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

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Abstract

Wheat stem rust, 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 received attention. It is important to clarify the effectiveness of resistance genes in a timely manner, especially for the purpose of using new resistance genes in wheat cultivars for durable-resistance. However, little is known about the stem rust resistance genes present in widely used wheat cultivars from Gansu. This study aimed to determine the resistance level at the seedling stage of the main wheat cultivars 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%)

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wheat varieties displayed resistance 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.

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

Introduction

Puccinia graminis f. sp. *tritici* Eriks. and E. Henn (*Pgt*) causes one of the most potentially destructive wheat diseases, seriously threatening world grain production (Pardey et al., 2013). Disease-resistance breeding to control wheat stem rust is economic, effective, and protective of the 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 wheat–rye chromosome arm translocation (Rouse et al., 2012). However, a new race Ug99 virulent to *Sr31* was identified in Uganda and classifed as TTKS by the North American Nomenclature System of *Pgt* in 1999 (Pretorius et al., 2000). Ug99 has broad 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

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mode of spread is similar to that of a virulent stripe rust pathogen race to *Yr9* predicted by Geographic Information System of CIMMYT (CIMMYT. 2007).

A new race TTTTF with 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 the epidemic in 2017. 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 (FAO, 2017). In view of this, in February 2017, 'Nature' highlighted the potential threat to European wheat production of this race (Bhattacharya, 2017). Therefore, the spread of Ug99 and TTTTF, and their variants, threaten 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, durable resistance to stem rust has been re-emphasized with the occurrence and spread of new 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 genes in cultivars.

Materials and Methods

Wheat cultivars and near-isogenic lines

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A total of 76 wheat cultivars in Gansu Province were provided by Dr. Fangping Yang from the Wheat Research Institute, Gansu Academy of Agricultural Sciences. Molecular markers linked to five *Sr* genes were tested: *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38*. Forty-five lines with *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 included 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 cultivars 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’)

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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 a genomic DNA extraction kit (http://www.sangon.com/, China). The DNA quality was examined by 1.2% (w/v) agarose gels 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 $mmol \cdot L^{-1}$ deoxyribonucleoside triphosphates, 2.5 µL of 10× buffer (Mg^{2+}), 0.2 µL of 5 $U \cdot \mu L^{-1}$ Taq polymerase, 1 µL of 10 $\mu mol \cdot L^{-1}$ of each primer, and 2 µL of 30 $ng \cdot \mu L^{-1}$ DNA. De-ionized water was used to achieve 25 µL volumes. Condition of PCR amplification were 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,

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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 01-29) showed different resistance levels (ITs 0, ;, ;1, 1+, and 2) to the three 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).

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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 expected wheat genetic stocks. 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.

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***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

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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).

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_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 Club (LC) and the tested cultivars, indicating that these cultivars lacked *Sr24*.

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_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* displayed 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 band was amplified in wheat lines with *Sr26* (Mago et al., 2005). Marker Sr26#43 was used to detect this fragment in tested wheat cultivars. No any visible band was detected, suggesting that these varieties do not carry *Sr26*, as expected.

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***Sr31* screening**

Two markers, SCSS30.2$_{576}$ and Iag95 linked to resistance gene *Sr31*, were used for detecting this locus. SCSS30.2$_{576}$ amplified a 576-bp fragment and marker Iag95 amplified an 1100-bp PCR fragment in *Sr31*-carrying lines such as *Sr31*/6*LMPG and Siouxland (Fig. 2). No fragment was amplified in the negative control Little Club. These two markers were used to detect *Sr31* in the tested cultivars. The result showed that these two fragments were detected in wheat cultivars Ganchun 25, Longchun 25, Longchun 23, Longchun 26, Ganchun 24, Yinchun 9, Longchun 31, Dingxi 41, Jinchun 5, Gansu 26, Longchun 27, Zhangchun 21, Xifeng 27, Lantian 26, Lantian 14, Zhongliang 22, Lantian 10, Tianxuan 39, Longjian 301, Longyu 2, Longjian P430, Longyuan 034, Gandong 017, 863-13, and Tian 01-29, indicating that these 25 cultivars carried *Sr31* (Table 5).

***Sr38* screening**

The *Lr37-Sr38-Yr17* rust resistance gene cluster was transferred to the short arm of bread wheat chromosome 2AS to from a segment of *Triticum ventricosum* (Tausch) Cess. chromosome 2NS (Helguera et al., 2003). 2NS-specific primer VENTRIUP-LN2 and 2AS-specific primer URIC-LN2 were developed to detect this rust resistance gene cluster in commercial wheat cultivars and 262-bp and 285-bp PCR products were amplified in wheat lines carrying *Lr37-Sr38-Yr17*, whereas none of these amplification products were found in negative control LC (without *Lr37-Sr38-Yr17*). Both 262-bp and 285-bp PCR fragments were amplified in nine wheat cultivars Dingxi 38, Jinchun 5, Gansu 26, Linmai 33, Jiuchun 6, Hangxuan 1,

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Lantian 14, Zhongliang 22, and Lantian 10 in this study, suggesting that these wheat cultivars carried *Sr38* (Table 5).

Discussion

The broad-spectrum wheat stem rust resistance gene *Sr2* confers adult-plant resistant to stem rust and is located on chromosome 3BS. It originated in tetraploid Yaroslav emmer (*T. dicoccum*) and later was transferred to the susceptible bread wheat "Marquis" in the 1920s (McFadden,1930). Several varieties with *Sr2* were cultivated worldwide (Singh et al., 2011). Markers Xgwm533 and csSr2 were used to detect *Sr2* in wheat cultivars from Gansu. However, marker csSr2 failed to predict *Sr2*. Only marker Xgwm533 amplified 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 was introgressed into wheat lines (McIntosh, Wellings & Park, 1995). *Sr24* was ineffective to some variants of Ug99 but is 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, developed by Mago et al. (2005) were used to detect the gene in Gansu wheat cultivars in this study. Surprisingly, no wheat cultivars carried this gene. However, it is reported that Chinese wheat cultivars in other

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provinces carry *Sr24* (Li et al., 2016b; Cao et al., 2007).

In Australia, *Sr26* has been released in the cultivar Eagle since 1971 (Martin, 1971). Later, other major cultivars including Flinders, Harrier, Kite, Takari, and Sunelg, were cultivated. Lines containing the *Sr26* fragment are resistant to new stem rust pathogen races such as Ug99 and its associated strains. None of the cultivars had *Sr26* in the present study, as expected, and similar results were observed in our previous study (Li et al., 2016a).

The stem rust resistance gene *Sr31* on 1BL/1RS was transferred from to 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 the 1BL/1RS translocation such as "Kavkaz" and "Luofu" were widely used in wheat breeding in Gansu Province, revealing the origin of *Sr31* in these wheat varieties.

Rust resistance gene cluster *Yr17-Lr37-Sr38* was initially transferred into the winter bread wheat line "VPM1" from *T. ventricosum* (Maia, 1967) and was located in a 2NS/2AS translocation (Bariana & McIntosh 1993; Cao et al., 2007). PCR assays

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

Conclusion

Breeding resistant cultivars is an economic and effective way to protect wheat from disease. After the development of molecular technology, molecular marker detection and utilization in wheat cultivars improved the disease resistance in a relatively short time, leading to increased crop production. The molecular markers linked to *Sr2*, *Sr24*, *Sr26*, *Sr31*, and *Sr38* were used to detect the occurrence of these genes in 75 major wheat cultivars (lines) in Gansu Province in this study. The results showed that 35 tested cultivars might carry one of these genes. This information can be used in wheat breeding in the future.

Acknowledgments

We appreciate very much to Dr. Fangping Yang at Wheat Research Institute, Gansu Academy of Agricultural Sciences for providing the wheat cultivars.

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