5 R	5 R
34	Shunmai 1718	32S/Gabo	30 MS	30 MS	20 R	10 R
35	Yunhan 618	Yunhan 92-18/Xinchun 9	40 MS	40 MS	10 R	10 R
36	Jimai 738 strong gluten	Gao 9618/Liangxing 99	100 S	90 S	10 R	5 R
37	Jishi 02-1 strong gluten	-	80 MS	50 MS	20 R	30 R
38	Yingzao 2018 strong gluten	-	30 R	20 R	20 R	5 R
39	Ke 2009 strong gluten	-	20 R	5 R	10 R	10 R
40	Shiyou 20 strong gluten	Ji 935-352/Jinan 17	10 R	5 R	5 R	5 R
41	Heng S29	Heng 98-5229 systematic breeding	5 R	5 R	10 R	5 R
42	Kenong 2011	Kn 9204/Pubing material PZW-9	30 R	20 R	10 R	10 R
43	Xingmai 27	-	10 R	10 R	10 R	5 R
44	Shannong 28	Jimai 22/6125	5 R	5 R	10 MS	20 MS
45	Jimai 120	ISENGRAIN/Shi 20-6091	60 R	50 R	20 R	40 R
46	Little club	-	90 S	80 S	90 S	90 S

[a] IRs, in combination with severity, were scored at the adult-plant stage in the field tests, following the descriptions of Roelfs et al. [31], where R is resistant, MR is moderately resistant, MS is moderately susceptible, and S is susceptible.

Table 5. Susceptibility and resistance proportion of 45 wheat cultivars to 2 races, 34MRGQM and 21C3CTHTM, at adult-plant stage during 2021 and 2022.

Races	Immune		Resistance—Moderately Resistant		Moderately Susceptible—Susceptible	
	2021	2022	2021	2022	2021	2022
34MRGQM	8 (17.8) [a]	5 (11.1)	29 (64.4)	32 (71.1)	8 (17.8)	8 (17.8)
21C3CTHTM	16 (35.6)	13 (28.9)	23 (51.1)	26 (57.8)	6 (13.3)	6 (13.3)
All races	5 (7.7)	3 (4.6)	28 (43.1)	30 (66.7)	12 (18.5)	12 (18.5)

[a] 8 (17.8): The number outside parentheses represents the number of cultivars, and the number inside parentheses represents the percentage of that number.

4. Discussion

Sr5 serves as the main gene for standard identification of *Pgt* in China. It can distinguish races of *Pgt* in almost all countries [30]. To date, based on the virulence of *Pgt* toward this gene, all wheat stem rust isolates in China can be classified into either 34 race groups (e.g., 34C0MKGSM and 34C0MRGQM) with virulence to *Sr5* or 21 race groups (e.g., 21C3CTHTM and 21C3CKHQM) without virulence to *Sr5* [33]. Although *Sr5* exhibits susceptibility to Ug99, it has shown excellent resistance to the Chinese-dominated 21C3 race. In this study, five wheat cultivars were postulated to contain *Sr5*, accounting for 11.1% of the experimental materials. In the field experiment, wheat cultivars containing *Sr5* showed high resistance to 21C3CTHTM, with disease severity levels below or equal to 30%. As an important resistance gene in wheat in China, *Sr5* also plays a vital role in the accumulation of lignin and callose in wheat tissues following stem rust infection [34].

Sr19, originated from the 2B chromosome of *Triticum aestivum* L., is characterized by the absence of resistance to Ug99. In this study, among the 45 wheat cultivars examined, the presence of *Sr19* was identified in a single cultivar (Han 5316). At the adult-plant stage, Han 5316 exhibited robust resistance (5-20R) to two dominant races in China, namely 34MRGQM and 21C3CTHTM. At the seedling stage, Han 5316 presented low ITs (0-2) to nine races in China, with only three ITs to 34MTGSM. The IT of the wheat cultivar Han 5316 containing *Sr19* to the Chinese race 34C3MTGQM was determined to be one. This is contrary to the results reported in previous studies, suggesting that Han 5316 may possess an additional resistance gene conferring resistance to 34C3MTGQM.

Sr22 is derived from *Triticum monococcum* L., presenting excellent resistance to the new race Ug99 of *Pgt*, as well as the predominant race groups 21C3 and 34C2 in China. Located

on the 7AL chromosome, *Sr22* is a temperature-sensitive gene whose resistance level increases with decreasing temperature, which makes it difficult to identify the existence of this gene. In this study, only one of the 45 wheat cultivars was identified to possess *Sr22* (Shimai 15). In the field experiment, Shimai 15 presented resistance to races 34MRGQM and 21C3CTHTM, with disease severity levels below or equal to 30%. In wheat cultivar identification within other regions, only four of the eighteen main cultivated materials in Heilongjiang Province were found to contain *Sr22* [35]. Similarly, Li et al. employed Xcfa2019-specific primers to detect 283 wheat cultivars in China, including 20 cultivars resistant to Ug99 from CIMMYT [24]. Their investigation revealed that none of these cultivars contained the resistance gene *Sr22*. This suggests the absence of this gene in the Chinese wheat cultivars during the breeding process.

Sr30, derived from the long arm of the 5D chromosome of *T. aestivum* L. [36], exhibits discriminatory capabilities against specific races of *Pgt* (34C4 and 34C5) from other races in the 34-race group in China [20]. Specifically, *Sr30* demonstrates susceptibility to races 34C4 and 34C5 while presenting resistance against races 34, 34C1, 34C2, and 34C3. Although *Sr30* lacks resistance to Ug99, it serves as the main disease resistance gene in wheat cultivars (lines) in Yunnan Province, China, and presents robust resistance to other races in China [37]. In this study, only one of the 45 wheat cultivars tested (Shiyou 20 strong gluten) was identified to contain *Sr30*.

Sr31 currently stands as the most widely used resistance gene for stem rust. Since the 1960s, cultivars of the Soviet Union and Romania containing *Sr31* have been introduced and extensively employed in wheat breeding programs across China. Although *Sr31* is susceptible to Ug99, it still exhibits excellent resistance to all *Pgt* races in China. To mitigate the threat of Ug99 and its cultivars to wheat production, the identification of disease-resistant germplasms has become paramount [20]. Therefore, this study aimed to identify the *Sr* gene present in the main cultivars cultivated in Shandong, Hebei, and Shanxi provinces using molecular markers. The results indicate that 23 of the 45 wheat cultivars contained *Sr31*, which accounted for 41.8% of the experimental materials. The wheat cultivars containing *Sr31* were resistant to 10 races in China at the seedling stage, while exhibiting MR or R responses to two dominant races in China (34MRGQM and 21C3CTHTM) at the adult-plant stage. Our results are consistent with those of numerous previous studies, suggesting that wheat cultivars in most areas of China may contain the *Sr31* gene [24]. Further pedigree analysis confirmed that the presence of *Sr31* in these cultivars could be derived from Avorara, Lovrin 10, Lovlin 13, and Lumai 14, carrying the linkage gene cluster *Sr31-Yr9-Lr26-Pm8*.

SrTmp is derived from *Triumph*, located on the 6DS chromosomes near *SrCad* and *Sr42*. This genetic positioning imparts resistance to Ug99. Unlike *Sr42, SrTmp* is resistant to races QCCJB, RKQSC, QTHJF, and TTKSK, whereas *Sr42* is resistant only to races RKQSC and TTKSK [38]. The presence of this gene can be detected in diverse wheat cultivars in certain countries such as Europe, India, United States, Ethiopia, Pakistan [20]. The gene not only demonstrates robust resistance to Ug99 and several Ug99 variants but also indicates excellent resistance to numerous races of *Pgt* in China. In this study, four wheat cultivars (Jimai 22, Jinan 17, Jimai 19, and Shannong 28) contained *SrTmp*, accounting for 8.8% of the experimental materials. Pedigree tracing analysis revealed that the presence of *SrTmp* in these cultivars can originate from Youbaomai, which contained the dwarf resistance gene *Rht2*, the stem-rust resistance gene *SrTmp*, as well as leaf-rust resistance genes *Lr1* and *Lr35*. In the field experiment, the lines containing *SrTmp* exhibited high resistance to the predominant race 34MRGQM in China, with disease severity levels below 30%.

The rust-resistant gene cluster *Yr17-Lr37-Sr38*, originating from *Aegilops obliquus* (*T. ventricosum*) and first transferred to the bread wheat cultivar "VPM1" [39], is located in the 2NS/2AS fragment. The gene cluster *Sr38-Yr17-Lr37* has excellent resistance to wheat stem rust, stripe rust, and leaf rust. The restriction fragment length marker cMWG682 can be used to detect the presence of 2NS/2AS fragments in wheat [39]. In this study, *Sr38* was detected in 1 of the 45 wheat samples. Although *Sr38* has lost its resistance to Ug99,

there have been no reports of *Pgt* races exhibiting virulence toward this gene in China. Consequently, *Sr38* has demonstrated excellent resistance to most races in China. In this study, the wheat cultivar containing *Sr38* showed low ITs (0–2) in response to the 10 tested races in China at the seedling stage. At the adult plant stage, the two predominant races in China (34MRGQM and 21C3CTHTM) presented resistance to diseases (R). Therefore, to enhance resistance against wheat stem rust in China, it is imperative to incorporate the Ug99-resistant gene into the breeding process.

In this study, *Sr* genes present in 45 wheat cultivars from Shandong, Shanxi, and Hebei provinces were identified using molecular markers and gene derivation techniques. As a result, seven *Sr* genes were detected, including *Sr5*, *Sr19*, *Sr22*, *Sr30*, *Sr31*, *Sr38*, and *SrTmp*, among which *Sr5* and *Sr31* emerged as the most prevalent genes applied in breeding programs. Although these genes lack resistance to Ug99, they exhibited excellent resistance to most *Pgt* races in China. Evaluation of the resistance of these cultivars (lines) to races 34MRGQM and 21C3CTHTM at both seedling and adult-plant stages demonstrated robust resistance. Except for Cangmai 119, all other resistant cultivars (lines) showed resistance to races 34MRGQM and 21C3CTHTM at the seedling stage (ITs: 0–2) and exhibited R to MR infection responses at the adult-plant stage, with relatively low severity. Conversely, cultivars showing susceptibility (ITs: 3–4) during the seedling stage also displayed MS to S infection responses during the adult-plant stage. Despite showing low ITs in races 34MRGQM and 21C3CTHTM at the seedling stage, Cangmai 119 exhibited S infection responses at the adult-plant stage. This suggests that Cangmai 119 may contain a temperature-sensitive gene that expresses resistance to low temperatures and is susceptible to high temperatures. In summary, wheat cultivars (lines) from the Henan, Hebei, Shandong, and Shanxi provinces exhibited substantial resistance to the tested races of *Pgt*. The findings of this study provide valuable resistance source materials and theoretical support for strategically organizing resistance genes regionally as well as for the selection and breeding of resistant cultivars in China.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14020198/s1, Table S1: Supplemental information on wheat cultivar; Table S2: Weather conditions in Shenyang from 30 May to 30 June 2021 and 2022.

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