Determination of the Role of *Berberis* spp. in Wheat Stem Rust in China

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Abstract

Zhao, J., Zhao, S. L., Chen, X. M., Wang, Z. Y., Wang, L., Yao, J. N., Chen, W., Huang, L. L., and Kang, Z. S. 2015. Determination of the role of *Berberis* spp. in wheat stem rust in China. *Plant Dis.* 99:1113-1117.

Previous studies on the relationship of barberry (*Berberis* spp.) and wheat stem rust suggested that, although some barberry species can serve as alternate hosts for the stem rust fungus *Puccinia graminis* f. sp. *tritici*, barberry plants play no role in wheat stem rust development and virulence variation of *P. graminis* f. sp. *tritici* in China. In the present study, severe rust infections on *Berberis shensiana*, *B. brachypoda*, *B. potaninii*, *B. soulieana*, and *B. aggregata* were observed during field surveys in 2011 and 2012. Through artificial inoculation of wheat seedlings ('Mingxian 169') under greenhouse conditions with aeciospores from naturally infected barberry plants, 185 isolates of *P. graminis* f. sp. *tritici* were obtained. From the 27 selected isolates that were tested on a set of wheat genotypes used to differentiate *P. graminis* f. sp. *tritici* races in China, 18

races were identified, of which 8 races were new and others were of Chinese 21 and 34 race groups. In addition to the information of virulence or avirulence patterns on the Chinese differentials, none of the races were virulent to resistance gene *Sr31*. The virulence frequencies based on individual *Sr* genes or differentials ranged from 0 to 96%. Using molecular markers for *P. graminis* f. sp. *tritici*, 75 of 4,036 samples of single aecia were identified as *P. graminis* f. sp. *tritici*. The low detectable rate of *P. graminis* f. sp. *tritici* was verified based on both infection and molecular marker tests. This observation may be correlated with the low levels of wheat stem rust in the surveyed regions. This study shows that *P. graminis* f. sp. *tritici* can produce new races through sexual reproduction on *Berberis* spp. in China.

Stem rust (or black rust), caused by *Puccinia graminis* f. sp. *tritici* Erikss. & Henning, is one of the most destructive diseases of wheat (*Triticum aestivum* L.) worldwide (Singh et al. 2006). In China, nine remarkably severe epidemics of stem rust occurred in the spring-wheat-growing region in northeastern China and winter-wheat-growing regions, including southeastern China, the Yangtze River region, and the Huai River region from the 1920s to the 1960s, which resulted in significant yield losses in the epidemic years (Li and Zeng 2002). For example, 1 million kg of wheat grain was lost due to the outbreak of stem rust in Jiangsu and Anhui provinces in 1956. The extensive growth of resistant cultivars over the world has dramatically diminished damage caused by *P. graminis* f. sp. *tritici* as a significant factor in wheat-growing areas (Roelfs et al. 1992). In China, likewise, great efforts have been made to successfully control wheat stem rust by developing wheat cultivars with resistance since the 1970s (Zhuang 2003). Among the stem rust (*Sr*) resistance genes, *Sr31* has been mostly used in wheat cultivars in China because the gene is linked to *Yr9* for resistance to stripe rust and *Pm8* to powdery mildew. As the TTKS (or Ug99) race group has emerged in eastern Africa and spread since 1998 (Jin and Sing 2006; Jin et al. 2007), wheat production has become vulnerable to the new virulent races in many wheat-growing countries, including China (He et al. 2008).

Previous studies have demonstrated that the sexual stage of *P. graminis* f. sp. *tritici* through infection on barberry plants (*Berberis* spp.) not only generated new races but also led to diverse virulence variations of *P. graminis* f. sp. *tritici* populations (Jin 2011; Johnson et al. 1932; Lekontseva et al. 2006; Martens et al. 1989; Newton et al. 1930; Rouse et al. 2009; Stakman et al. 1930; Stakman and Rodenhiser 1958; Waterhouse 1929; Wilcoxson and Paharia 1958). Moreover,

initial infection of the stem rust pathogen on wheat crops can be triggered by aeciospores in regions where susceptible barberry and wheat plants coexist, such as many regions in Australia (Waterhouse 1934), Canada (Johnson and Green 1951), Ethiopia (Lekontseva et al. 2006), France (Guyot and Massenot 1955), Italy (Sibilia 1936), Spain (Urrías and Cañamas 1952), and the United States (Stakman 1919; Stakman et al. 1934, 1958). For this reason, eradication of susceptible barberry plants by legislation was practiced to uncover significant benefits: reduction of initial inoculum, decrease in the number of races, and stabilization of the pathogen populations in many countries, especially in Europe and North America (Jin 2011; Roelfs 1982; Stakman 1919; Zadoks and Bouwman 1985).

With respect to the association of barberry to wheat stem rust in China, little is known about whether barberry plays a role in providing initial inoculum to trigger primary infection on wheat, or in generating new races of *P. graminis* f. sp. *tritici*. Wang (1955) obtained *P. graminis* f. sp. *tritici* uredinial pustules on wheat 'Wugong 7774', after inoculation using aeciospores from *Berberis amurensis* in the wild in Hebei Province. However, Zhang et al. (1957) challenged the results according to their continuous 3-year investigation in the same region, and suggested that the infection on Wugong 7774 wheat by Wang (1955) might not be caused by aeciospores from barberry but by urediniospores from wheat falling on aecia on barberry leaves. In Qinghai Province, aecia were remarkably abundant on seven species of barberry, including *B. verna*, *B. henryana*, *B. thunbergii*, *B. dubia*, *B. kansuensis*, *B. diaphana*, and an unidentified *Berberis* sp. in spring, but aecial samples failed to produce infection on five susceptible wheat cultivars (Wheat Rust Research Group, Northwest Institute of Agricultural Sciences 1958). Zeng and Xue (1963) reported that, although *B. amurensis* serves as an alternate host for *P. graminis* f. sp. *tritici*, it does not have any effect on epidemics of wheat stem rust in the middle part of northeastern China, and pointed out that the stem rust fungus did not generate diverse races via *B. amurensis*. Although Wang et al. (1958) reported that *P. graminis* f. sp. *tritici* growing on *B. amurensis* generated two new races (races 9 and 15) by artificial inoculation under laboratory conditions, aeciospores from serious rust-infected *B. amurensis* were not successful

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Accepted for publication 23 November 2014.

<http://dx.doi.org/10.1094/PDIS-09-14-0928-RE>
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for producing infection on wheat after inoculation in the middle part of northeastern China. Therefore, it was inferred that *B. amurensis* plays little to no role in epidemics of wheat stem rust in this region.

In the present study, abundant aecia were observed on several *Berberis* spp. in Gansu and Shaanxi provinces in China during the surveys in 2011 and 2012. Aecial collections were used to inoculate wheat. The objectives of the present study were to isolate *P. graminis* f. sp. *tritici* from aecia of barberry and identify *P. graminis* f. sp. *tritici*

racess from isolates derived from aeciospores produced on barberry plants using a set of wheat genotypes that are used to differentiate Chinese *P. graminis* f. sp. *tritici* races and lines carrying important *Sr* genes, including *Sr31*.

Materials and Methods

Sample collection and inoculation. During our previous investigations, we found that *B. brachypoda*, *B. potaninii*, *B. soulieana*,

Table 1. Samples of aecia produced on *Berberis* spp. infected by rust fungi in Gansu and Shaanxi provinces in 2011 and 2012, and identification of aeciospores as *Puccinia graminis* f. sp. *tritici* by inoculation on ‘Mingxian 169’ wheat in the greenhouse and by polymerase chain reaction (PCR) amplification using primer pairs *Puccinia* internal transcribed spacer (ITS)3 (5'-ACATCGATGAAGAACACAGT-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')

<i>Berberis</i> spp.	Location	Number of							
		Inoculation				PCR			
		Aecia		<i>P. graminis</i> f. sp. <i>tritici</i> isolates ^a		Aecia		<i>P. graminis</i> f. sp. <i>tritici</i> aecia	
		2011	2012	2011	2012	2011	2012	2011	2012
<i>B. aggregata</i>	Qishou, Tianshui, Gansu	648	370	4	15	500	270	4	11
<i>B. brachypoda</i>	Gangu, Tianshui, Gansu	545	836	13	23	500	510	1	16
<i>B. potaninii</i>	Honghuapu, Feng County, Shaanxi	742	64	3	2	ND ^b	206	ND	5
<i>B. shensiiana</i>	Yingge, Taibai, Shaanxi	323	1643	0	66	500	630	6	2
<i>B. soulieana</i>	Jiangluo, Hui County, Gansu	458	1024	2	57	500	420	12	18
Total		2,716	3,937	22	163	2,000	2,036	23	52

^a Isolates indicated uredinia of *P. graminis* f. sp. *tritici* produced on wheat leaves inoculated with aeciospores from a single aecium on *Berberis* spp.

^b ND = no data.

Table 2. Virulence of 27 randomly selected isolates of *Puccinia graminis* f. sp. *tritici* recovered from *Berberis* spp. infected by rust fungi in nature in 2011 and 2012 tested on the Chinese set of wheat differentials consisting of 12 *Sr* single-gene lines and 6 wheat genotype cultivars as supplementary differentials

Number	<i>Berberis</i> spp.	Chinese differentials ^a									
		<i>Sr</i> single-gene line									
		<i>Sr5</i>	<i>Sr21</i>	<i>Sr9e</i>	<i>Sr7b</i>	<i>Sr11</i>	<i>Sr6</i>	<i>Sr8a</i>	<i>Sr9g</i>	<i>Sr36</i>	<i>Sr9b</i>
1	<i>B. aggregata</i>	L	L	L	H	H	L	H	H	H	H
2	<i>B. aggregata</i>	H	L	L	H	H	H	H	H	H	L
3	<i>B. aggregata</i>	H	H	L	H	L	H	H	H	L	L
4	<i>B. aggregata</i>	H	H	L	L	H	H	H	L	H	H
5	<i>B. aggregata</i>	L	L	L	L	H	H	H	H	L	L
6	<i>B. aggregata</i>	H	H	L	L	L	H	H	H	L	L
7	<i>B. aggregata</i>	H	H	L	H	H	H	H	H	L	L
8	<i>B. aggregata</i>	H	H	L	H	H	L	H	H	L	L
9	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
10	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
11	<i>B. brachypoda</i>	H	H	L	L	H	H	L	H	L	H
12	<i>B. brachypoda</i>	L	L	L	L	H	H	H	H	L	H
13	<i>B. brachypoda</i>	H	H	L	L	H	H	H	H	L	H
14	<i>B. brachypoda</i>	L	L	L	H	H	H	H	H	L	L
15	<i>B. brachypoda</i>	H	H	L	H	H	H	H	H	H	H
16	<i>B. brachypoda</i>	L	H	L	H	L	H	H	H	H	L
17	<i>B. brachypoda</i>	L	H	L	H	H	H	H	H	H	H
18	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
19	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
20	<i>B. brachypoda</i>	L	H	L	L	L	H	H	H	H	L
21	<i>B. brachypoda</i>	H	L	L	H	H	H	H	H	H	H
22	<i>B. brachypoda</i>	H	H	L	H	H	H	H	H	L	L
23	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
24	<i>B. brachypoda</i>	H	L	L	H	L	H	H	H	L	H
25	<i>B. potaninii</i>	H	H	L	H	H	H	H	H	H	L
26	<i>B. potaninii</i>	H	L	L	H	L	H	H	H	H	H
27	<i>B. potaninii</i>	H	L	L	L	H	H	H	H	H	L
Virulence frequency (%)		74.1	48.1	0.0	70.4	66.7	92.6	96.3	96.3	96.3	55.6

(continued on next page)

^a Chinese differentials for different *P. graminis* f. sp. *tritici* races include 12 *Sr*-single gene lines and 6 supplementary genotypes: *Sr5* = ISr5-Ra, *Sr21* = *Triticum monococon* derivative, *Sr9e* = Verstein, *Sr7b* = ISr7b-Ra, *Sr11* = ISr11-Ra, *Sr6* = ISr6-Ra, *Sr8a* = ISr8-Ra, *Sr9g* = CnSSr9g, *Sr36* = W2691SrTt-1, *Sr9b* = W2691Sr9b, *Sr30* = BtSr30Wst, *Sr17* = Combination VII, Einkorn (Ein., *Sr21*), Mianzi 52 (M52, *Sr17*, *SrMz*, and more undetermined resistance stem rust genes), Minnesota 2761 (M2761, unknown gene), Huadong 6 (H6), Rulofen (Rul., unknown gene), and Orofen (Oro., unknown gene) based on Chen et al. (1994), Roelfs and Martens (1988), and Zhang et al. (1994). ‘L’ indicates low (infection type [IT] 0 to 2) and ‘H’ indicates high (IT 3 to 4), based on the 0 to 4 scale described by Stakman et al. (1962).

^b The *Sr31* single-gene line (Sr31/6*LMG) was included to determine any possible ‘Ug99’ like races.

^c The race was the determined race type of known races or race group of *Puccinia graminis* f. sp. *tritici*.

B. aggregata, and *B. shensiensis* grow extensively in Gansu and Shaanxi provinces, and plants of these species were heavily infected by rust fungi (unpublished data). Aecia generated on plants of different *Berberis* spp. were similar in color but varied in sizes. Samples of leaves bearing aecia were collected from rust-infected barberry plants in Gansu and Shaanxi (Table 1). Single leaf samples were kept in paper bags (15 by 5 by 10 cm) in a box with ice packs during surveys, then stored at 4°C. Aeciospores from a single aecium were used to make an aeciospore suspension with two drops of sterile water (approximate 0.1 ml) and inoculated on seedlings of 'Mingxian 169' wheat using the method described by Yao et al. (1993). The inoculated wheat plants were incubated at 18°C for 16 h in the dark and for 8 h with slight light, and moved to a growth chamber with temperatures set to approximately 20 to 25°C. Uredinial sporulation was observed 10 days after inoculation.

Evaluation of races for virulence. Isolates of *P. graminis* f. sp. *tritici* derived from barberry plants were inoculated on Mingxian 169 for increasing urediniospores, and on a set of differentials (kindly provided by Dr. Yuanying Cao at Shenyang Agricultural University, Shenyang, Liaoning, China) listed in Table 2 for identification of *P. graminis* f. sp. *tritici* races using the conditions as described above. The set of Chinese *P. graminis* f. sp. *tritici* differentials consists of 12 *Sr* single-gene lines and 6 wheat cultivars used as supplementary differentials (Chen et al. 2007). In addition, a wheat line with *Sr31* was also included in the tests to determine its response to the *P. graminis* f. sp. *tritici* isolates. Infection types were scored based on the 0-to-4 scale described by Stakman et al. (1962) at 10 days after inoculation. Infection types 0 to 2 were treated as resistant and marked as low (L) and 3 to 4 as susceptible and marked as high (H) infection type. If an isolate produced a virulence pattern different from any previously reported race, the test was repeated to confirm the virulence pattern.

Identification of *P. graminis* f. sp. *tritici* using polymerase chain reaction amplification and sequencing. To identify *P. graminis*

f. sp. *tritici* on barberry leaves bearing aecia, aecial cups produced on single aecium were cut and put into a 2-ml plastic tube with two steel beads (5 mm in diameter) to pulverize using a TissueLyser (Qiagen, Hilden, Germany). Total DNA of an aecium sample was extracted according to the method described by Chen et al. (1993), with slight modification. The fine powder was dispersed in 100 µl of extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 mM NaCl; and 55 mM cetyltrimethylammonium bromide) and gently mixed. Then, 20 µl of 20% preheated sodium dodecyl sulfate (final concentration 1%) in a water bath with temperature set at 65°C was added to the tube. The solution was mixed using a vortex (Genie 2T; Scientific Industries, Inc., NY) and incubated for 30 min at 65°C. Proteins were denatured and removed by adding 500 µl of Tris-HCl saturated phenol/chloroform/isoamyl (25:24:1, vol/vol/vol), and the purification step was repeated as needed. Liquid phases were separated by centrifuging for 10 min at 12,000 rpm and 4°C, the top aqueous phase was transferred to a clean tube, and 2.5 volumes (about 300 µl) of cold 95% ethanol was added. After 30 min of incubation at 4°C, the solution was centrifuged for 10 min at 12,000 rpm and 4°C to precipitate the nucleic acid. The DNA pellet was rinsed twice with cold 70% ethanol, dried in a clean bench, and dissolved in 50 µl of Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). The DNA samples were checked on 0.8% agarose gel by electrophoresis for visualization.

A pair of primers, *Puccinia* internal transcribed spacer (ITS)3 (5'-ACATCGATGAAGAACACAGT-3') and ITS4 (5'-TCCTCCGC TTATTGATATGC-3'), designed by Drs. Meinan Wang and Xianming Chen (United States Department of Agriculture-Agricultural Research Service and Washington States University) to differentiate *P. graminis* f. sp. *tritici*, *P. striiformis* f. sp. *tritici*, and *P. triticina* (the causal agent of wheat leaf rust), was used to detect *P. graminis* f. sp. *tritici*. Based on polymerase chain reaction (PCR) amplification, *P. graminis* f. sp. *tritici*-positive samples with bands similar in size to a positive check were reamplified. Approximately 100 µl of PCR product was sequenced by Beijing Aoke Dingsheng Bio-

Table 2. (continued from preceding page)

Number	<i>Berberis</i> spp.	Chinese differentials ^a									Race or race group ^c
		<i>Sr</i> single-gene line		Supplementary							
		<i>Sr30</i>	<i>Sr17</i>	Ein.	M52	M2761	H6	Rul.	Oro.	<i>Sr31</i> ^b	
1	<i>B. aggregata</i>	L	H	L	H	H	H	H	L	L	New race 1
2	<i>B. aggregata</i>	L	L	L	L	H	H	L	L	L	New race 2
3	<i>B. aggregata</i>	L	L	L	L	L	H	L	L	L	34
4	<i>B. aggregata</i>	H	L	L	L	L	H	L	L	L	34
5	<i>B. aggregata</i>	L	L	L	L	L	H	L	L	L	34
6	<i>B. aggregata</i>	L	L	L	L	L	H	L	L	L	34
7	<i>B. aggregata</i>	L	L	L	L	L	H	L	L	L	34M
8	<i>B. aggregata</i>	L	L	L	H	L	H	L	L	L	21C3
9	<i>B. brachypoda</i>	L	H	L	L	L	L	L	L	L	34MKH
10	<i>B. brachypoda</i>	L	L	L	L	L	L	L	L	L	34MKG
11	<i>B. brachypoda</i>	L	L	L	H	L	H	L	H	L	New race 3
12	<i>B. brachypoda</i>	H	L	L	L	L	H	L	L	L	34
13	<i>B. brachypoda</i>	L	L	L	H	L	H	L	H	L	New race 4
14	<i>B. brachypoda</i>	L	H	L	L	H	H	L	L	L	21C3CPH
15	<i>B. brachypoda</i>	H	H	L	H	H	H	H	L	L	New race 5
16	<i>B. brachypoda</i>	L	L	L	H	L	H	L	L	L	21C3
17	<i>B. brachypoda</i>	L	L	L	L	H	H	L	L	L	New race 6
18	<i>B. brachypoda</i>	L	L	L	L	L	L	L	L	L	34MKG
19	<i>B. brachypoda</i>	L	L	L	L	L	L	L	L	L	34MKG
20	<i>B. brachypoda</i>	H	H	L	L	L	H	L	L	L	34
21	<i>B. brachypoda</i>	L	L	L	L	L	L	L	L	L	34MKR
22	<i>B. brachypoda</i>	L	L	L	H	L	H	L	H	L	New race 7
23	<i>B. brachypoda</i>	L	H	L	H	L	L	L	L	L	34C1MKH
24	<i>B. brachypoda</i>	L	H	L	H	L	H	L	L	L	34C1MKH
25	<i>B. potaninii</i>	H	H	L	H	H	H	H	L	L	New race 8
26	<i>B. potaninii</i>	L	H	L	L	L	L	L	L	L	34C2MKR
27	<i>B. potaninii</i>	L	H	L	H	H	H	L	L	L	34C2
Virulence frequency (%)		18.5	37.0	0.0	40.7	25.9	74.1	7.4	11.1	0.0	...

tech Ltd. after checking on 1% agarose gel for successful amplification. The ITS sequence of selected *P. graminis* f. sp. *tritici*-positive samples were aligned for homology with *P. graminis* f. sp. *tritici* and other *Puccinia* fungi data at the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov/>).

Results

Rust infection on *Berberis* spp. and *P. graminis* f. sp. *tritici* isolates.

In 2011, from 2,716 aecia that were inoculated on leaves of Mingxian 169 wheat, 22 uredinial isolates typical of *P. graminis* f. sp. *tritici* were obtained from individual aecia, accounting for 0.81% of the single-aecium samples tested. Except for the aecial samples from *B. shensiana* that failed to generate uredinia, aecia collected from all four other species (*B. aggregata*, *B. brachypoda*, *B. potaninii*, and *B. soulieana*) produced *P. graminis* isolates (Table 1). In 2012, of 3,937 aecia collected from the five *Berberis* spp., 163 (4.1%) were *P. graminis* f. sp. *tritici*. The average of acquisition rate of *P. graminis* f. sp. *tritici* using artificial inoculation under greenhouse conditions was 2.78%.

Identification of *P. graminis* f. sp. *tritici* by PCR amplification.

P. graminis f. sp. *tritici* was identified from aecial samples collected from four *Berberis* spp. (*B. brachypoda*, *B. soulieana*, *B. aggregata*, and *B. shensiana*) but not *B. potaninii*. From a total of 2,000 aecia in 2011, 23 (1.15%) were detected as *P. graminis* f. sp. *tritici* (Table 1). In 2012, 52 (2.25%) of 2,036 aecia were identified as *P. graminis* f. sp. *tritici* (Table 1). Although the detectable rate of *P. graminis* f. sp. *tritici* in 2012 was higher than that of 2011, these rates were very low. The detected rates from *B. brachypoda*, *B. soulieana*, and *B. aggregata* were slightly higher than that of *B. shensiana*. The average detectable rate by the PCR method of the 2 years was 1.86%, which was lower than the 2.78% by inoculation. No matter which method was used, the rate of *P. graminis* f. sp. *tritici* infection was quite low, indicating that barberry infection by *P. graminis* f. sp. *tritici* is relatively infrequent in the sampled regions compared with other *Puccinia* spp. infection. The sequences of aecial samples, which were submitted to GenBank in our previous study (Zhao et al. 2013), had >95% homologies with those of *P. graminis* f. sp. *tritici* in the NCBI database but had <90% homologies with other *Puccinia* spp. This result, together with typical signs of stem rust produced on wheat plants, showed that the aecia from barberry plants and their derived uredinial isolates were *P. graminis* f. sp. *tritici*.

***P. graminis* f. sp. *tritici* races.** From the 185 *P. graminis* f. sp. *tritici* isolates derived from the aecia on plants of five *Berberis* spp., 27 were randomly selected to test for virulence on the set of wheat differentials (Table 2). The 27 isolates were identified as 18 races, of which 8 races with 16 isolates were assigned to the race 34 group and 2 races with 3 isolates to the race 21 group. The remaining eight isolates were different from each other and also different from the previously identified races (Cao et al. 1996; Chen et al. 1994, 2007; Han et al. 2010; Huang et al. 1984; Wu 1987; Yao et al. 1993, 1997) and, therefore, were identified as eight new races.

Virulence frequencies. Virulences to *Sr5*, *Sr7b*, *Sr11*, *Sr6*, *Sr8a*, *Sr9g*, *Sr36*, and genotype ‘Huadong 6’ were highly frequent (>60%); those to *Sr21*, *Sr9b*, *Sr17*, *M52*, and *M2761* were intermediate (20 to 60%); and those to *Sr30*, ‘Rulofen’, and ‘Orofen’ were low (<20%). All 27 tested isolates were avirulent to *Sr9e*, ‘Einkorn’, and *Sr31* (Table 2). Therefore, no Ug99-like races were detected.

Discussion

Based on previous reports, 83 species of genus *Berberis*, 7 species of genus *Mahonia*, and 1 hybrid of $\times$ *Mahoberberis* (hybrid between *Mahonia* spp. and *Berberis* spp.) were susceptible to *P. graminis* f. sp. *tritici*; and 51 species of *Berberis*, 12 species of *Mahonia*, and 3 species of $\times$ *Mahoberberis* were resistant to *P. graminis* (Roelfs 1985). *B. aggregata*, *B. brachypoda*, and *B. soulieana* have been identified to serve as alternate hosts of *P. graminis* f. sp. *tritici* (Roelfs 1985). In the present study, more than 100 isolates of *P. graminis* f. sp. *tritici* were obtained from the three *Berberis* spp., indicating that these *Berberis* spp. play a role in sexual reproduction of *P. graminis* f. sp. *tritici*

under natural conditions, at least in the areas surveyed in the present study. Before the present study, there was no report that *B. shensiana* serves as an alternate host of *P. graminis* f. sp. *tritici*. In the present study, we obtained 70 *P. graminis* f. sp. *tritici* isolates from *B. shensiana*, showing that this species is susceptible to stem rust. According to the study by Roelfs (1985), *B. potaninii* is resistant to *P. graminis* f. sp. *tritici*. However, in the present study, we identified 10 aecia as *P. graminis* f. sp. *tritici* from samples on *B. potaninii*, indicating that the species is susceptible to stem rust. The difference could be due to different plants of the *Berberis* spp. or different virulences of the isolates.

Previous studies suggested that some *Berberis* spp. can serve as alternate hosts for rust fungi on grasses, and some grasses can serve as auxiliary hosts of *P. graminis* f. sp. *tritici* in China. Zhang et al. (1957) reported *B. poiretii* to be an alternate host of *P. culmicola*, with its uredinial stage on *Elymus ciliaris* var. *ciliaris* (synonym: *Agropyron ciliaris*). Wang et al. (1958) suggested *E. sibiricus* (synonym: *Clinelymus sibiricus*) as an auxiliary host of *P. graminis* f. sp. *tritici*. In the present study, some of the aecia collected from barberry plants could be stem rust or stripe rust fungi on grasses because they could not infect Mingxian 169. More studies are needed to identify all rust species and formae speciales and their relative frequencies on *Berberis* spp.

In the recent years, some races of the 21C3 (21C3CTH, 21C3CFH, and 21C3CPH) and 34 (34MKG) race groups were detected, while other races (21C3CKH, 21C3CKR, and 21C3CTR) of the 21C3 race group, which occurred in high frequencies in the early 1990s (Yao et al. 1993), were not detected (Han et al. 2010) in wheat fields. In the present study, most isolates tested were of the 21 or 34 race groups. The 34 race group was more predominant than the 21 race group, which was in contrast to the previous reports on race groups from isolates collected from wheat (Han et al. 2010; Yao et al. 1997). This result was inconsistent with the previous reports that race groups 34 and 21 were predominant in recent decades in China (Chen et al. 1994; Han et al. 2010; Huang et al. 1984; Yao et al. 1997) (Y. Y. Cao, personal communication) (Table 2). Races that are virulent to Rulofen and Orofen wheat were first identified in the late 1970s (Cao et al. 1996; Huang et al. 1984). However, no races virulent to Rulofen or Orofen were detected after the 1970s (Han et al. 2010; Wang et al. 1958; Yao et al. 1993, 1997) (Y. Y. Cao, personal communication). Importantly, Rulofen and Orofen are good resistance resources to be used for breeding wheat cultivars against leaf rust and stem rust. Therefore, races virulent to Rulofen and Orofen detected on barberry plants are potential threats to wheat production in China.

The present study demonstrates that *Berberis* spp. can generate new races under natural conditions and may play a role in virulence variation of the *P. graminis* f. sp. *tritici* population in China. This study may change the previous thought that barberry does not play a role in generating virulence variation in the *P. graminis* f. sp. *tritici* population in China (Wang et al. 1958; Zhang et al. 1957; Zeng and Xue 1963). However, more studies are needed to determine the direct relationship of a sexually reproduced population with the populations of *P. graminis* f. sp. *tritici* in epidemic regions of wheat stem rust, because the present study was conducted in Gansu and Shaanxi provinces, where the disease is not a major problem.

In the present study, we observed differences between Einkorn (*Sr21*) and the *Sr21* single-gene line in reaction to the *P. graminis* f. sp. *tritici* isolates. Such differences were also observed in the past decades (Y. Y. Cao, personal communication). Wu (1987) reported virulence of races 17 and 19 on Einkorn but did not detect virulence to the *Sr21* gene line in the tests of more than 10,000 isolates collected from wheat fields from 1959 to 1985. A significant virulence frequency (59.5%) of the *Sr21* (‘W3586’ wheat) virulence was reported by Chen et al. (1994) but not in the collections of 2007 to 2008 (Han et al. 2010). The differences indicate that Einkorn and the *Sr21* “single-gene” line may have additional race-specific resistance genes, which may not be detected by races in other countries. Further studies are needed to test the hypothesis.

The present study shows that *P. graminis* f. sp. *tritici* is able to infect several *Berberis* spp. and the pathogen is able to generate more diverse races on alternate hosts in Gansu and Shaanxi provinces of northwestern China. Stem rust occurs in these provinces but usually does not cause significant damage on wheat, which may be due to the

fact that the winter wheat is harvested early (primarily in June). The early harvest may not leave enough time for stem rust to develop into large-scale epidemics. In addition, infection of barberry plants by *P. graminis* f. sp. *tritici* is low, as detected in the present study. The low infection on barberry that generates low amounts of inoculum for infection of wheat and the short growth period of wheat crops may explain the relatively low level of stem rust in the region compared with stripe rust, the most destructive disease of wheat crops in the region. In a previous study, we also found that barberry infection by the stripe rust pathogen (*P. striiformis* f. sp. *tritici*) was also low (Zhao et al. 2013). The low frequencies of both *P. graminis* f. sp. *tritici* and *P. striiformis* f. sp. *tritici* indicate that other *Puccinia* spp. and formae speciales may infect barberry plants, as discussed above. More studies are needed to determine these species and their relationships to rusts on grasses, as well as the role of *P. graminis* f. sp. *tritici* aeciospores generated on barberry plants in the annual stem rust occurrence on cereal crops.

Acknowledgments

This study was supported by the National Basic Research Program of China (number 2013CB127700), the Earmarked Fund for Modern Agroindustry Technology Research System (number CARS-3-1-11), the Nature Science Foundation of China (number 31271986), International Science & Technology Cooperation Program of China (number 2011DFG32990), and the 111 Project from the Ministry of Education of China (B07049).

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