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First report on race and virulence characterization of *Puccinia graminis* f. sp. *avenae* and resistance of oat cultivars in China

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Abstract The economic importance of oat stem rust is becoming more recognized with the increases of oat cultivated acreage and the disease damage in China. However, there is still no information on races of the pathogen and resistance of oat cultivars. In 2012 and 2013, 26 *Puccinia graminis* f. sp. *avenae* (*Pga*) isolates were obtained from 11 oat stem rust samples collected from oat fields in Hebei province, one of China's major oat growing provinces. The isolates were tested on 16 *Pg* single-gene lines that have been used to differentiate *Pga* races in North America. From the isolates, three races, TKR, TJM and TKM, were identified at 61.6, 30.8 and 7.6 % frequencies, respectively. Virulences to resistance genes *Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg12*, *Pg16* and the gene(s) in the universally susceptible variety "Marvellous" were detected at 100 % frequency, to *Pg10* at 69.2, to *Pg13* at 61.6, to *Pg-a* at 61.6 %, and to the gene(s) in Rodney 0 at 84.6 %. No virulence to *Pg6* and *Pg15* was observed. The above races were used to evaluate the resistance in oat cultivars as seedlings grown in a greenhouse. Of 35 oat cultivars tested, only 13 (37.1 %) were resistant to all three races. This is the first study to identify *Pga* races and oat stem rust resistance in Chinese oat cultivars.

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Introduction

Oat (*Avena sativa* L.) is one of the important cereal crops grown worldwide (Gold et al. 2005), generally divided into hulled oat (with lemma and palea) and naked oat (bare grain/groat) (Zhao et al. 2007a, b). China is the site of origin of naked oat and its cultivation can be traced back to 5000 years ago (Qu et al. 2006). Naked oat is the popular type planted in China while hulled oat is grown in other countries (Guo et al. 2012; Zhen and Zhang 2012). According to statistical data in 2013, China is among the top ten world's oat producers, namely EU, Russia, Canada, Australia, United States, Ukraine, Chile and China (USDA. Foreign Agricultural Service, World Agriculture Production, October 2014). Used as high-quality forage and healthy food, oat is considered as a high value crop. Oat cultivated acreage has rapidly increased to 1.0 million ha, mainly grown in Shanxi, Hebei, Inner-Mongolia, and Jilin as well as other provinces (Qu et al. 2006; Zhao et al. 2007a, b.

The productivity and quality of oat can be greatly affected by oat stem rust (Fig. 1) which is caused by the obligate biotrophic fungus *P. graminis* Per. f. sp. *avenae* Eriks. & E. Henn. (*Pga*) (Stakman et al. 1923). The first four races of *Pga* were identified using oat cultivar differentials: Victory, White Tartar and Monarch in North America in 1923 (Stakman et al. 1923) and the fifth race or race five was identified with differentials

Fig. 1 The natural occurrence of oat stem rust in a field in Baicheng, Jilin province, in the years 2008–2009 (provided by Leng Yanrui, Baicheng Academy of Agricultural Science)

White Tartar, Richland and Jostrain in 1925 (Fetch and Dunsmore 2003). Since then, race identification for *Pga* has become routine in some countries (Harder 1994; Keiper et al. 2005). Severe epidemics of oat stem rust occurred frequently in North America in the early 1900s (Fetch 2003) and also in western Canada in the 1940s (Martens 1978). Recent epidemics in Manitoba and eastern Saskatchewan occurred in 1977 and 2002 and caused estimated yield losses of 35 and 5~10 %, respectively (Fetch 2003). In China, there were no published reports on oat stem rust before the present study. In 2008 and 2009, severe epidemics of oat stem rust occurred in the main oat producing areas in Baicheng, Jilin Province, and in 2012 and 2013, similar epidemics occurred in Zhangjiakou, Hebei province. The disease caused yield losses of approximately 10~15 %.

The recent severe epidemics of oat stem rust in China should be basically resulted from lack of the knowledge of the rust resistance of oat cultivars and the information on race or virulence of oat stem rust population. Therefore the objectives of this study were to characterize the races and virulence pathotypes of the *Pga* population using the oat differentials and to evaluate the resistance of the commonly planted oat cultivars.

Materials and methods

Collection of oat stem rust and oat cultivars

Of a total of 11 oat stem rust samples, four were collected in 2012 and provided by Hebei North University and seven were collected from production fields in September, 2013 also in Zhangjiakou (altitudes 1200–2000 m), Hebei Province. After naturally drying at room temperature, the samples were kept at 4 °C for later use.

Thirty-five oat cultivars were obtained with the help of local research institutes of plant protection (Baicheng Academy of Agricultural Sciences, Gansu Academy of Agricultural Sciences, Shanxi Academy of Agricultural Sciences, Inner Mongolia Academy of Agricultural Sciences). These oat cultivars included the major cultivars used in the major oat production areas in China.

Procedures of isolation and multiplication of single-uredium isolates

Single-uredium isolates were obtained using a previously described method (Huang et al. 1989) as it follows: Seeds of the universally susceptible variety “Marvellous” were sown in 12 cm diameter clay pots. When the primary leaves were fully expanded or when the seedlings were about 7 days old, the leaves were cut off from the seedlings and put onto two layers of filter paper wetted with 40 mg l^{-1} 6-benzylaminopurine (BA) solution used to keep detached leaves green in 15 cm diameter petri dishes. Both ends of the detached leaves in the petri dishes were immobolized with glass stripes. Urediospores of single uredia on the hydrated samples were scratched and smeared onto the detached leaves using whittled-flat toothpicks. The inoculated leaves were then moistened with an atomizer containing 0.05 % Tween 20. The dishes were covered with the lids and placed in an incubation chamber for 20 h dark at 16~20 °C, transferred to a culture room with 14 h (light)/10 h (dark) at 18~20±1 °C. Visible uredinia (diameter, 0.5~0.7 mm) appeared about 6 days after inoculation. The isolation or purification of single pustules was

conducted using the above procedures, repeated three times to obtain a purified single pustule. In order to obtain sufficient urediospores for later race identification use, the multiplication of the purified single pustules also was done following the above method.

Differentials and designation of races

Twelve single gene lines *Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg6*, *Pg8*, *Pg9* (Rodney-Pg9 background), *Pg10*, *Pg12*, *Pg13*, *Pg15* and *Pg16* were included in the differential host system, and *Pg9-R* (*Pg9* in Rosen's Mutant background), *Pg-a*, Rodney 0 and Marvelous (no known resistance genes) were used as supplementary differentials. These differentials, listed in Table 1, were provided by Dr. J. H. Yuan, Hebei North University (from Dr. Y. Jin, Cereal Disease Laboratory, USDA-ARS, St. Paul, MN).

The letter code nomenclature utilizes 12 single-gene *Pg* differential lines, with three subsets of four lines organized into a hexadecimal system that has 16 possible combinations of low (L) or high (H) reaction for each letter (Table 2). Each isolate was designated with a three letter race code based on its reaction on the differential hosts (Fetch and Jin 2007). The infection types (ITs) of the subsets were represented by the first letter, the second and third letters. For instance, high ITs on the four *Pg* genes in a set are assigned the letter 'T', while low ITs on the four hosts, are assigned a letter 'B'. Hence, if an isolate produces high infection types (susceptible reactions) on the 12 *Pg* genes, the race will be assigned the three letter code 'TTT'. An isolate which produces low ITs (resistant reactions) on the 12 *Pg* genes has a race code 'BBB'.

Inoculation of oat stem rust differential hosts

Sixteen *Pg* single-gene differentials were orderly planted in 12 cm diameter porcelain pots. Four holes were evenly dug into the growing medium in each pot and 5–6 seeds for each differential were sown in a hole. Marvellous was used as the susceptible control to ascertain the success of inoculation to each differential host set. Inoculation, incubation and disease development conditions were the same as previously described above. Fourteen days after inoculation, ITs were assessed following the 0–4 scale described by Stewart and Roberts (1970). ITs were grouped into two categories, namely, the ITs 0,;, 1, 1+, 2 and 2+ were considered as low (resistance) while the ITs 3-, 3, 3+ and 4, were considered as high (susceptible) (Stewart and Roberts 1970).

All the experiments were carried out in the College of Plant Protection, Shenyang Agricultural University.

Table 1 Background and origin of differentials and their low infection types caused by *P. graminis* f. sp. *avenae*

Code	Line	Source	*Pg* gene	Low infection type
ORS 1	Rodney-Pg1	06AB	*Pg1*	2
ORS 2	Rodney-Pg2	06AB	*Pg2*	2
ORS 3	Rodney-Pg3	08AB	*Pg3*	1,X
ORS 4	Rodney-Pg4	06AB	*Pg4*	1
ORS 5	CI 6956	04Winnipeg	*Pg6*	0;1-
ORS 6	Rodney-Pg8	06AB	*Pg8*	2
ORS 7	Rodney-Pg9	06AB	*Pg9*	2,2+
ORS 8	Illinois hulless	06AB	*Pg10*	2,3C
ORS 9	CI 8250 (Kyto)	08AB	*Pg12*	0:,1-
ORS 10	Rodney-Pg13	06AB	*Pg13*	2-
ORS 11	Rodney-Pg15	06AB	*Pg15*	1+
ORS 12	Rodney-Pg16	07 Canada	*Pg16*	1+
ORS 13	Rodney-Pg-a	09AB	*Pg-a*	0;,;
ORS 14	Rosen's Mutant	08AB	*Pg9*	2-
ORS 15	Rodney 0	08AB		4
ORS 16	Marvellous			4

* Provided by Dr. J. H. Yuan, Hebei North University (from Dr. Y. Jin, Cereal Disease Laboratory, USDA-ARS, St. Paul, MN)

Table 2 Letter code designations for races of *P. graminis* f. sp. *avenae* using 12 differential lines in three ordered subsets of lines each

Code	Subset	Classification of infection types(ITs)*			
	1	*Pg1*	*Pg2*	*Pg3*	*Pg4*
	2	*Pg6*	*Pg8*	*Pg9*	*Pg10*
	3	*Pg12*	*Pg13*	*Pg15*	*Pg16*
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

* Classification of infection types: *L* low/resistant and *H* high/susceptible (Roelfs and Martens 1988)

Results

Race identification

Twenty-six isolates were obtained from 11 accessions. Three races, namely TKR, TJM and TKM, of *Pga* were characterized and the occurrence frequencies were 61.6, 30.8 and 7.6 %, respectively, as shown in Table 3. TKR was predominant and TJM was less -dominant. It was worth noting the special infection types (Fig. 2) produced on oat lines with *Pg10* by some isolates of TKR, where the epidermis of the primary leaves was ruptured by uredinia and outward rolled. The pustule sizes were big enough to be scored into IT three or four (Fig. 2) and some pustules began to coalesce, which we scored as susceptible ITs though there was some resistant feature or chlorosis around uredinia (Fig. 2).

The virulence formulae of three races, namely, TKR, TJM, and TKM, to the 16 *Pg* genes showed that *Pg6* and *Pg15* were highly effective to all of the races identified in this study. *Pg10*, *Pg13*, and *Pg-a* were moderately resistant while *Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg12*, *Pg16*, *Pg9-R* and Marvellous were highly susceptible to all of the races. Races TJM and TKM showed opposite ITs on *Pg10, ie.*, TKM was virulent to *Pg10* while TJM, avirulent. Similarly, races TKM and TKR were opposite in their virulence to *Pg13* and *Pg-a*, TKR being virulent to *Pg13* and avirulent to *Pg-a*, but TKM was avirulent to *Pg13* and virulent to *Pg-a*. Furthermore, race TKR was virulent to *Pg10* and *Pg13*, and avirulent to *Pg-a*, while TJM was virulent to *Pg-a* and avirulent to *Pg10* and *Pg13*.

Virulence frequencies of the 26 isolates to the16 differential hosts

Virulences to resistance genes *Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg12*, *Pg16* and the gene(s) in Marvellous were detected at 100 % frequency; to *Pg10* at 69.2, *Pg13* at 61.6, *Pg-a* at 61.6 %, and the gene(s) in Rodney 0 at 84.6 %; and no virulence to *Pg6* and *Pg15* was detected (Table 4).

Table 3 Infection type on *P. graminis* f. sp. *avenae* genes and frequency for 3 races of oat stem rust

Race	Infection types on *Pg* genes*																No. of isolates	Frequency (%)
	1	*2*	*3*	*4*	*6*	*8*	*9*	*10*	*12*	*13*	*15*	*16*	*a*	9-R	R*	M*		
TJM	4	4	4	3^+	0	4	4	2	4	1	1^+	4	4	4C	4	4	8	30.8
TKR	4	4	4	3^+	0	4	4	4 N	4	3	1^+	4	1^+	4	3	4	16	61.6
TKM	4	4	4	4	0	4	4	3	4	1^+C	2	4	3	3	4	4	2	7.6

* Infection types(ITs): are based on a 0-to-4 scale where ITs of 0,;, 1, and 2 are indicative of a resistant (*low*) response and ITs of 3 or 4 of a susceptible (*high*) response; Symbols + and – indicate slightly larger and smaller pustule sizes, respectively;N is for necrotic flecks; C is for chlorotic flecks (Stewart and Roberts 1970); R is for Rodney 0; M is for Marvellous

Fig. 2 Infection types on *Pg10* produced by four different isolates of race TKR. Note: Ym8, Ym11, Ym13 and Ym26 were single pustule codes

Reaction of oat cultivars to the oat stem rust races

The resistance test results are shown in Table 5. Thirteen (37.1 %) of the 35 tested oat cultivars showed different resistance levels to races TKR, TKM and TJM at the seedling stage. Of thirteen resistant varieties, twelve were highly resistant (ITs;1,1) and one immune. The remaining 22 (62.9 %) were all moderately to highly susceptible (Table 5).

Discussion

The races of *Pga* in China

In this study, 26 single-uredium isolates of *Pga* obtained from one of China's major oat production regions (Hebei province), were tested following the way adopted in North America, i.e., using the single *Pg* gene differentials of oat and the three-letter Pga-code nomenclature system (Fetch and Jin 2007). Three races, TKR, TJM and TKM, were found. This is the first published report for the physiological races and virulence of *Pga* in China. All of the reported races in North America have no virulence on gene *Pg10* and only races NA1 and NA70 have virulence on *Pg6* (Fetch and Jin 2007). In China, among the 26 isolates tested, none had virulence to *Pg6* and *Pg15* while up to 69.2 % showed virulence on *Pg10*. Therefore, it is obvious that races and virulence of *Pga* in China and North America are considerably different, similar to the wheat stem rust pathogen, *P. graminis* f. sp. *tritici*, in China and North America (Cao and Chen 2010).

The virulence of oat stem rust races and effective *Pg* genes in China

According to this study, *Pg6* and *Pg15* were resistant to all Chinese isolates tested. The studies conducted in the Rust Laboratory, previously located in Winnipeg, Canada, showed that *Pg6* is useful for differentiating isolates of *Pga* in Canada. Most North American races were

Table 4 Virulence frequency of 26 *P. graminis* f. sp. *avenae* isolates on 16 differentials

Pg gene	Virulence frequency (%)	*Pg* gene	Virulence frequency (%)	*Pg* gene	Virulence frequency (%)	*Pg* gene	Virulence frequency (%)
1	100	*6*	0	*12*	100	*a*	61.6
2	100	*8*	100	*13*	61.6	*9-R*	100
3	100	*9*	100	*15*	0	Rodney 0	84.6
4	100	*10*	69.2	*16*	100	Marvellous	100

Table 5 Reaction of oat cultivars to three races of *P. graminis* f. sp. *avenae*

Cultivars	TKR	TKM	TJM	Cultivars	TKR	TKM	TJM	Cultivars	TKR	TKM	TJM
Bayou 8	4	4	4	Bayan 4	4	3	3	Baiyan 12	4	4	4
Bayou 13	3+	4	4	ZNY-200	4	4	4	Baiyan 13	;1	;	1+
STK	;	4	;	Baiyan1	;	;	;	Baiyan 14	4	4	4
Bayan 6	4	3+	4	Baiyan 2	;	;	;	Baiyan 15	;1	;	;1-
Bayan 5	4	4	3+	Baiyan 3	;	0	;	Yanke1	4	3+	3
Bayou 3	3	4	4	Baiyan 4	;	0	1+	Keyan 2	3+	4	4
Huahan 2	4	4	4	Baiyan 5	0	0	0	Neiyan5	4	4	4
Bayan 4	4	3	4	Baiyan 7	4	4	4	Mengyan1	3+	3+	3+
Pin16	;1-	3	;	Baiyan 8	;1=	;	;	Caoyou1	4	4	4
Bayou 5	4	4	4	Baiyan 9	0	;	1	Pinyan1	3-	3	3+
Bayan1	;	;1	;1	Baiyan 10	;1	;	;	Pinyan 2	3	3+	3+
Bayou 9	;	0	;	Baiyan 11	;	0	;	Marvelous	4	4	4

* ITs of 0,;, 1, and 2 are indicative of a resistant (*low*) response and ITs of 3 or 4 of a susceptible (*high*) response; Symbols + and – indicate slightly larger and smaller pustule sizes, respectively (Stewart and Roberts 1970)

avirulent to *Pg6* while the virulence to Saia (*Pg6*) was frequent in Australia (Adhikari et al. 2000). *Pg15* is ineffective to most North American races, but in our study, *Pg15* was immune or nearly immune (';' or ';1=') to most isolates.. *Pg10*, *Pg13*, *Pg-a* and Rodney had different levels of resistance to the three identified races, while *Pg1*, *Pg2*, *Pg3*, *Pg4*, *Pg8*, *Pg9*, *Pg12*, *Pg16*, *Pg9-R* and Marvellous were ineffective. Most of the *Pg* genes were ineffective to the tested isolates, indicating that the Chinese *Pga* population has a relatively wide spectrum of virulence.

Resistance level of Chinese oat cultivars

The seedling resistance genes are usually effective at the adult plant stage and confer a strong resistance response (Singh et al. 2008). In this study, seedling resistance evaluation of 35 cultivars was carried out using the three races we obtained. Only 13 varieties, including Bayan 1, Bayou 9 and Baiyan derivatives have different degrees of resistance and 22 oat cultivars were highly susceptible, with nearly 100 % severity. The results show that the majority of the cultivars are susceptible to oat stem rust. Thus, it is urgent to develop oat varieties with resistance to stem rust (Zhao et al. 2007a, b). The information of the races, virulence patterns and resistant cultivars identified in the study will be useful for breeding for stem rust resistant oat varieties. As a pioneering study on the disease in China, this report can only cover a relatively small number of oat stem rust samples collected from one major oat growing region. Therefore it is necessary to keep on monitoring the disease and characterizing virulence and races from a large number of rust samples from all major oat-growing regions in China.

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