Stem rust seedling resistance genes evaluation and identification of *Sr2*, *Sr24*, *Sr26*, *Sr31* and *Sr38* in wheat lines from Gansu Province in China (#19842)

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Stem rust seedling resistance genes evaluation and identification of *Sr2*, *Sr24*, *Sr26*, *Sr31* and *Sr38* in wheat lines from Gansu Province in China

Tian Ya Li [Corresp., 1], **Xiao Feng Xu** [1], **Yue Gao** [1], **Zi Yuan Wang** [1], **Yu Chen Ma** [1], **Shuo Yang** [1], **Yuan Yin Cao** [1], **Yuan Hu Xuan** [1]

[1] College of Plant Protection, Shenyang Agricultural University, Shenyang, China

Corresponding Author: Tian Ya Li
Email address: litianya11@syau.edu.cn

Stem rust is caused by *Puccinia granimis* f. sp. *tritici*, severely affects wheat production, but it has been effectively controlled in China since the 1970s. However, the appearance and spread of wheat stem rust races Ug99 (virulence to *Sr31*) and TTTTF (virulence to the cultivars carrying *Sr9e* and *Sr13*) have caught breeders attention for developing resistance lines to stem rust of wheat. It is important to clarify resistance genes timely, especially digging a new resistance gene in wheat cultivars for durable-resistance of wheat breeding. However, little is known about the stem rust resistance genes of widely used wheat cultivars (lines) from Gansu. This study aimed to determine the resistance level at the seedling stage of main wheat cultivars (lines) in Gansu Province. A secondary objective was to assess the prevalence of *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* using molecular markers. The results of the present study indicated that 42 (66.0%) tested wheat varieties showed different resistant grade to all the tested races of *Puccinia graminis* f. sp. *tritici.* The molecular marker analysis showed that 13 out of 75 major wheat cultivars likely carried *Sr2*; 25 wheat cultivars likely carried *Sr31*; and 9 wheat cultivars likely carried *Sr38*. No cultivar was found to have *Sr26*, as expected. Surprisingly, no wheat cultivars carried *Sr24*. The results might enable the development of appropriate strategies to breed varieties resistant to stem rust.

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Xiao Feng Xu[#], Yue Gao[#], Zi Yuan Wang[#], Yu Chen Ma, Shuo Yang, Yuan Yin Cao, Yuan Hu Xuan*, Tian Ya Li*

College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning, China

[#]These authors contributed equally to this work.

*Corresponding authors

Phone/Fax: +86 24 8834 2056, litianya11@syau.edu.cn (Tian Ya Li) or

Phone/Fax: +86 24 8834 2056, xuanyuanhu115@syau.edu.cn (Yuan Hu Xuan)

Abstract

Stem rust is caused by *Puccinia granimis* f. sp. *tritici*, severely affects wheat production, but it has been effectively controlled in China since the 1970s. However, the appearance and spread of wheat stem rust races Ug99 (virulence to *Sr31*) and TTTTF (virulence to the cultivars carrying *Sr9e* and *Sr13*) have caught breeders attention for developing resistance lines to stem rust of wheat. It is important to clarify resistance genes timely, especially digging a new resistance gene in wheat cultivars for durable-resistance of wheat breeding. However, little is known about the stem rust resistance genes of widely used wheat cultivars (lines) from Gansu. This study aimed to determine the resistance level at the seedling stage of main wheat cultivars (lines) in Gansu Province. A secondary objective was to assess the prevalence of *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* using molecular markers. The results of the present study indicated that 42 (66.0%) tested wheat varieties showed different resistant grade to all the tested races of *Puccinia graminis* f. sp. *tritici.*

The molecular marker analysis showed that 13 out of 75 major wheat cultivars likely carried *Sr2*; 25 wheat cultivars likely carried *Sr31*; and 9 wheat cultivars likely carried *Sr38*. No cultivar was found to have *Sr26*, as expected. Surprisingly, no wheat cultivars carried *Sr24*. Ch results might enable the development of appropriate strategies to breed varieties resistant to stem rust.

Key words: Wheat Stem rust; marker; resistance genes; wheat cultivar

Introduction

Puccinia graminis f. sp. *tritici* Ereks. and E. Henn (*Pgt*) occupies a large area of airborne disease with high specificity and causes one of the most potentially destructive wheat diseases, seriously threatening the grain production safety in the world (Pardey et al., 2013). Disease-resistant breeding to control wheat stem rust is economic, effective, and protective for the ecological environment, and has been proved to be the best control method by repeated practice (Goutam et al., 2015). Wheat stem rust has been effectively controlled with the wide use of resistance gene *Sr31* from a 1BL/1RS ectopic system of wheat–rye (Rouse et al., 2012). However, a new race Ug99 virulent to *Sr31* was identified in Uganda and named as TTKS by a reference of the North American Race Vulnerability Nomenclature System of *Pgt* in 1999 (Pretorius et al., 2000). Ug99 has strong virulence, and mutates and spreads quickly. Since 1999, 13 variants of Ug99 have been found in 13 countries (FAO, 2017). Recently, Ug99 has been monitored in Egypt, which is the main wheat production area of the Middle East, revealing that its mode of spread is similar to that of a virulent race to *Yr9* predicted by Geographic Information System of CIMMYT, namely

East Africa–West Asia–South Asia–East Asia (CIMMYT. 2007).

When the world is trying to control the spread and epidemic of the new race Ug99 and its variants causing global panic, another new race TTTTF having associated virulence to *Sr9e* and *Sr13* attacked thousands of hectares of durum wheat in Sicily, Italy, in 2016, resulting in the largest burst of wheat stem rust in Europe since the 1950s (Bhattacharya, 2017). The large number of spores produced by TTTTF may continue to infect and spread in 2017. If the environmental conditions are appropriate, the disease outbreak and epidemic may occur again. Moreover, the researchers from the Global Rust Research Center shared a major concern in the warning report that TTTTF could infect not only durum wheat and bread wheat but also dozens of laboratory-grown strains of wheat, including hardy varieties that are usually highly resistant to diseases (FAO, 2017). In view of this, in February 2017, 'Nature' focused on the potential threat to European wheat production of this race (Bhattacharya, 2017). Therefore, the spread of Ug99 and TTTTF, and their variants, threating the wheat production safety in China.

Gansu Province, located in the northwest of China, plays a significant role in the epidemic and spread of wheat stem rust in China (Cao, 1994). Resistance-breeding for this disease has not been a primary objective because it has been effectively controlled in China since the 1970s (Wu et al., 2014). However, great importance has been attached again to durable resistance to stem rust in wheat breeding with the occurrence and spread of races of *Pgt*. It is necessary to analyze the resistance genes in wheat cultivars (lines) from Gansu Province, and the information provided here will be important for developing potentially durable combinations of stem rust resistance cultivars.

Materials and Methods

Wheat cultivars and near-isogenic lines

A total of 76 tested main productive wheat cultivars (lines) in Gansu Province were provided by Dr. Fangping Yang from the Wheat Research Institute, Gansu Academy of Agricultural Sciences.

Five *Sr* genes were tested: *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38*. Forty-five *Sr* genes were used to confirm the validity of these molecular markers. The near-isogenic lines carrying these resistance genes were provided by Dr. Yue Jin from USDA-ARS, Cereal Disease Laboratory, University of Minnesota, USA.

The tested *Pgt* races include the dominant 21C3CTHTM and 34MKGQM, and 34C3RTGQM (a new race identified from the alternative host *Berberis*). These races were named according to the methods described in a published study (Li et al., 2016b). The full names of the races and their virulence/avirulence patterns are shown in Table 1. They were isolated and identified by the Plant Immunity Institute, Shenyang Agricultural University, China.

Seedling resistance evaluation

The whole cultivars (lines) were planted in porcelain pots with a 12-cm-diameter. Seven days later, the leaves were moistened by water with 0.1% Tween 20 using an atomizer and then sprayed with 1 g of fresh urediniospores and dried talc in a ratio of 1:20 (v:v). The inoculated seedlings were transferred to a greenhouse with the temperature in a range of 18 to 22 ± 1°C. Three biological replicates of the seedling assays were performed for each *Pgt* race. After 14 days of inoculation, the infection types (ITs) were recorded using the 0–4 IT scale (Stakman,

Stewart & Loegering, 1962). ITs were then grouped into low ('0', ';', '1', '1+', '2', '2+', and X) and high ('3–', '3', '3+', and '4') infection types. The ITs used in this study are shown in Fig. 1.

DNA extraction

DNA was extracted from young leaves of 10-day-old seedlings using the genomic DNA extraction kit (http://www.sangon.com/, China). The DNA quality was examined by 1.2% (w/v) agarose gel and DNA quantification was performed using the NanoDrop-1000 version 3.3.1 spectrophotometer.

Polymerase chain reaction (PCR)-specific primers were synthesized by Shanghai Biotech Biotech Co., Ltd, China (Table 2). PCR amplifications were carried out in 25 μL volume, including 0.5 μL of 10 mmol·L^{-1} deoxyribonucleoside triphosphates, 2.5 μL of 10× buffer (Mg^{2+}), 0.2 μL of 5 U·μL^{-1} Taq polymerase, 1 μL of 10 μmol·L^{-1} of each primer, and 2 μL of 30 ng·μL^{-1} DNA. De-ionized water was used to achieve 25 μL volume. Condition of PCR amplification was as follows: 94°C for 4 min, 30 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 1 min, followed by the final extension at 72°C for 8 min; other specific conditions were as described in previous studies (Table 1).

Results

Wheat seedling resistance

The resistance test results of 75 main wheat cultivars in Gansu to the races 21C3CTHTM, 34MKGQM, and 34C3RTGQM are shown in Table 3. Forty-two (66.0%) of the 75 tested wheat cultivars (Ningchun 39, Dingfeng 10, Ganchun 25, Longchun 25, Longchun 23, Longchun 26, Longchun 22, Ganchun 24, Yinchun 9, Longchun 31, Longchun 28, Dingxi 38, Dingxi 41,

Wuchun 4, Jinchun 5, Gansu 26, Linmai 33, Jiuchun 6, Longchun 27, Linmai 34, Dingfeng 12, Zhangchun 21, Wuchun 6, Xifeng 27, Lantian 26, Hangxuan 1, Lantian 14, Xifeng 20, Longyu 4, Zhongliang 22, Lantian 10, Tianxuan 39, Lantian 30, Longnan 2000-8-2-1, Longjian 301, Longyu 2, Longjian P430, Lan 092, Longyuan 034, Gandong 017, 863-13, Tian 01-2(9) showed different resistance levels (ITs 0, ;, ;1, 1+, and 2) to these races at the seedling stage. The remaining 33 (44.0%) wheat cultivars showed varying levels of susceptibility (ITs 3, 3–, 3+, and 4) (Table 3).

Validity of the markers

Five specific PCR markers closely linked with resistance genes *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* were validated using 45 single differentials carrying known resistance genes to further study the validity of the markers. Table 4 shows that these three markers amplified only specific bands in the known gene linked with them. For example, primer SCSS30.2$_{576}$ amplified only 576-bp specific bands in Siouxland, Sisson, Sr31/6*LMPG, and Federation*4/Kavl, while in other wheat lines without *Sr31*, no bands were amplified, indicating that these markers are able to be well applied for the molecular detection of the five resistance genes.

***Sr2* screening**

A DNA marker was developed to accurately predict *Sr2* in diverse wheat germplasm for the partial resistance of *Sr2* is very difficult to screen under field conditions (Mago et al., 2011). Two markers, Xgwm533 and csSr2, were used to detect *Sr2* in wheat cultivars of Gansu Province. A specific PCR band with 120-bp in size was amplified with marker Xgwm533, but no PCR product was amplified using marker csSr2 in Hope with *Sr2*. A similar 120-bp band was

detected in Longchun 26, Wuchun 8, Ganchun 24, Yinchun 9, Ganchun 21, Longchun 33, Jiuchun 6, Longchun 27, Dingfeng 12, Wuchun 6, Lantian 14, Zhongliang 18, and 01-426e-1 in this study, indicating that the 13 tested cultivars carried *Sr2* (Table 5).

_Sr24_ screening

Two markers, Sr24#12 and Sr24#50, were developed to detect *Sr24*, located on chromosome 3DL (Mago et al., 2005) in Agent- or 1BS in Amigo-derived lines. These two markers were applied to detect *Sr24* existence in the 75 major wheat cultivars (lines) of Gansu Province in this study. The results showed that marker *Sr24*#12 amplified a 500-bp specific band and marker *Sr24*#50 amplified an approximately 200-bp specific band in the *Sr24* control Lc*Sr24*Ag. No PCR fragment was amplified in Little Culb (LC) and tested cultivars, indicating that these cultivars lacked *Sr24*.

_Sr26_ screening

Sr26 was transferred into the long arm of wheat chromosome 6A from *Thinopyrum ponticum* (Mago et al., 2005). Although the cultivars carrying *Sr26* show resistance to all the dominant *Pgt* races in China, it is not utilized in wheat breeding. A dominant STS marker Sr26#43 was developed for detecting this wheat stem rust resistance gene and a 207-bp specific band was amplified in wheat with *Sr26* (Mago et al., 2005). Marker Sr26#43 was used to detect this fragment in tested wheat cultivars (lines). No any visible band was detected, suggesting that these varieties do not carry *Sr26*, as expected.

_Sr31_ screening

Two markers, SCSS30.2$_{576}$ and Iag95 linked to resistance gene *Sr31*, were used for detecting this

148 locus. SCSS30.2$_{576}$ amplified a 576-bp fragment and marker Iag 95 amplified an 1100-bp PCR
149 fragment in *Sr31*-carrying lines such as *Sr31*/6*LMPG and Siouxland (Fig. 2). No fragment was
150 amplified in the negative control Little Club. These two markers were used to detect *Sr31* in the
151 tested cultivars. The result showed that these two fragments were detected in wheat cultivars
152 Ganchun 25, Longchun 25, Longchun 23, Longchun 26, Ganchun 24, Yinchun 9, Longchun 31,
153 Dingxi 41, Jinchun 5, Gansu 26, Longchun 27, Zhangchun 21, Xifeng 27, Lantian 26, Lantian 14,
154 Zhongliang 22, Lantian 10, Tianxuan 39, Longjian 301, Longyu 2, Longjian P430, Longyuan
155 034, Gandong 017, 863-13, and Tian 01-29, indicating that the 25 tested cultivars carried *Sr31*
156 (Table 5).

157 **_Sr38_ screening**

158 The *Lr37-Sr38-Yr17* rust resistance gene cluster was transferred to the short arm of bread wheat
159 chromosome 2AS from a segment of *Triticum ventricosum* (Tausch) Cess. chromosome 2NS
160 (Helguera et al., 2003). 2NS-specific primer VENTRIUP-LN2 and 2AS-specific primer URIC-
161 LN2 were developed to detect this rust resistance genes cluster in commercial wheat cultivars in
162 2003 and 262-bp and 285-bp PCR products were amplified in wheat line carrying *Lr37-Sr38-*
163 *Yr17*, respectively, while none of these amplification products were found in negative control LC
164 (without *Lr37-Sr38-Yr17*). Both 262-bp and 285-bp PCR fragments were amplified in nine
165 wheat cultivars Dingxi 38, Jinchun 5, Gansu 26, Linmai 33, Jiuchun 6, Hangxuan 1, Lantian 14,
166 Zhongliang 22, and Lantian 10 in this study, suggesting that these wheat cultivars (lines) carried
167 *Sr38* (Table 5).

168

Discussion

The stem rust broad-spectrum resistance gene *Sr2* which refers the adult-plant resistant to wheat stem rust and is located on chromosome 3BS. It was originated in tetraploid Yaroslav emmer (*T. dicoccum*) and later transferred to the susceptible bread wheat "Marquis" in the 1920s (McFadden,1930). ,use extended the cultivation of several resistant lines in worldwide (Singh et al., 2011). Because of an effective function of *Sr2* in stem rust resistance, markers Xgwm533 and csSr2 were used to detect *Sr2* in wheat cultivars from Gansu. However, marker csSr2 failed to predict *Sr2*. Only marker Xgwm533 primed a 120-bp band in the positive control and 13 tested cultivars, but the 120-bp band also occurred in many North American and CIMMYT lines which are considered not to have *Sr2*. Therefore, it is difficult to conclude that all the accessions that showed a 120-bp fragment size for this marker carry *Sr2*.

The stem rust resistance gene *Sr24* is completely associated with leaf rust resistance gene *Lr24*. It has been widely used in wheat breeding programs worldwide, since it introgressed into wheat lines (McIntosh, Wellings & Park, 1995). *Sr24* was ineffective to the variants of Ug99 but effective to the new race TTTTF (Bhattacharya 2017) and many *Pgt* races in China (Han, Cao & Sun, 2010). Therefore, two markers, Sr24#12 and Sr24#50, screened by Mago et al. (2005) were used to detect the gene in Gansu wheat cultivars (lines) in this study. Surprisingly, no wheat cultivars carried this gene. However, it is reported that Chinese wheat cultivars carry *Sr24* (Li et al., 2016b; Cao et al., 2007), the cause of which needs further investigation.

In Australia, *Sr26* has been released with the cultivar Eagle since 1971 (Martin, 1971). Later, the other major cultivars including Flinders, Harrier, Kite, Takari, Sunelg, and Avocet carrying

Sr26 were cultured lines contain *Sr26* fragment effectively resistant to new stem rust races such as Ug99 and its associated strains, but it rarely used in breeding programs (Yu et al., 2010). None of the cultivars (lines) had *Sr26* in the present study, as expected, and the similar results were observed in our precious study (Li et al., 2016a).

The stem rust resistance gene *Sr31* was became a 1BL/1RS, which was transferred from the bread wheat from "Petkus" rye (Graybosch, 2001). Since then a higher number of wheat cultivars carrying *Sr31* have been released in global wheat breeding (Das et al., 2006). It is reported that more than 60% (1.3 × 107 hm^2) of the total wheat planting areas carried this translocation in China (Jiang et al., 2007). Although the gene is ineffective to Ug99 and related variants, it is also an effective gene against all *Pgt* races in China and the new race TTTTF. Molecular marker detection showed that 25 wheat cultivars carried *Sr31*. All these cultivars (lines) produced resistance ITs (0, ;, ;1, 1+, and 2) to all tested *Pgt* races, as expected. Moreover, pedigree tracking indicated that resistant materials carrying 1BL/1RS translocation such as "Kavkaz" and "Luofu" lines were widely used in wheat breeding in Gansu Province, revealing the origin of resistance genes in these wheat varieties.

Rust resistance gene cluster *Yr17-Lr37-Sr38* was initially transferred into a winter bread wheat "VPM1" from *T. ventricosum* (Maia, 1967) and was located in a 2NS/2AS(Bariana & McIntosh 1993; Cao et al., 2007). PCR assays using restriction fragment length marker cMWG682 were developed for selecting the 2NS/2AS translocation in wheat cultivars (Helguera et al., 2003). *Sr38* became susceptible to new races related to Ug99 but no virulent *Pgt* race to *Sr38* has been found in China. The results showed that nine wheat cultivars were able to carry the gene. The

211 resistance of these cultivars against the tested *Pgt* races might be attributed to this gene.

212 **Conclusion**

213 Breed resistant cultivars is an economic and effective way to protect wheat from disease;
214 however, the process took longer than conventional methods. Later developing of molecular
215 technology, using molecular marker detection and utilization in main cultivars improved the
216 disease-resistant cultivars in a relatively short time, leading to increased crop production. The
217 molecular genetic markers *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* were used to detect the occurrence of
218 these genes in 75 major wheat cultivars (lines) in Gansu Province in this study. The results
219 showed that 35 tested cultivars might carry one of these genes. This information can be used in
220 wheat breeding in the future.

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223 Agricultural Sciences for providing the wheat cultivars.

224

225 **Reference**

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Table 1(on next page)

Table 1.

Virulence/avirulence patterns of 3 races of *P. graminis* f. sp. *tritici.*

1

Race	Ineffective *Sr* genes	Effective *Sr* genes
21C3CTHTM	*6*, *7b*, *8a*, *9a*, *9b*, *9d*, *9f*, *9g*, *10*, *11*, *12*, *13*, *14*, *15*, *16*, *17*, *18*, *24*, *28*, *29*, *34*, *35*, *Tmp*, *McN*	*5*, *9e*, *19*, *20*, *21*, *22*, *23*, *25*, *26*, *27*, *30*, *31*, *32*, *33*, *36*, *37*, *38*, *47*
34MKGQM	*5*, *6*, *7b*, *8a*, *9a*, *9b*, *9d*, *9f*, *9g*, *12*, *15*, *16*, *20*, *24*, *27*, *28, 29*, *McN*	*9e*, *10*, *11*, *13*, *14*, *17*, *18*, *19*, *21*, *22*, *23*, *25*, *26*, *30*, *31*, *32*, *33*, *34*, *35*, *36*, *37*, *38*, *47*, *Tmp*
34C3RKGQM	*5*, *6*, *7b*, *8a*, *9a*, *9b*, *9d*, *9f*, *9g*, *12*, *16*, *19*, *21*, *23*, *24*, *27*, *28*, *29*, *McN*	*9e*, *10*, *11*, *13*, *14*, *15*, *17*, *18*, *20*, *22*, *25*, *26*, *30*, *31*, *32*, *33*, *34*, *35*, *36*, *37*, *38*, *47*, *Tmp*

2

Table 2(on next page)

Table 2.

The primer linked to resistance genes *Sr2*, *Sr24*, *Sr26*, *Sr31* and *Sr38*.

1

Genes	Marker	Forward primer	Reverse primer	References
Sr2	Xgwm533	5′-GTTGCTTTAGGGGAAAAGCC	5′-AAGGCGAATCAAACGGAATA	Hayden et al. 2004
	csSr2	5′-CAAGGGTTGCTAGGATTGGAAAAC	5′-AGATAACTCTTATGATCTTACATTTTTCTG	Mago et al. 2011
Sr24	Sr24#12	5′-CACCCGTGACATGCTCGTA	5′-AACAGGAAATGAGCAACGATGT	Mago et al. 2005
	Sr24#50	5′-CCCAGCATCGGTGAAAGAA	5′-ATGCGGAGCCTTCACATTTT	Mago et al. 2005
Sr26	Sr26#43	5′-AATCGTCCACATTGGCTTCT	5′-CGCAACAAAATCATGCACTA	Mago et al. 2005
Sr31	SCSS30.2$_{576}$	5′-GTCCGACAATACGAACGATT	5′-CCGACAATACGAACGCCTTG	Das et al. 2006
	Iag95	5’-CTCTGTGGATAGTTACTTGATCGA	5’-CCTAGAACATGCATGGCTGTTACA	Mago et al. 2002
Sr38	VENTRIUP-LN2	5’-AGGGGCTACTGACCAAGGCT	5’-TGCAGCTACAGCAGTATGTACACAAAA	Helguera et al. 2003
	URIC-LN2	5’GGTCGCCCTGGCTTGCACCT	5’TGCAGCTACAGCAGTATGTACACAAAA	Helguera et al. 2003

2

Table 3(on next page)

Table 3.

Resistant proportion of 75 wheat cultivars to wheat stem rust.

1

Races	Susceptible		Resistance	
	Number of cultivars	Percentage/%	Number of cultivars	Percentage/%
21C3CTHTM	28	37.3	47	62.7
34MKGQM	30	40.0	45	60.0
34C3RKGQM	26	34.7	49	65.3
All tested races	33	44.0	42	56.0

2

Table 4(on next page)

Table 4.

Amplification results for the known *Sr* genes by markers.

Table 4. Amplification results for the known *Sr* genes by markers

Line	*Sr* Gene	Source	Xgwm533	csSr2	Sr24#12	Sr24#50	Sr26#43	SCSS30.2$_{576}$	Iag95	VENTRI UP-LN2	URIC -LN2
ISr5-Ra	*5*	11Aberdeen	-	-	-	-	-	-	-	-	-
CnS_T_mono_der	*21*	11Aberdeen	-	-	-	-	-	-	-	-	-
Vernstine	*9e*	11Aberdeen	-	-	-	-	-	-	-	-	-
ISr7b-Ra	*7b*	11Aberdeen	-	-	-	-	-	-	-	-	-
IS11-Ra	*11*	11GH	-	-	-	-	-	-	-	-	-
ISr-Ra	*6*	11GH	-	-	-	-	-	-	-	-	-
ISr8a-Ra	*8a*	11Aberdeen	-	-	-	-	-	-	-	-	-
CnSr9g	*9g*	10Aberdeen	-	-	-	-	-	-	-	-	-
W2691SrTt-1	*36*	11GH	-	-	-	-	-	-	-	-	-
W2691Sr9b	*9b*	11Aberdeen	-	-	-	-	-	-	-	-	-
BtS30Wst	*30*	11Aberdeen	-	-	-	-	-	-	-	-	-
Combination Ⅶ	*17+13*	11Aberdeen	-	-	-	-	-	-	-	-	-
ISr9a-Ra	*9a*	11Aberdeen	-	-	-	-	-	-	-	-	-
ISr9d-Ra	*9d*	11Aberdeen	-	-	-	-	-	-	-	-	-
W2691Sr10	*10*	11Aberdeen	-	-	-	-	-	-	-	-	-
CnsSrTmp	*Tmp*	11Aberdeen	-	-	-	-	-	-	-	-	-
LcSr24Ag	*24*	11Aberdeen	-	-	+	+	-	-	-	-	-
Sr31/6*LMPG	*31*	11Aberdeen	-	-	-	-	-	+	+	-	-
Trident	*38*	11Aberdeen	-	-	-	-	-	-	-	+	+
McNair 701	*McN*	Griffey 2010	-	-	-	-	-	-	-	-	-
Line E	-	09AB	-	-	-	-	-	-	-	-	-
Acme	*9g*	09AB	-	-	-	-	-	-	-	-	-
Siouxland	*24+31*	2011 Baenzinger	-	-	+	+	-	+	+	-	-
Sisson	*31+36*	Griffey 2010	-	-	-	-	-	+	+	-	-
SwSr22T.B.	*22*	12GH	-	-	-	-	-	-	-	-	-
Agatha/9*LMPG	*25*	08AB	-	-	-	-	-	-	-	-	-
Eagle	*26*	10AB	-	-	-	-	+	-	-	-	-
73,214,3-1/9*LMH?	*27*	08AB	-	-	-	-	-	-	-	-	-
Federation*4/Kavl	*31*	10AB	-	-	-	-	-	+	+	-	-
ER 5155	*32*	10AB	-	-	-	-	-	-	-	-	-
Tetra Canthatch/A?	*33*	09AB	-	-	-	-	-	-	-	-	-
Mq(2)5XG2919	*35*	10AB	-	-	-	-	-	-	-	-	-
W3563	*37*	09Aberd	-	-	-	-	-	-	-	-	-
L6082	*39*	10AB	-	-	-	-	-	-	-	-	-
L6088	*40*	10AB	-	-	-	-	-	-	-	-	-
TAF 2	*44*	10AB	-	-	-	-	-	-	-	-	-
DAS15	*47*	10AB	-	-	-	-	-	-	-	-	-
Satu	*Satu*	09Aberd	-	-	-	-	-	-	-	-	-
TAM 107-1	*1A.1R*	12GH	-	-	-	-	-	-	-	-	-
Fed*3/Gabo*21BI	*R*	10AB	-	-	-	-	-	-	-	-	-
Iumillo	*9g,12,+*	09GH	-	-	-	-	-	-	-	-	-
Leeds	*9e,13,+*		-	-	-	-	-	-	-	-	-
Hope	*2*		-	-	+	-	-	-	-	-	-
ST464	*13*	08GH	-	-	-	-	-	-	-	-	-
Q21861	*Rpg1,4,5*	04NewZealand	-	-	-	-	-	-	-	-	-

Table 5(on next page)

Table 5.

Seedling infection types to races 21C3CTHQM, 34MKGQM and 34C3RTGQM of *Puccinia graminis* f. sp. *tritici* and molecular detection of resistance genes *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* on 75 wheat cultivars (lines).

Table 5. Seedling infection types to races 21C3CTHQM, 34MKGQM and 34C3RTGQM of *Puccinia graminis* f. sp. *tritici* and molecular detection of resistance genes *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* on the 75 wheat cultivars (lines)

Cultivars (lines)	Infection types			*Sr2*		*Sr24*		*Sr26*	*Sr31*		*Sr38*	
	21C3 CTHQM	34 MKGQM	34C3 RTGQM	Xgwm533	csSr2	Sr24#12	Sr24#50	Sr26#43	SCSS30.2$_{576}$	Iag95	VENTRI UP-LN2	URIC-LN2
Ningchun 39	0	0	0	-	-	-	-	-	-	-	-	-
Dingfeng 10	0	0	0	-	-	-	-	-	-	-	-	-
Linmai 32	4	4	3	-	-	-	-	-	-	-	-	-
Wuchun8	1+	3-	0	+	-	-	-	-	-	-	-	-
Wuchun 7	4	4	4	-	-	-	-	-	-	-	-	-
Dingxi 41	;	0	0	-	-	-	-	-	+	+	-	-
Longchun 31	0	0	0	-	-	-	-	-	+	+	-	-
Longchun 22	0	0	0	-	-	-	-	-	-	-	-	-
Ganchun 25	0	0	0	-	-	-	-	-	+	+	-	-
Longchun 25	;	2	2	-	-	-	-	-	+	+	-	-
Longchun 23	0	0	0	-	-	-	-	-	+	+	-	-
Longchun 26	0	0	0	+	-	-	-	-	+	+	-	-
Ganchun 24	0	0	0	+	-	-	-	-	+	+	-	-
Yinchun 9	0	0	0	+	-	-	-	-	+	+	-	-
Longchun 28	;	0	;	-	-	-	-	-	-	-	-	-
Wuchun 5	1+	3	;	-	-	-	-	-	-	-	-	-
Ganchun 20	4	4	4	-	-	-	-	-	-	-	-	-
Ningchun 4	4	3-	4	-	-	-	-	-	-	-	-	-
Linmai 35	4	4	4	-	-	-	-	-	-	-	-	-
Lianghan 2	4	4	2	-	-	-	-	-	-	-	-	-
Dingxi 38	;1	1	0	-	-	-	-	-	-	-	+	+
Ganchun 21	4	1	4	+	-	-	-	-	-	-	-	-
Dingxi 40	4	4	4	-	-	-	-	-	-	-	-	-
Wuchun 4	0	0	0	-	-	-	-	-	-	-	-	-
Wuchun 3	4	4	4	-	-	-	-	-	-	-	-	-
Jinchun 5	;	0	0	-	-	-	-	-	+	+	+	+
Gansu 26	1+	1	1	-	-	-	-	-	+	+	+	+
Linmai 33	1	;	1	-	-	-	-	-	-	-	+	+
Longchun 33	4	3	4	+	-	-	-	-	-	-	-	-
Jiuchun 6	;	0	1+	+	-	-	-	-	-	-	+	+
Longchun 27	1	1	;1	+	-	-	-	-	+	+	-	-
Linmai 34	0	0	2	-	-	-	-	-	-	-	-	-
Dingfeng 12	0	2	1	+	-	-	-	-	-	-	-	-
Dingfeng 16	4	2	4	-	-	-	-	-	-	-	-	-
Zhangchun 21	1	0	1+	-	-	-	-	-	+	+	-	-
Wuchun 6	0	;	0	+	-	-	-	-	-	-	-	-
Lantian 23	3+	4	0	-	-	-	-	-	-	-	-	-
Lantian 19	4	4	4	-	-	-	-	-	-	-	-	-
Lantian 25	3+	4	0	-	-	-	-	-	-	-	-	-
Lantian 13	;	3	0	-	-	-	-	-	-	-	-	-
Xifeng 27	;	1	;	-	-	-	-	-	+	+	-	-
Lantian 26	0	1	1	-	-	-	-	-	+	+	-	-
Longjian 101	4	4	4	-	-	-	-	-	-	-	-	-
Hangxuan 1	0	0	0	-	-	-	-	-	-	-	+	+
Lantian 14	0	0	0	+	-	-	-	-	+	+	+	+
Lantian 31	0	3-	2	-	-	-	-	-	-	-	-	-
Pingliang 42	;1	3-	4	-	-	-	-	-	-	-	-	-
Xifeng 20	1	2	1	-	-	-	-	-	-	-	-	-
Longyu 4	0	2	1	-	-	-	-	-	-	-	-	-
Changwu 131	4	4	4	-	-	-	-	-	-	-	-	-
Zhongliang 18	4	0	4	+	-	-	-	-	-	-	-	-
Zhongliang 22	0	;1	0	-	-	-	-	-	+	+	+	+
Lantian 10	;	0	1	-	-	-	-	-	+	+	+	+
Tianxuan 39	1	;1	0	-	-	-	-	-	+	+	-	-
Huandong 6	4	4	4	-	-	-	-	-	-	-	-	-
Longjian 196	4	4	4	-	-	-	-	-	-	-	-	-

Lantian 30	1	2	1	-	-	-	-	-	-	-	-	-
Longnan 2000-8-2-1	0	0	1	-	-	-	-	-	-	-	-	-
Longjian 301	1+	;	1	-	-	-	-	-	+	+	-	-
Longyu 2	0	1	1	-	-	-	-	-	+	+	-	-
Longjian P430	0	;	1	-	-	-	-	-	+	+	-	-
Longjian 103	4	4	4	-	-	-	-	-	-	-	-	-
Lantian 29	4	4	4	-	-	-	-	-	-	-	-	-
Lan 092	0	1-	1	-	-	-	-	-	-	-	-	-
Qingnong 1	4	4	4	-	-	-	-	-	-	-	-	-
Pingyuan 50	3+	4	4	-	-	-	-	-	-	-	-	-
Longyuan 034	0	0	0	-	-	-	-	-	+	+	-	-
Lan 05-9-1-4	4	4	4	-	-	-	-	-	-	-	-	-
Gandong 017	0	2	0	-	-	-	-	-	+	+	-	-
Longjian 19	4	4	4	-	-	-	-	-	-	-	-	-
Lantian 24	4	4	4	-	-	-	-	-	-	-	-	-
863-13	0	0	0	-	-	-	-	-	+	+	-	-
01-426e-1	3+	3	4	+	-	-	-	-	-	-	-	-
Tian 01-29	;	2	;	-	-	-	-	-	+	+	-	-
Tian 01-104	4	4	4	-	-	-	-	-	-	-	-	-

5

Figure 1

Fig. 1.

Infection types used in this study.

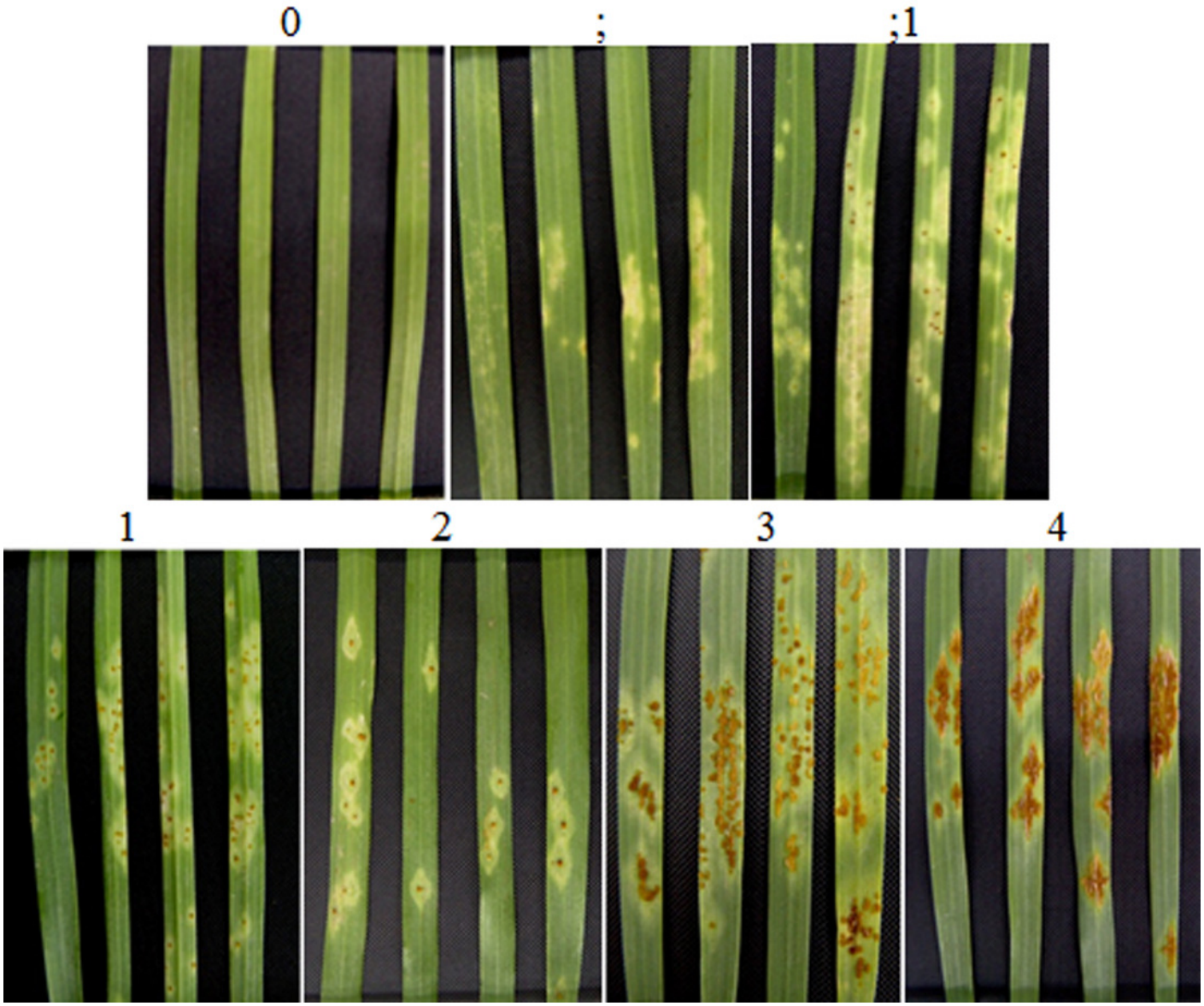

Figure 2

Fig. 2.

Amplification result for parts of wheat varieties with SCSS30.2$_{576}$ and Iag95. Lane 1-11, Monogenic *Sr31*, Little Club, Wuchun 7, Dingxi 41, Longchun 31, Longchun 22, Ganchun 25, Longchun 25, Longchun 23, Longchun 26, Ganchun 24, Yinchun 9, 'M' indicates 2000 bp DNA ladder and black arrow indicates the position of the specific band.