



# Stripe rust resistance to a burgeoning *Puccinia striiformis* f. sp. *tritici* race CYR34 in current Chinese wheat cultivars for breeding and research

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**Abstract** Stripe (yellow) rust is one of the most destructive diseases in wheat production. More than 80 stripe rust resistance (*Yr*) genes have been officially named, however *Yr26* gene has lost resistance to CYR34 (V26) since 2011. In this study, we evaluated resistance of 692 elite wheat cultivars from China to stripe rust in adult plant stage and resistance to CYR32, CYR33, and CYR34 *Pst* races in seedling stages. *Yr26* was deduced in 692 cultivars by WE173 and WE33 molecular marks. The result showed that 45 (7%) entries had all-stage resistance, 79 (11%) entries had adult-plant resistance, and 568 (82%) entries were susceptible to one or more stripe rust races. Besides,

48 (81%) entries in over-summering region were resistant to CYR34, 4 (10%) in over-wintering region, 121 (20%) in spring epidemic region. And 43 entries across China were detected to have *Yr26* gene.

**Keywords** Stripe rust · CYR34/V26 · *Yr26* · Wheat

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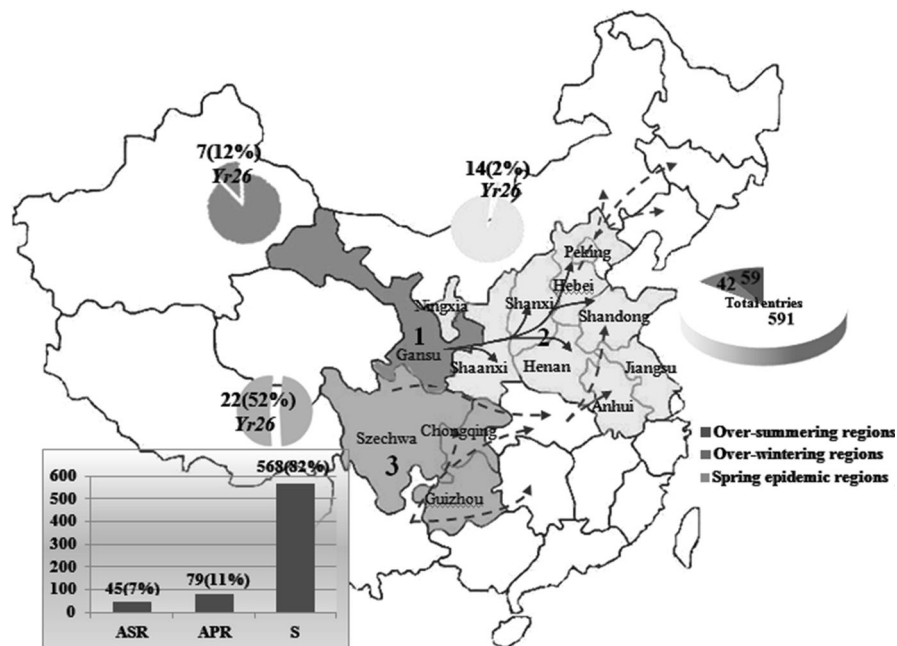
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## Introduction

Stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive diseases in wheat (Lei et al. 2017; Prins et al. 2016; Zheng et al. 2008). It spreads mainly by long-distance dispersal resulted from seasonal wind among continents. In 1979, one virulent stripe rust race spread into Australia, then more than 20 races derived in Australia and 10 years later the uredospore of which spread to New Zealand from eastern Australia probably by wind (Hovmøller et al. 2016; Wu et al. 2016; Wellings et al. 2003; Brown and Hovmoller 2002). China is a main wheat producing region in the world and plays a significant role in stripe rust dispersal (Zeng and Luo 2006; Brown and Hovmoller 2002). In China, severe epidemics in 1950, 1964, 1990, and 2002 lead to yield losses of 6.0, 3.2, 1.8, and 1.3 million tons, respectively (Liu et al. 2012; Chen et al. 2007).

According to the regular epidemic of stripe rust, areas suffering from stripe rust in China are divided into three regions (Fig. 1) including (1): over-



**Fig. 1** (1) Profile of regions and provinces where wheat entries from 59 entries from over-summering region, 32 entries from over-wintering region, and 591 from spring epidemic regions. And 1, 2, 3 in this and subsequent figures orderly represent over-summering region, over-wintering region, and spring epidemic region. (2) Resistance evaluation of wheat entries to stripe rust in China. 45 (7%) of all wheat entries were ASR, 79 (11%) were

APR, and 568 (82%) were S. (3) Detection of *Yr26* gene in wheat entries of China. 7 (12%) wheat entries carried *Yr26* gene in over-summering region (1), 22 (52%) in over-wintering region (2), and 14 (2%) in spring epidemic region. Epidemic traces were cited from Brown JKM and Hovmoller MS (Brown and Hovmoller 2002; Li and Zeng 2002)

summering region where the climate in cool in summer for stripe rust to overcome high temperature, such as south of Gansu, Chongqing and so on, (2): over-wintering region, south of Jiexiu (Shanxi), Shijiazhuang (Hebei) and Dezhou (Shandong) where the climate is warm in winter for stripe rust to overwinter, (3): spring epidemic region where the stripe rust pandemic in spring including the vast Southwest, North China, Middle and lower Yangtze River wheat product regions in China. In autumn, stripe rust in region 1 spread to region 2 and 3, but can't overwinter in most of region 3. Because temperature in most north of region 3, such as Zhangjiakou, wheat region of Inner Mongolia, is too low and the seedlings of wheat providing host for stripe rust are blighted (Liang et al. 2013; Zeng et al. 2014; Zeng and Luo 2006). In spring of the next year, stripe rust spread to regions 2 and 3 causing massive loss of wheat. Besides, the variation of stripe rust showed a gradually decreasing tendency from region 1 to region 2 and the variation of stripe

rust is the most abundant in region 1 (Singh et al. 2014; Li and Zeng 2002).

Stripe rust has strong adaptive capacity, and it can change virulent races in several years (Ksenia et al. 2017; Liu et al. 2016; Cheng et al. 2006). The periodic outbreak and the diversity of stripe rust are the main factors causing of the resistance to stripe rust in wheat cultivars lost (Han et al. 2012). Up to now, more than 80 genes (*Yr* genes) conferring resistance to stripe rust in wheat have been reported and most of them have been used for breeding programs of stripe rust resisting (Yuan et al. 2018; Zeng et al. 2014; Han et al. 2015). The most significant, effective and low-cost approach to restrain stripe rust is distributing *Yr* genes rationally in different wheat regions (Yuan et al. 2014; Zhou et al. 2014; Zeng et al. 2007; Khanna et al. 2005). Currently, cultivars with *Yr26* has been deployed nationally since *Yr26* was identified in a series of wheat-Haynaldia villosa 6AL/6VS translocation lines, such as 92R137, 92R149, and 92R078.

(Han et al. 2015; Ma et al. 2001; Chen et al. 1995). Whereas, a new race, virulent-*Yr26* pathotype (CYR34/V26) (Wang et al. 2017), was firstly isolated in Sichuan province of China in 2008 (Yuan et al. 2014; He et al. 2011; Liu et al. 2010). In 2010, *Yr26*-virulent races were detected at low frequencies (Liu et al. 2010). In 2014, frequency of V26 (CYR34), in 236 standard samples of different virulent races from Gansu, was up to 16.1% (Wang et al. 2014). With gradually epidemic of CYR34, the effect of *Yr26* restricting stripe rust is losing, so it's necessary to evaluate the resistance of cultivars to CYR34 before CYR34 bringing more lost in wheat production.

Therefore, the objective of the study was to evaluate resistance of 692 Chinese elite wheat cultivars to CYR32, CYR33 and CYR34 *Pst* races for controlling stripe rust. Identification of *Yr* genes in the cultivars is important for gene deployment and have an immense aid to wheat breeders managing their time selecting the resistant cultivars for breeding research.

## Materials and methods

With the object to evaluate the resistance of cultivars to CYR34 in China and provide theoretical basic for predicting epidemic of CYR34 and breeding research, we collected 692 wheat entries and a burgeoning *Puccinia striiformis* f. sp. *tritici* race CYR34 and two current epidemic races CYR32 and CYR33 for our research.

### Wheat plant materials

Six hundred ninety-two main wheat cultivars (437 leading cultivars and 255 advanced lines) were collected from 13 provinces (municipalities) (Fig. 1) in China (59 from over-summering region, 42 from over-wintering region, and 591 from spring epidemic region). MX169 was used as susceptible control in seedling test, Xiaoyan22 in field test and 92R137 as positive control in detection of *Yr26*.

### *Pst* races materials

CYR32, CYR33, CYR34 provided by Institute of Plant Pathology of Northwest Agriculture & Forestry University were used in seedling tests in greenhouse.

Mixture of CYR32, CYR33 and CYR34 were used to test adult plant resistance in Yangling by inoculating adult plant artificially and in Tianshui Gansu adult plant were inoculated naturally. The virulence/avirulence patterns of the races were confirmed by testing on 'Avocet' near-isogenic wheat lines used to differentiate *Pst* races (Table 1).

### Seedling tests

Seedling test was conducted in the greenhouse of Northwest Agriculture & Forestry University. Ten seeds of each entry were sowed in a 10 cm<sup>3</sup> cube pot using substrate especially for culture of seedling, and each entry made three repetitions for inoculating three stripe rust races separately. Then place entries inoculated the same race together and cultivar them at uniform condition. At two-leaf stage, the leaves of seedlings of 692 entries were inoculated by the mixture of urediniospores and talcum powder (1:20). Then the seedlings were put in dark and moist at 10 °C for 24 h, finally cultivar the seedlings at 10–18 °C with 16 h light (About 10,000 lx of fluorescent light)/8 h dark. Infection types (ITs) were recorded 20 days post inoculation using 0 to 9 scale described by Line and Qayoum (1992). Plants with ITs of 0 to 6 were considered resistant to the inoculated race, and 7 to 9 were considered susceptible. Seedlings inoculated different races were cultivated in different greenhouse for preventing interference among races.

### Resistance test of wheat entries in fields

Approximately 60 seeds of each the 692 entries were sown manually in a 0.80 m long row spaced 0.4 m in Tianshui, Gansu province and Yangling, Shaanxi province by complete random block design with two replications in 2015 cropping season, end of September. MX169 was sown every two meters in all pathways for efficient infection of stripe rust. Before sowing, plots were fertilized with N: P: K according to recommended ratio. Adult plants were inoculated artificially in Yangling and had natural stripe rust infection in Tianshui. In the middle and late May of 2016, Infection types (IT) and disease severity (DS) were recorded at least twice when the susceptible check had at least 30% severity using the Peterson et al. (1948) scale. Disease severity was assessed visually using the percentage of diseased leaf area

**Table 1** Virulence (V) and avirulence (A) of CYR32, CYR33 and CYR34 races used in this study and their virulence spectra on *Yr* single-gene lines and *Yr26* donor line in seedling stage (Wu et al. 2016)

Race name	Virulence or avirulence formula on <i>Yr</i> genes
CYR32	V: <i>Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr18, Yr27, Yr28, Yr29, Yr31, Yr43, Yr44, YrExp2, YrSP</i> A: <i>Yr5, Yr10, Yr15, Yr24, Yr26, Yr32, YrTr1</i>
CYR33	V: <i>Yr1, Yr6, Yr7, Yr9, Yr17, Yr18, Yr28, Yr29, Yr31, Yr43, Yr44, YrExp2, YrSP</i> A: <i>Yr5, Yr8, Yr10, Yr15, Yr24, Yr26, Yr27, Yr32, YrTr1</i>
CYR34	V: <i>Yr1, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr18, Yr24, Yr26, Yr27, Yr28, Yr29, Yr31, Yr43, Yr44, YrExp2, YrSP</i> A: <i>Yr5, Yr15, Yr32, YrTr1</i>

covered by stripe rust. The highest IT and DS were used to determine the reaction of the entries in the last assessment (Wu et al. 2016).

#### DNA extraction of the entries

The entries were sowed as described in “Seedling test” for efficiently isolating genomic DNA. When plants reached two-leaf stage, 100 mg leaf tissue of each entry was collected in 1.5 ml centrifuge tube, then the tube was closed and frozen in liquid nitrogen immediately. Finally, the genomic DNA of each entry was extracted by the improved method by Song et al (1994).

#### Detection of *Yr26*

WE173 and WE33 (Table 2) primer pairs synthesized by Sangon Biotech Co, Ltd. (Shanghai, China) were used to identify *Yr26* by Polymerase Chain Reaction (PCR) in Bio-Rad®S1000 Thermal Cycler With 96-WellFast Reaction Module. Both PCR capacity of WE173 and WE33 were 15 µl, containing 100 ng DNA template strand, Mg-free 1 × buffer, 2 mM Mg Cl<sub>2</sub>, 0.2 mM dNTP bought from Roche (German), 0.75 U TaqDNA polymerase, 0.6 Mm/each primer. MgCl<sub>2</sub>, 10 × PCR buffer and Taq DNA polymerase were bought from Fermentas of Canada. The PCR program and gel electrophoresis (Table 3) were used

as described by Lagudah et al. (2006) and Wang et al. (2008).

PCR products of WE173 and WE33 were detected by polyacrylamide gel electrophoresis (PAGE). 3 µl formamide loading buffer was added into each PCR product, then the mixture was denaturized at 94 °C and then immediately placed in ice for 5 min. 5 µl mixture was loaded in 5% denaturing polyacrylamide gel. The gel was silver stained and scanned as described by An et al (2009). Avocet (AVS) and 92R137 were used as Negative and positive controls, respectively.

## Results

#### Stripe rust resistance

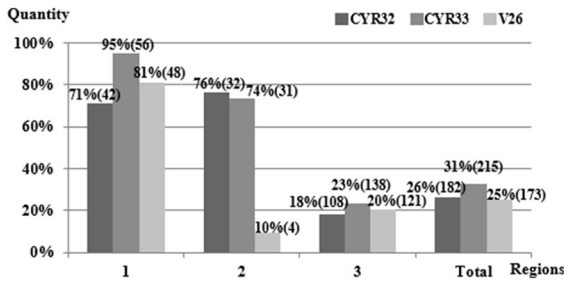
Stripe rust resistance test showed that 568 (about 82%) entries were susceptible. In seedling stage, 519 (75%) entries from three regions and 21 (50% entries of this region) entries from variation central were susceptible to CYR34. Entries resistant to individual races (Fig. 2), two races (CYR32 and CYR33) (Fig. 3), or three races (Fig. 3) in separate regions were summed. As showed in Fig. 2, the ratios of wheat entries resistant to CYR32 in over-summering (1), over-wintering (2) and spring epidemic region (3) were

**Table 2** WE173 and WE33 primer pairs for identifying *Yr26*

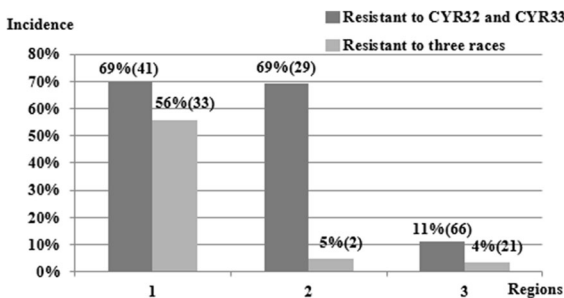
Primer name	Primer sequence (5′–3′)	Annealing temp. (°C)	Fragment size (bp)
WE173	GGGACAAGGGGAGTTGAAGC	55	+ 451
	GAGAGTTCCAAGCAGAACAC		– 730
WE33	TAAACCAAGTCCCCAAA	55	+ About 450
	GGAGTCCATCTTACCGA		

**Table 3** PCR programs of WE173 and WE33

Initialization (94 °C) min	Denaturation (94 °C) S	Annealing, °C, S	Extension (72 °C) S	No. of cycles	Final elongation (72 °C) min
4	30	55.30	60	40	10
4	30	55.60	60	35	10



**Fig. 2** Ratios of wheat entries resistant to individual virulence in separate regions. In over-summering (1), ratios of wheat entries resistant to CYR32, CYR33, CYR34 were orderly 71% (42), 95% (56), 81% (48); in over-wintering region (2) were 76% (32), 74% (31), 13% (4); in spring epidemic region were 18% (108), 23% (138), 20% (121); and totally were 26% (182), 31% (215), 25% (173)



**Fig. 3** Statistics of resistance to multiple races. In regions 1, 2, 3 ratios of wheat entries resistant to two epidemic virulent races CYR32 and CYR33 are separately 69% (41), 69% (29), 11% (66), and resistant to the three races are 56% (33), 5% (2), 4% (21)

separately 71%, 76%, 18%, to CYR33 were 95%, 74%, 23%, and to CYR34 were 81%, 10%, 20%. In addition, ratios (Fig. 3) of wheat entries resistant to CYR32 and CYR33 in region 1, 2 and 3 were 69%, 69%, 11% and to these three virulence races were 56%, 5%, 4%. The purple bar in Fig. 2 demonstrated that the resistance level of wheat entries to CYR34 in over-wintering region and epidemic region are low. The total ratio (Fig. 2) of entries resistant to CYR32,

CYR33 and CYR34 were individually 26%, 33%, 25%, which revealed that resistance level of wheat to stripe rust in China is low in seedling stage.

Among (Fig. 1) all wheat entries, 45 (7%) entries were all-stage resistance (ASR), 79 (11%) were adult-plant resistance (APR) and 568 (82%) were susceptible to one or more races (S), which suggested that resistance of wheat to stripe rust in China are rare and exploring cultivars with APR or ASR should be enhanced. The detail ITs of seedling test, ITs and severities of adult plants, and PCR detection results were listed in electronic supplementary material Table 4.

#### Identification of *Yr26* gene

Because of the gradually strengthened threaten of CYR34 and the change of *Yr26* from resistant to susceptible to CYR34, *Yr26* gene in 692 wheat entries from China was detected to clarify which wheat entry and region is more liable to be threatened by CYR34 and provide theoretical basic for taking preventive measures. In 92R137, a 450-bp positive band can be detected by WE33 primer pair, and a 500-bp positive band by WE173. In Avocet (AVS), a 750-bp band can be detected by WE173. In 692 experimental wheat entries, 7 (12%) (Fig. 1) entries from over-summering region, 22 (52%) from over-wintering region and 14 (2%) from spring epidemic region were derived to carry *Yr26* gene due to positive bands of both WE33 and WE173 primer pairs in gels. In total 43 (6%) wheat entries were detected to carry *Yr26* gene, which indicated that *Yr26* genes are still distributed in China, and especially in over-wintering region about half of entries have *Yr26* gene. Besides, 7 (12%) entries in over-summering region own *Yr26* gene, which can provide host and make screening effect in the central area of stripe rust for epidemic of CYR34. In addition, these entries (electronic supplementary material in Table 4) should be treated seriously for preventing the pandemic of CYR34 and when distributing stripe rust resistant genes.



## Discussion

Stripe rust, which has abundant virulent variant races, is one of the most destructive diseases to wheat. Exploring wheat germplasm resources with durable resistance to stripe rust and deploying different genes in different regions are economical-effective ways to resist it (Bai et al. 2014; Lan et al. 2014). Virulent-*Yr26* pathotype CYR34 has been a potentially epidemic virulent race since 2008. Predicting epidemic of stripe rust and take precautions is essential for resisting stripe rust (Li and Zeng 2002).

Statistics result showed that entries resistant to these three races demonstrated declining tendency from over-summering region (1), where the varieties of virulence stripe rust is most diverse in China, to over-wintering region (2) and then to spring epidemic region (3), except to CYR32 was raised from region 1 to region 2, which is accord with the theory that distributing disease-resistant entries to resist the dispersal of stripe rust nearby variation central (Bai et al. 2014). Stripe rust overcome winter mainly in over-wintering region, and Fig. 2 showed that in this region ratios of entries resistant to CYR32 were 76%, to CYR33 were 74%, and to these both epidemic races was 69% (Fig. 3), which indicate that distributing wheat entries have certain effect to resist stripe rust. Notably, in region 2 when statistics brought in the resistance to CYR34, the percentage suddenly decrease to 5% (2 entries). Besides, 52% entries (Fig. 1) have *Yr26* gene in region 2, indicating that *Yr26* gene susceptible to CYR34 is still distributed in over-wintering region which is according with previous study (Li et al. 2017). So wheat entries in region 2 are liable to suffer from CYR34, which will provide host for CYR34 in seedling stage and improve quantity of CYR34 for spring epidemic of the next year, finally cause wheat infected by CYR34 in eastern wheat regions. In addition, in spring epidemic region only 4% (Fig. 3) entries were resistant to these three races in seedling stage simultaneously, and wheat is planted in large scale in this region, so significant loss of wheat production will be caused when CYR34 epidemic in this region. Therefore, the combined result demonstrated that over-wintering region and spring epidemic region are suffering the potential threaten of CYR34 in China.

Combining seedling test and adult plant resistance test, the result demonstrated that 568 (82%) (Fig. 1)

wheat entries were susceptible to stripe rust, containing 4 (7%) from region 1, 28 (67%) from region 2, 536 (91%) from region 3. It suggested that stripe rust is seriously threatening wheat production in China, especially in spring epidemic region. Besides, wheat susceptible to stripe rust in over-summering and over-wintering regions may act as screener to filtrate novel virulent races, which will generate potential risk to wheat production. So it is urgent to exploit novel *Yr* genes and resistance resources, especially resources with adult plant resistance to restrain stripe rust in China. And breeders should properly manage their time to select the resistant cultivars for breeding.

*Yr26* gene was detected in 692 wheat entries collected from China and 42 entries were detected to have *Yr26* gene. Wheat entries owning *Yr26* in over-wintering region takes a great proportion (Fig. 1), on which CYR34 may survival in cold winter and epidemic to eastern wheat regions to threaten wheat production. Besides, 12% (Fig. 1) wheat entries carrying *Yr26* gene in variable region of stripe rust may screen CYR34 race and improve the fundamental quantity of CYR34 for its epidemic. In brief, wheat in China is facing great risk of CYR34 and in some areas has already lost great production by the invasion of CYR34. And wheat cultivars with *Yr26* gene which has lost resistance to CYR34 is still distributing frequently in China, which should be taken seriously when breed novel resistant entries and distribute resistant genes.

Genes in entries detected to carry *Yr26* genes but resistant to CYR34 need further study to be confirmed. Distribution of wheat cultivars has changed many times due to the epidemic of different stripe rust races, and the prevail of CYR34 may also influence the distribution of wheat cultivars in China. Comprehensively, resistant level of wheat cultivars to stripe rust in China is low, screening new genes conferring resistance to stripe rust in wheat and exploit resistance resources are needed.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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