

RESEARCH

Open Access



Three formae speciales of *Puccinia striiformis* were identified as heteroecious rusts based on completion of sexual cycle on *Berberis* spp. under artificial inoculation

Shujie Huang[†], Shuxia Zuo[†], Dan Zheng, Yao Liu, Zhimin Du, Zhensheng Kang* and Jie Zhao*

Abstract

Puccinia striiformis Westend. is an obligate biotrophic parasite that could infect wheat and grasses to cause stripe rusts. *P. striiformis*, a species of rust fungi, is divided into several formae speciales based on host specialization, including *P. striiformis* f. sp. *tritici*, *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *elymi*, *P. striiformis* f. sp. *agropyri*, and *P. striiformis* f. sp. *secalis*. Among the five forms of *P. striiformis*, sexual stage was confirmed only for the wheat form of the rust, *P. striiformis* f. sp. *tritici*, but not known for the rest four forms. In the present study, we investigated the host specialization of three forms of *P. striiformis* including *P. striiformis* f. sp. *agropyri*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *hordei*, compared the morphology of their urediniospores and teliospores, and analyzed the molecular phylogenetic tree of the three forms of *P. striiformis* and other related species in the genus *Puccinia*. Each of the three forms was able to infect barberry to complete pycnial and aecial stages under artificial inoculation. Further inoculations of grass, barley cv. Guoluo and wheat cv. Mingxian 169, highly susceptible to *P. striiformis* f. sp. *tritici*, using the resultant aeciospores showed that the aeciospores could successfully infect their respective hosts and produce uredinia with high infection types, with the exception on wheat cv. Mingxian 169 where only necrosis was observed. The results suggested that *Berberis* spp. were alternate hosts for the three formae speciales of *P. striiformis*, and confirmed that the three rusts were macrocyclic and heteroecious rust fungi completing the whole life cycle on primary hosts and alternate hosts. Our study provides a basis for studying pathogenic variation of the rust fungi through sexual hybridization between formae speciales of *P. striiformis*.

Keywords: *Puccinia striiformis*, Grass, Barley, Stripe rust, Alternate host, Sexual reproduction, Barberry

Background

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend., is a common fungal disease damaging cereal crops and gramineous grasses (Liu and Hambleton 2010). The disease is spread by air-borne urediniospores for hundreds to thousands kilometers (Zadoks 1961; Brown and Hovmøller 2002; Hovmøller et al. 2002). Huge yield losses of crops resulted from epidemics of stripe rust often occurred in temperate areas where the summer is cool and the moisture is adequate for dew formation (Rapilly 1979;

Li and Shang 1989; Zadoks and van den Bosch 1994). Wheat stripe rust is an important wheat disease in many countries, especially in China (Wan et al. 2007), the United States (Chen 2005), and some new stripe rust-invaded territories including Australia (Murray et al. 1994; Wellings 2007), New Zealand (Beresford 1982), and South Africa (Boshoff et al. 2002).

Stripe rust epidemics are often caused by new virulent races of *P. striiformis*. In China, seven displacements of major wheat cultivars nationwide have happened since 1950 because of continuous emergence of new races (Wan et al. 2007). Recently, a few of new highly virulent races, V26 virulent to *Yr24* (= *Yr26* + *YrCH42*) (Liu et al. 2010), G22–9 virulent to Guinong 22 (*Yr10*), Chuanmai 42 (*YrCH42*), 93R137 (*Yr26*) and Moro (*Yr10*) (Jia et al.

* Correspondence: kangzs@nwfau.edu.cn; jiezhao@nwfau.edu.cn

[†]Shujie Huang and Shuxia Zuo contributed equally to this work as co-first authors.

State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, China



2012), and *avrYr10/24/26/ch42* virulent to *Yr10*, *Yr24*, *Yr26* and *YrCH42* (Kuang et al. 2013), have been identified and found to evolve continuously to produce more new races. The new races caused 'break down' of the resistance of major wheat cultivars possessing the specific *Yr* genes.

However, lack of sexual stage has made it difficult to study virulence variation of the rust fungi. Grasses and cereals harbor a massive of rust fungi, containing more than 380 species in genera of *Puccinia* and *Uromyces*, but only some of them are heteroecious (Cummins 1971). Most of the rust fungi do not have known alternate hosts for sexual reproduction. *P. striiformis* is a complex group of rust fungi and was first reported to have five formae speciales, namely *P. striiformis* f. sp. *tritici* (on wheat), *P. striiformis* f. sp. *hordei* (on barley), *P. striiformis* f. sp. *agropyri* (on *Agropyron* spp.), *P. striiformis* f. sp. *elymi* (on *Elymus* spp.) and *P. striiformis* f. sp. *secalis* (on rye), based on host specialization by Eriksson and Henning (1894). Subsequently, other scientists reported four different formae speciales, including *P. striiformis* f. sp. *dactylidis* on orchard grass (Manners 1960; Tollenaar 1967), *P. striiformis* f. sp. *poae* on Kentucky bluegrass (Tollenaar 1967), *P. striiformis* f. sp. *leymi* on *Leymus secalinus* (Niu et al. 1991), and *P. striiformis* f. sp. *pseudo-hordei* on wild barley grass (*Hordeum* spp.) (Wellings et al. 2000). In recent years, based on combined methods of spore morphology and sequences of internal transcribed spacer (ITS) and beta-tubulin DNA regions, the stripe rust fungi infecting bluegrass and orchard grass were renamed as *P. pseudostriiformis* M. Abbasi, Hedjaroude, and M. Scholle, and *P. striiformoides* M. Abbasi, Hedjaroude, and M. Scholle, respectively (Abbasi et al. 2004; Liu and Hambleton 2010). Liu and Hambleton (2010) suggested that the *P. striiformis* on grass species in genera of *Aegilops*, *Elymus*, *Hordeum*, and *Triticum*, should be recognized as *P. striiformis* sensu stricto. Until recently, alternate hosts for *P. striiformis* f. sp. *tritici* causing wheat stripe rust and for *P. pseudostriiformis* (syn. *P. striiformis* f. sp. *poae*) on *Poa pratensis* has been reported (Jin et al. 2010), but the alternate hosts for other forms of *P. striiformis* have not been known. Therefore, the objective of the present study is to test whether barberry can serve as aecial hosts for the three forms of *P. striiformis*, *P. striiformis* f. sp. *agropyri* (*Psa*), *P. striiformis* f. sp. *elymi* (*Pse*) and *P. striiformis* f. sp. *hordei* (*Psh*).

Results

Host specialization of *P. striiformis* formae speciales

Fifteen days post inoculation (dpi) of the single uredinium-derived isolates of the three *P. striiformis* formae speciales on their respective original hosts, heavy sporulation was observed on *A. cristatum* (Fig. 1a), *E. cylindricus* (Fig. 1b), and barley cv.

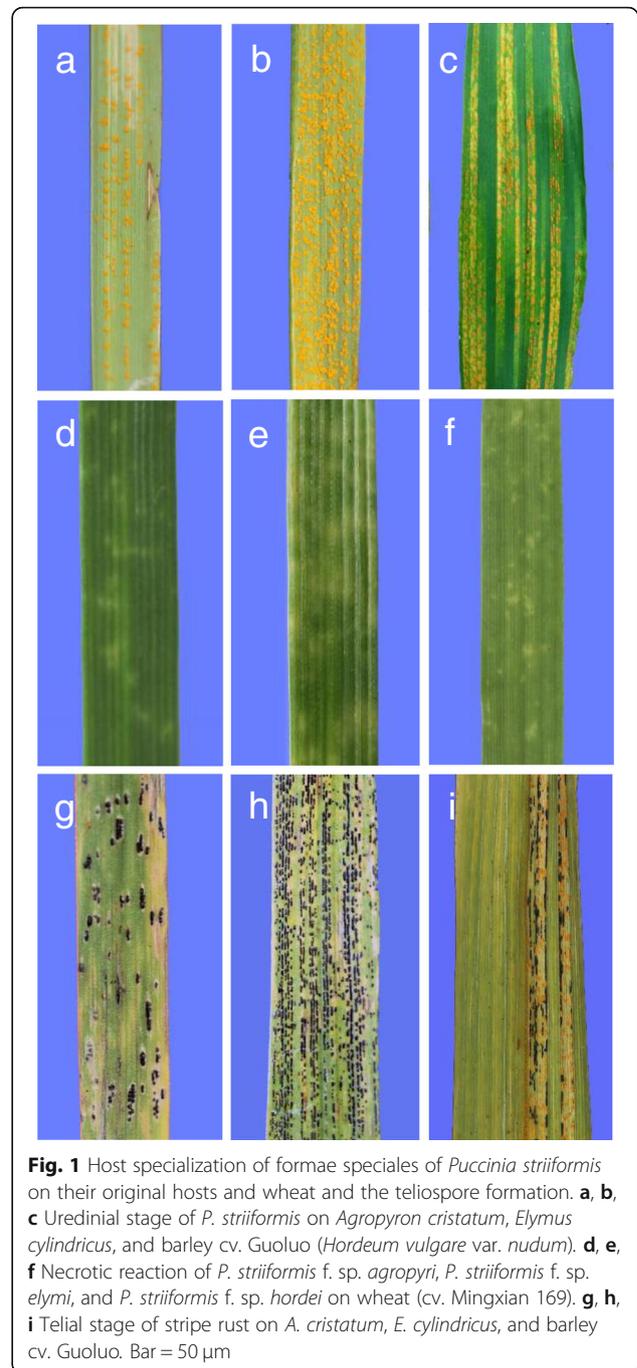


Fig. 1 Host specialization of formae speciales of *Puccinia striiformis* on their original hosts and wheat and the teliospore formation. **a, b, c** Uredinal stage of *P. striiformis* on *Agropyron cristatum*, *Elymus cylindricus*, and barley cv. Guoluo (*Hordeum vulgare* var. *nudum*). **d, e, f** Necrotic reaction of *P. striiformis* f. sp. *agropyri*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *hordei* on wheat (cv. Mingxian 169). **g, h, i** Telial stage of stripe rust on *A. cristatum*, *E. cylindricus*, and barley cv. Guoluo. Bar = 50 μ m

Guoluo (Fig. 1c), but not on wheat cv. Mingxian 169. Instead, necrosis lesions were produced on leaves of Mingxian 169 (Fig. 1d, e and f). Therefore, the three stripe rust pathogens infecting *A. cristatum*, *E. cylindricus*, and barley cv. Guoluo were determined to be *P. striiformis* f. sp. *agropyri*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *hordei*, respectively. Subsequently, the three rust forms produced telia about 25 dpi (Fig. 1g, h and i).

Morphology of three formae speciales of *P. striiformis*

To further characterize the three forms of *P. striiformis*, morphology of their urediniospores and teliospores were assessed under a light microscope. Urediniospores of *P. striiformis* f. sp. *agropyri* on *A. cristatum* were near round in shape, single-celled, light orange in color, $23\text{--}34 \times 21\text{--}30 \mu\text{m}$ (mean $28 \times 24 \mu\text{m}$) in size (Fig. 2a). Teliospores were two-celled, single-celled occasionally, without or with a short stalk. The apical cell of teliospores was domed or inclined, $17\text{--}32 \times 15\text{--}28 \mu\text{m}$ (mean $26 \times 21 \mu\text{m}$). The lower cells of teliospores were $21\text{--}37 \times 12\text{--}21 \mu\text{m}$ (mean $27 \times 16 \mu\text{m}$). Teliospore stalks were (0-) $1\text{--}6$ (-18) μm (mean $4 \mu\text{m}$ in length). The upper cells were brown, and darker than the lower cells (Fig. 2b).

Urediniospores of *P. striiformis* f. sp. *elymi* on *E. cylindricus* were fresh orange-colored, single-celled, near

round or occasionally oblong in shape, $21\text{--}29 \times 19\text{--}26 \mu\text{m}$ (mean $24 \times 22 \mu\text{m}$) in size (Fig. 2c). The apical cells of teliospores were dark brown, flat or inclined, $19\text{--}32 \times 14\text{--}19 \mu\text{m}$ (mean $23 \times 17 \mu\text{m}$) in size. The lower cells of teliospores were $21\text{--}32 \times 9\text{--}16 \mu\text{m}$ (mean $28 \times 11 \mu\text{m}$). Teliospore stalks were (0-) $0.3\text{--}0.7$ (1.3) μm long (Fig. 2d).

Urediniospores of *P. striiformis* f. sp. *hordei* on barley cv. Guoluo were light orange, single-celled, near round in shape, and were averaged $26 \mu\text{m}$ in diameter (Fig. 2e). Teliospores were light orange in color, two-celled, with a short stalk or without a stalk. The apical cells of teliospores were $27\text{--}30 \times 23\text{--}30 \mu\text{m}$ (mean $29 \times 24 \mu\text{m}$) in size. The lower cells of teliospores were $23\text{--}30 \times 21\text{--}25 \mu\text{m}$ (mean $26 \times 23 \mu\text{m}$). Teliospore stalks were (0-) $2.3\text{--}4.5$ (7.3) μm long (Fig. 2f).

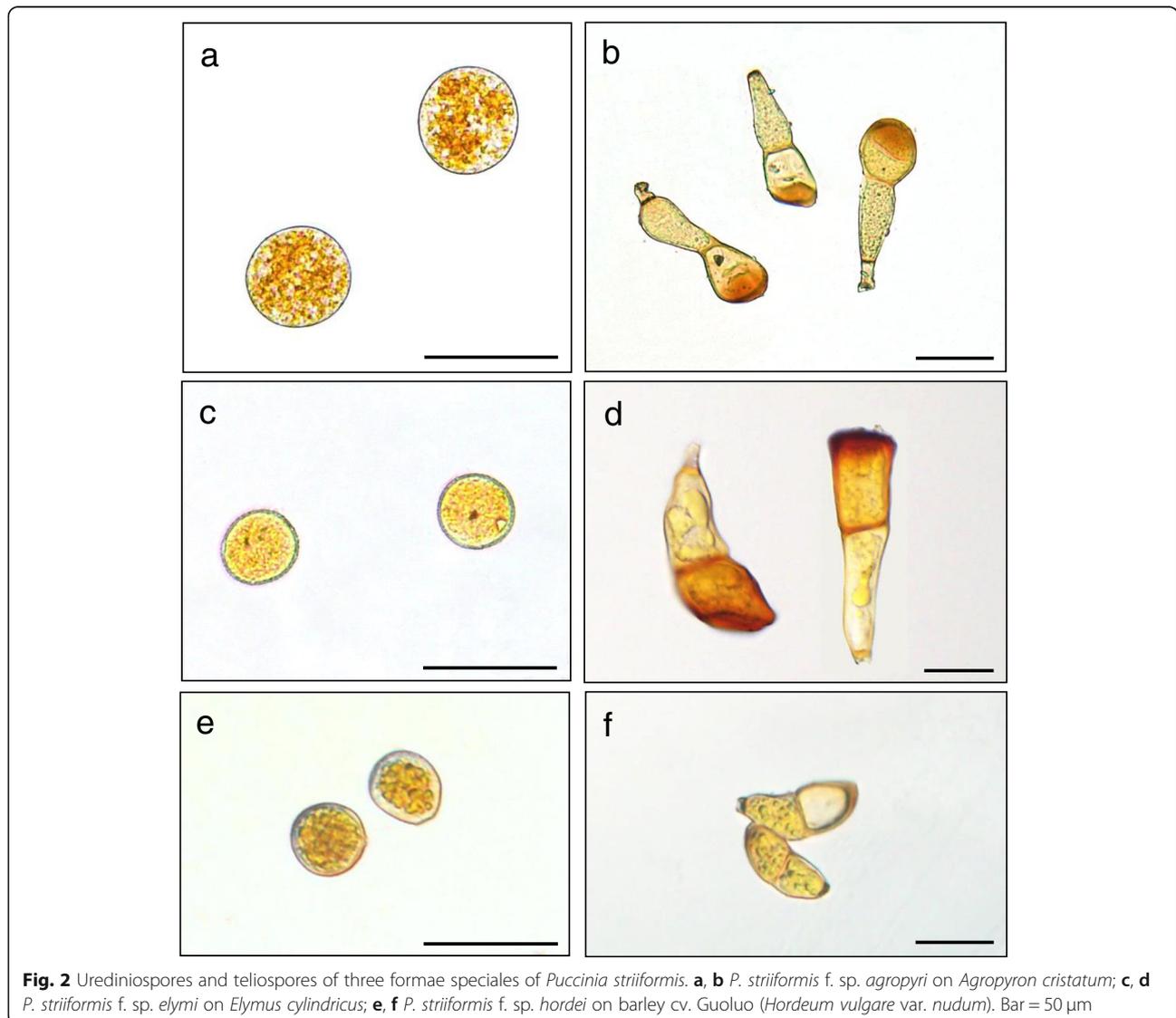


Fig. 2 Urediniospores and teliospores of three formae speciales of *Puccinia striiformis*. **a, b** *P. striiformis* f. sp. *agropyri* on *Agropyron cristatum*; **c, d** *P. striiformis* f. sp. *elymi* on *Elymus cylindricus*; **e, f** *P. striiformis* f. sp. *hordei* on barley cv. Guoluo (*Hordeum vulgare* var. *nudum*). Bar = $50 \mu\text{m}$

Sequence analysis

Alignment of ITS sequence of *Agropyron* form and *Elymus* form of *P. striiformis* as well as *P. striiformis* f. sp. *tritici* showed that they shared a high sequence similarity of 97.31% (Additional file 1: Figure S1), indicating that *Agropyron* form and *Elymus* form of rust fungi used in this study were *P. striiformis*. *Agropyron* form and *Elymus* form of *P. striiformis* presented a similarity of 95.05% and displayed obvious sequence difference in internal transcribed spacer region based on sequence alignment (Additional file 2: Figure S2). Phylogenetic tree of the three forms of *Puccinia striiformis* used in this study, namely *Psa* (GenBank accession No.: MK418351), *Pse* (GenBank accession No.:MK418452), and *Psh* (GenBank accession No.:MK414678) and reference *Puccinia* species submitted in NCBI database (Table 1) indicated that *Psh*, *Pse* and *Psa* were grouped into one subclade with reference *Puccinia striiformis* formae speciales, including *P. striiformis* f. sp. *hordei* on *Hordeum*, *P. striiformis* f. sp. *elymi* on *Elymus*, *P. striiformis* f. sp. *tritici* on *Triticum*, and *P. striiformis* f. sp. *agropyri* on *Aegilops*, but distinguished from *P. pseudostriformis*, *P. striiformoides*, *P. coronata* s. l., and *P. graminis* (Fig. 3). The sequence analysis, together with host specialization, showed that the three forms of *P. striiformis* used in the present study were *P. striiformis* f. sp. *hordei*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *agropyri*, respectively.

Table 1 List of *Puccinia* species used in the phylogenetic analysis

<i>Puccinia</i> species	Host	GenBank accession No.
<i>P. pseudostriformis</i>	<i>Poa pratensis</i>	HM057113 ^a
<i>P. gansensis</i>	<i>Achnatherum inebrians</i>	HM057115
<i>P. striiformis</i>	<i>Triticum aestivum</i>	HM057118
<i>P. striiformis</i>	<i>Aegilops ligustica</i>	HM057127
<i>P. striiformis</i>	<i>Hordeum</i> sp.	HM057128
<i>P. striiformis</i>	<i>Elymus elymoides</i>	HM057130
<i>P. striiformis</i>	<i>T. aestivum</i>	HM057135
<i>P. striiformis</i>	<i>H. comosum</i>	HM057136
<i>P. striiformoides</i>	<i>Dactylis glomerata</i>	HM057137
<i>P. coronata</i> s.l.	<i>Avena</i> sp.	HM057140
<i>P. graminis</i>	<i>T. aestivum</i>	HM057144
<i>P. poae-nemorialis</i>	<i>Koeleria litwinowii</i>	HM057155
<i>P. striiformis</i>	<i>Agropyron cristatum</i>	MK418351 ^b
<i>P. striiformis</i>	<i>E. cylindricus</i>	MK418452 ^b
<i>P. striiformis</i>	<i>H. vulgare</i> var. <i>nudum</i>	MK414678 ^b

^aData of HM series of *Puccinia* species were originated from Liu and Hambleton (2010). ^bEach of sequences of rust fungi used in this study was submitted to GenBank of NCBI Database (<https://www.ncbi.nlm.nih.gov/>) and was provided accession number, respectively

Pycnial and aecial production of the three formae speciales of *P. striiformis* on barberry

Seven to nine dpi, initial symptoms of tiny, light yellow lesions were visible at the early stage of the *Pst* infection on barberry leaves (*B. shensiana*). With the development of pycnia, lesions developed radially around the primary infection sites, showing dark yellow color in the centers of lesions (Fig. 4a, b and c). Simultaneously, massive pycniospores were produced inside a pycnium and moved upward to accumulate around the pycnial ostiole on the surface of barberry leaves, forming a transparent, oval drop of nectar. Nectars appeared yellow with a yellow background. After fertilization (spermatization), 13 to 16 dpi, orange, finger-shaped aecia emerged from the back side of a pycnial lesion (Fig. 4d, e and f), extending to several millimeters in length, generally 1 to 3 mm. The number of aecial cups (aecia) differed from one lesion to another.

Pathogenicity of aeciospores

Aeciospores of three formae speciales of *P. striiformis* produced on barberry were used to inoculate their respective hosts, *A. cristatum*, *E. cylindricus*, barley cv. Guoluo, as well as wheat cv. Mingxian 169. Eighteen days after aeciospore inoculation, yellow uredinia were produced on leaves of *A. cristatum*, *E. cylindricus*, and barley cv. Guoluo (Fig. 4g, h and i), but not on leaves of wheat cv. Mingxian 169 on which only necroses were observed (Fig. 4j, k and l). Massive uredinia lined in arrays between leaf veins of the two grasses, *A. cristatum* and *E. cylindricus*, showing typical symptoms of stripe rust. The *P. striiformis* f. sp. *hordei* uredinia were scattered on leaves of barley cv. Guoluo seedlings probably due to the lack of vein tissues. Thus, these three forms were able to infect barberry and the resulted aeciospores could infect the grass hosts from which they were collected and produce uredinia but not on other plants tested.

Discussion

Puccinia striiformis frequently produces variants. Rust fungi from wild grasses could possibly harbor higher genetic variation than those from crops because of the selection on crop host. Cheng et al. (2016) reported that grasses harbor more diverse pathotypes of *P. striiformis* than cereals based on pathogenicity and DNA polymorphism of urediniospore isolates collected from wheat, barley, rye, grasses, and triticale in the United States. Our study of the alternate host for the three forms of *P. striiformis* has led us to hypothesize that sexual genetic recombination may contribute to diversity of this stripe rust pathogen. Up to now, only the *tritici* form of *P. striiformis* has been verified to produce new variants through sexual reproduction (Tian et al. 2016;

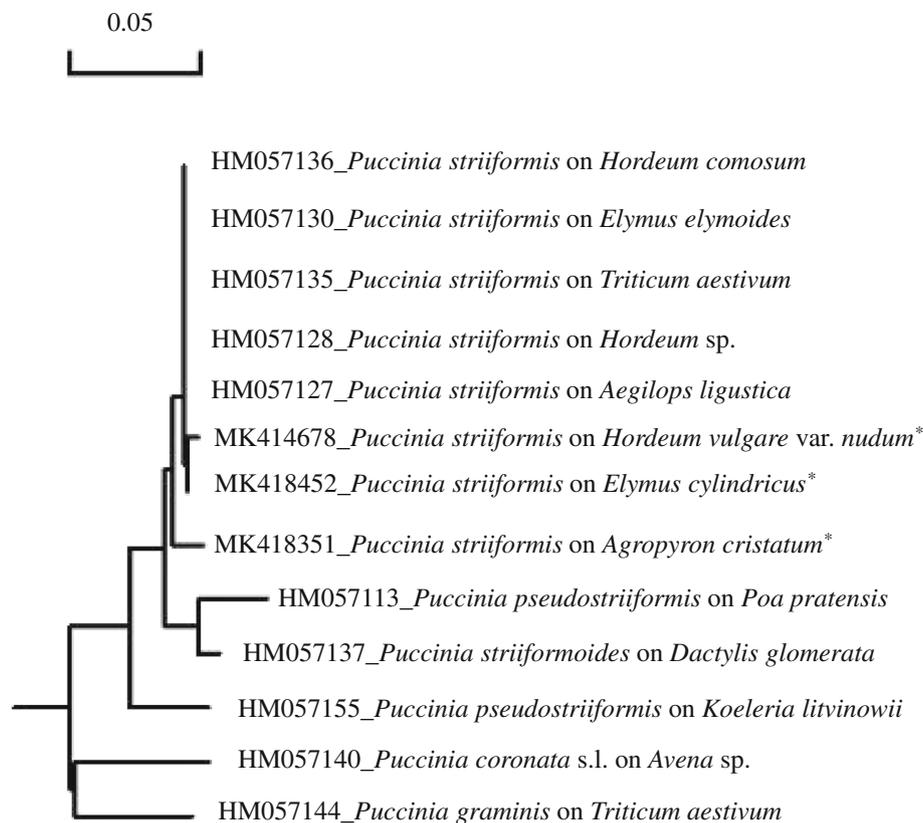


Fig. 3 Phylogenetic tree based on ITS sequences of three formae speciales of *Puccinia striiformis* and the related species in *Puccinia*. **P. striiformis* f. sp. *hordei* on *Hordeum vulgare* var. *nudum* (GenBank accession No.:MK414678), *P. striiformis* f. sp. *elymi* on *Elymus cylindricus* (GenBank accession No.:MK418452) and *P. striiformis* f. sp. *agropyri* on *Agropyron cristatum* (GenBank accession No.:MK418351). The related *Puccinia* species submitted at NCBI Database (<https://www.ncbi.nlm.nih.gov/>) were used as reference

Wang et al. 2018). Other forms of *P. striiformis* have not been reported with the exception of *P. striiformis* f. sp. *poae*, which is now considered as a different species, *P. pseudostriformis* (Abbasi et al. 2004; Liu and Hambleton 2010). In the present study, based on artificial inoculation and host specialization tests, we identified barberry (*B. shensiensis*) as a potential alternate host for f. sp. *elymi*, f. sp. *agropyri*, and f. sp. *hordei* of *P. striiformis*. Simultaneously, another barberry species, *B. aggregata* Schneid., native to China and collected from Tianshui of Gansu Province was used to test the susceptibility to the three formae speciales of *P. striiformis*. The results indicated that basidiospores of each of the three forms could also successfully infect *B. aggregata* and complete the pycnial and aecial stages. All the information suggested that, similar to *P. striiformis* f. sp. *tritici* and *P. pseudostriformis* (syn. *P. striiformis* f. sp. *poae*), the three forms of *P. striiformis* are heteroecious, macrocyclic rust fungi.

The determination of sexual stage of the three forms of *P. striiformis* facilitates studies on virulence variation through hybridization between different forms. Previous studies showed that hybridization of different formae

speciales of *P. graminis* can result in new races of *P. graminis* f. sp. *tritici*, the causal agent of wheat stem rust (Stakman et al. 1930). Johnson et al. (1932) made crosses of formae speciales, *P. graminis tritici* × *P. graminis secalis* and *P. graminis tritici* × *P. graminis agrostidi* on barberry plants, and obtained new variants from the progenies. *B. shensiensis* has been reported as an alternate host for *P. striiformis* f. sp. *tritici* (Zhao et al. 2013). In this study, *B. shensiensis* was identified as a potential alternate host for the three forms tested. It appears that barberry may serve as a common platform for different formae speciales to hybrid if simultaneous infections occur under natural conditions. Further studies are needed to test this hypothesis.

Uredinial hosts of *P. striiformis* are restricted in Gramineae family. The most susceptible genera include *Aegilops*, *Agropyron*, *Bromus*, *Elymus*, *Hordeum*, *Secale*, and *Triticum* (Stubbs 1985). Formae speciales differentiation of *P. striiformis* was primarily conducted based on host specialization (Eriksson 1894). The species of *P. striiformis* were divided into five formae speciales, namely *Pst* on wheat, *Psh* on barley, *Pse* on *Elymus*, *Pss*

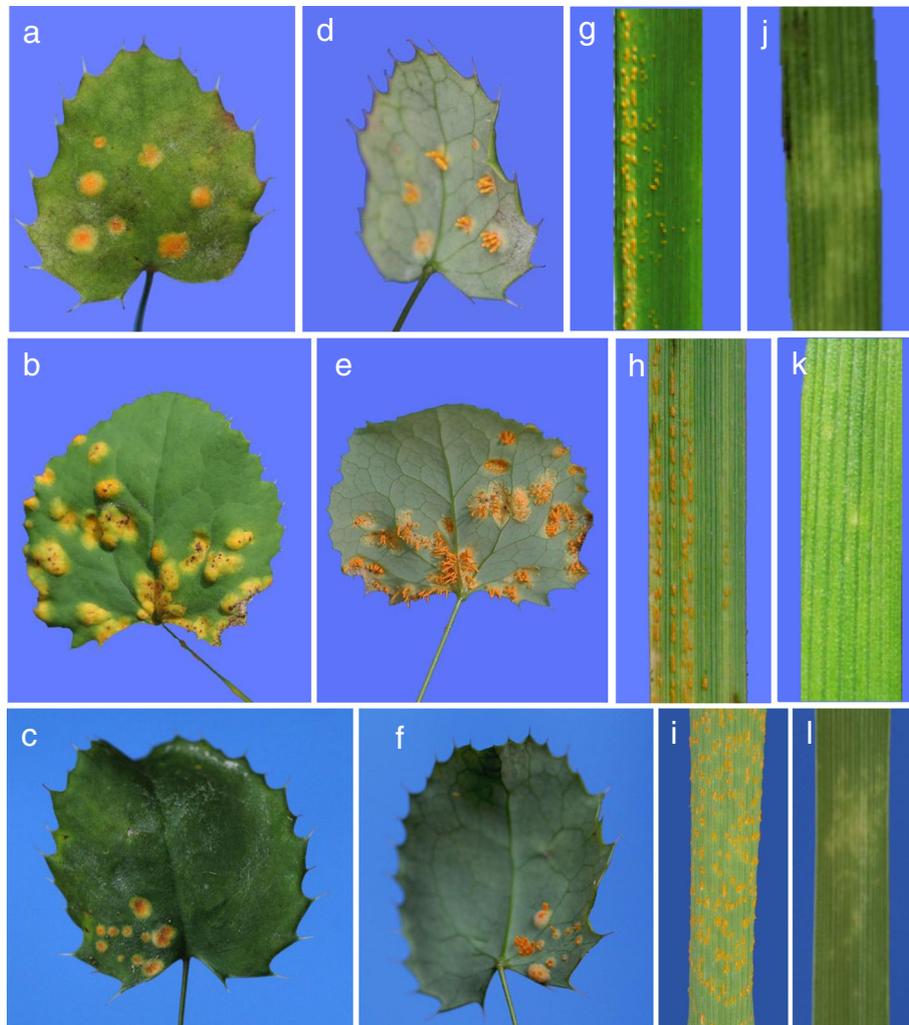


Fig. 4 Pycnial and aecial stages of each of formae speciales of *Puccinia striiformis* infecting *Berberis*. **a, b, c** Pycnial stage of *Puccinia striiformis* f. sp. *agropyri*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *hordei*, on barberry (*Berberis shensiiana*), respectively. **d, e, f** Aecial stage of *P. striiformis* f. sp. *agropyri*, *P. striiformis* f. sp. *elymi*, and *P. striiformis* f. sp. *hordei*, on barberry (*B. shensiiana*), respectively. **g, h, i** Aeciospore infections of the three formae speciales of *P. striiformis* on respective original hosts, *Elymus cylindricus*, *Agropyron cristatum*, and barley cv. Guoluo (*Hordeum vulgare* var. *nudum*), after inoculation, respectively. **j, k, l** Reaction of wheat cv. Mingxian 169 seedlings inoculated with aeciospores of each of the three forms

on rye, and *Psa* on *Agropyron*. Subsequently, four more forms of *P. striiformis*, including f. sp. *dactylidis*, f. sp. *poae*, f. sp. *leymi*, and f. sp. *pseudo-hordei*, were reported by several researchers (Manners 1960; Tollenaar 1967; Niu et al. 1991; Wellings et al. 2000). Based on host specialization of *P. striiformis*, f. sp. *hordei*, f. sp. *tritici*, and f. sp. *poae* and DNA markers, Chen et al. (1995) determined that the three forms were different from each other, but the wheat and barley forms were more related to each other than to the bluegrass form. The forma specialis level of *Pst* and *Psh* was also confirmed at the genome level (Xia et al. 2018). According to spore morphology and molecular data, Liu and Hambleton (2010) classified *P. striiformis* into four lineages, including *P. striiformis* on Triticeae, *P. striiformoides* on

Dactylis glomerata, *P. pseudostriiformis* on *Poa*, and *P. gansensis* on *Achnatherum inebrians*. For the first lineage, *P. striiformis*, has the widest host range in tribe Triticeae, including *Aegilops*, *Elymus*, *Hordeum*, and *Triticum*.

Wild grasses are the primary hosts for *P. striiformis* in the original center (Hassebrauk 1965). Rust fungi in Uredinales are speculated to experience profound changes in either morphological or biological traits a couple of million years ago (Anikster and Wahl 1979). This indicates that cereal rust pathogens, including *P. striiformis*, have evolved with cultivation of crops during dissemination of the rust pathogen from the original area to other regions (Stubbs 1985). Under the pressure of host selection, *P. striiformis* has been acquiring new

pathogenicity to produce different forms on crops and wild grass species. The present study may open the opportunity to study the genetic relationships between the different forms.

Conclusions

In the present study, we determined that barberries can serve as an alternate host for *P. striiformis* f. sp. *agropyri* on *Agropyron*, f. sp. *elymi* on *Elymus*, and f. sp. *hordei* on *Hordeum* based on host specialization, spore morphology, and artificial inoculation. The results showed that these forms have their own specific primary hosts and their basidiospores can infect barberry and produce pycnia and aecia. The resulted aeciospores of these forms can further infect their respective primary hosts but not wheat. Thus we showed that barberry can serve as an alternate host for these formae speciales of *P. striiformis*.

Methods

Plants

Plants and seeds of two grass species, *Agropyron cristatum* (L.) Gaertn. and *Elymus cylindricus* (Franch.) Honda, were collected in Tianshui, Gansu Province during September to October 2013, and cultivated in greenhouse. The two grass species were identified by Zhenhai Wu, a botanical researcher at College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, China (Additional file 3: Figure S3). Seeds of hulless barley cv. Guoluo (*Hordeum vulgare* L. var. *nudum* Hook) were kindly provided by Yuelin Peng at College of Plant and Pasture, Tibet University, Linzhi, Tibet, China. Young plants of barberry (*Berberis shensiiana* Ahrendt) were transplanted from Baoji, Shaanxi Province and grown in the greenhouse to produce new leaves for later use. Seeds of *A. cristatum*, *E. cylindricus*, barley cv. Guoluo, susceptible to the barley stripe rust pathogen (*P. striiformis* f. sp. *hordei*), and wheat cv. Mingxian 169, susceptible to all known races of *P. striiformis* f. sp. *tritici* (the causal pathogen of wheat stripe rust), were grown in pots filled with commercial mixed soil (Inner Mongolia Mengfei Biotech Co. Ltd., China). Two or three-leaf old wheat and barley cv. Guoluo seedlings and 5 or 6-leaf old grass seedlings were used for spore propagation and infection tests.

Inoculum production and host specialization testing

Stripe rust-infected leaf samples and plants of *A. cristatum* and *E. cylindricus*, were collected from Tianshui, Gansu in October, 2013 (Additional file 4: Figure S4). Leaf samples infected by the barley stripe rust fungus were collected in Linzhi, Tibet in June, 2013. To obtain pure stripe rust isolates of each of three forms of *P. striiformis*, a single uredinium produced on leaves of each of the two grass species and barley cv. Guoluo was picked to inoculate new leaves of the sample grass or barley cv.

Guoluo. To test host specialization, urediniospores of the single uredinium-derived isolates were inoculated on three to five leaves of each of the two grass species and barley cv. Guoluo, and wheat cv. Mingxian 169 seedlings, respectively. Simultaneously, race CYR32 of *P. striiformis* f. sp. *tritici* was used to inoculate seedlings of wheat cv. Mingxian 169 as a control in all the tests. Inoculated plants of grasses and wheat were incubated in the dark at 10 °C for 24 h in dew chamber (I-36D, Percival, U.S.A.), and then transferred to a growth chamber at 13 °C to 16 °C, and 16 h light and 8 h dark with light intensity of 8000 to 10,000 Lx. Reactions of the single uredinium isolates on grass and wheat plants were observed at 15 to 20 dpi according to a scale of 0 to 4 (Li and Shang 1989).

Teliospore preparation

To prepare teliospores of the stripe rust isolates on two grass species and barley cv. Guoluo, the urediniospore-infected plants were kept in a growth chamber under the same conditions as mentioned above. Leaves bearing telia were collected and kept in a desiccator for a couple of weeks to 1 month at room temperature for later use.

Morphological observation of urediniospores and teliospores

Based on the determination of each form of *P. striiformis* by tests of host specialization, morphological characteristics of urediniospores and teliospores were observed using a light microscope (DP72, Olympus, Japan). Size of urediniospores and teliospores were determined by measuring approximately 100 spores using software cellSens attached by the microscope apparatus.

PCR amplification and sequence analysis

To further determine *Agropyron* form and *Elymus* form of *P. striiformis*, genome DNA of urediniospores were extracted using a Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Tokyo, Japan) according to the manual operation guide. The genomic DNA were amplified with primers Rust2inv (Aime 2006) and ITS4 (White et al. 1990) in a PCR thermal cycler (Applied Biosystems, MA, USA), with *P. striiformis* f. sp. *tritici* race CYR32 as a positive control. Each of the targeted bands was cloned into a pMD-T simple vector (TaKaRa, Japan) after PCR products were electrophoresed in an agarose gel. The clones were sequenced by Yangling Tianrun Aoke Biotech Co. Ltd. Sequence alignment was performed among the two grass species forms of *P. striiformis* and *P. striiformis* f. sp. *tritici* race CYR32 using software DNAMAN (V 6.0, Lynnon). Sequences of the three forms of *P. striiformis* and that of the related *Puccinia* species retrieved from the NCBI database (<https://>

www.ncbi.nlm.nih.gov/) were used to construct phylogenetic tree for taxa.

Inoculation of rust fungi on barberry

To test whether barberry can serve as the aecial hosts for the three forms of *P. striiformis* on the grass species and barley cv. Guoluo, leaf segments bearing teliospores were placed on 2% (w/v) water agar medium in a Petri dish and kept at 16 °C in an incubator and periodically checked for teliospore germination. As teliospores began to germinate, a transparent cylinder made with an A4 paper-sized plastic film was used to enclose the young barberry plants and the dish was inverted to cover the top of the cylinder. The barberry plants were moved into an incubator for inoculation at 16 °C and kept there for 3 days before being moved to a growth chamber. Infections of the barberry plants were observed periodically.

Pathogenicity tests of aeciospores

Aeciospores were used to inoculate seedlings of wheat and grass species. The inoculated plants were incubated in a dew chamber after inoculation and then in a growth chamber under the same conditions as described above. Reactions of wheat and the three grass species were recorded at 15 to 20 dpi. The same scales as mentioned above were used to determine the pathogenicity of aeciospores.

Additional files

Additional file 1: Figure S1. Alignment of ITS sequence of *Puccinia striiformis* f. sp. *elymi*, *P. striiformis* f. sp. *agropyri*, and *P. striiformis* f. sp. *tritici*. (PPTX 67 kb)

Additional file 2: Figure S2. Alignment of ITS sequence of *Puccinia striiformis* f. sp. *elymi* and *P. striiformis* f. sp. *agropyri*. (PPTX 59 kb)

Additional file 3: Figure S3. Plants and heads of grass species collected in Tianshui, Gansu Province in 2013 used in this study. **a, b** *Agropyron cristatum* (L.) Gaertn. **c, d** *Elymus cylindricus* (Franch.) Honda. (PPTX 1859 kb)

Additional file 4: Figure S4. Natural production of uredinia and telia of *Puccinia striiformis* infecting two grass species. **a, b** *Agropyron cristatum*. **c, d** *Elymus cylindricus*. (PPTX 1184 kb)

Abbreviations

cv.: Cultivar; f. sp.: Forma specialis (pl. formae speciales); *Psa*: *Puccinia striiformis* f. sp. *agropyri*; *Pse*: *Puccinia striiformis* f. sp. *elymi*; *Psh*: *Puccinia striiformis* f. sp. *hordei*; spp.: Species

Acknowledgements

We thank Guorong Wei for providing urediniospores of race CYR32 of *Puccinia striiformis* f. sp. *tritici* used in this study. We also thank Dr. Chongjing Xia from Department of Plant Pathology, Washington State University, Pullman, US and Dr. Chunlei Tang from College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, China, for English language polishing.

Funding

This study was financially supported by National Key R&D Program of China (2018YFD0200500), the Natural Science Basic Research Plan in Shaanxi Province of China (2017JM3006), The Key Project of Science and Technology of Tibetan Autonomous Region, China (XZ201702NB15), the National Natural Science Foundation of China (31071641), and Program for New Century Excellent Talents in University (NCET-13-0490).

Availability of data and materials

Whole plants and seeds for these experiments were collected by Prof. Jie Zhao from Northwest A&F University. Fungal materials were provided by Institution of Plant Pathology, Northwest A&F University.

Authors' contributions

JZ and ZK designed the experiments of the study. SH, SZ, DZ, YL, and ZD conducted experiments. SH, ZK and JZ wrote the paper. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 28 October 2018 Accepted: 20 March 2019

Published online: 11 April 2019

References

- Abbasi M, Hedjaroude G, Scholler M, Goodwin SB. Taxonomy of *Puccinia striiformis* s. l. in Iran. *Rostaniha*. 2004;5(2):71–82 199–224.
- Aime MC. Toward resolving family-level relationships in rust fungi (Uredinales). *Mycoscience*. 2006;47(3):112–22.
- Anikster Y, Wahl I. Coevolution of the rust fungi on *Gramineae* and *Liliaceae* and their hosts. *Annu Rev Phytopathol*. 1979;17:367–403.
- Beresford RM. Stripe rust (*Puccinia striiformis*), a new disease of wheat in New Zealand. *Cereal Rusts Bull*. 1982;10(2):35–41.
- Boshoff WHP, Pretorius Z, van Niekerk BD. Establishment, distribution, and pathogenicity of *Puccinia striiformis* f. sp. *tritici* in South Africa. *Plant Dis*. 2002; 86(5):485–92.
- Brown JKM, Hovmøller MS. Epidemiology-aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*. 2002; 297(5581):537–41.
- Chen XM. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Can J Plant Pathol*. 2005;27(3):314–37.
- Chen XM, Line RF, Leung H. Virulence and polymorphic DNA relationships of *Puccinia striiformis* f. sp. *hordei* to other rusts. *Phytopathology*. 1995;85(11): 1335–42.
- Cheng P, Chen XM, See RD. Grass hosts harbor more diverse isolates of *Puccinia striiformis* than cereal crops. *Phytopathology*. 2016;106(4):362–71.
- Cummins GB. The rust fungi of cereals, grasses and bamboos. New York: Springer-Verlag New York Inc; 1971.
- Eriksson J. Über die spezialisierung der parasitismus bei den getreiderostpilzen. *Ber Deut Bot Ges*. 1894;12:292–331.
- Eriksson J, Henning E. Die Hauptresultate einer neuen Untersuchung über die Getreiderostpilze. *Z Pflanzenkr*. 1894;4:197–203.
- Hassebrauk K. Nomenklatur, geographische, Verbreitung und Wirtsbereich des Gelbrostes, *Puccinia striiformis* West. *Mitt Biol Bundesanst Land-Forstwirsch Berl-Dahl*. 1965;116:1–75.
- Hovmøller MS, Justesen AF, Brown JKM. Clonality and long-distance migration of *Puccinia striiformis* f. sp. *tritici* in north-West Europe. *Plant Pathol*. 2002;51(1): 24–32.
- Jia QZ, Huang Q, Cao SQ, Zhang B, Wang XM, Jin SL. Discovery of new stripe rust strain infected to China's major wheat resistant material Guinong 22 and its preliminary pathogenicity analysis. *Gansu Agr Sci Techn*. 2012;1:3–5 (in Chinese).
- Jin Y, Szabo L, Carson M. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* spp. as an alternate host. *Phytopathology*. 2010;100(5):432–5.
- Johnson T, Newton M, Brown AM. Hybridization of *Puccinia graminis tritici* with *Puccinia graminis secalis* and *Puccinia graminis agrostidis*. *Sci Agric*. 1932;13(3): 141–53.
- Kuang WJ, Zhang GS, Zhang ZY, Ji HL, Shen L, Ni JY, et al. Studies on virulence of *avrYr10/24/26/ch42* mutants of *Puccinia striiformis* f. sp. *tritici*. *Southwest Chin J Agr Sci*. 2013;26(6):2323–31 (in Chinese).
- Li ZQ, Shang HS. Wheat rusts and its control. Shanghai: Shanghai Scientific & Technical Publishers; 1989.

- Liu M, Hambleton S. Taxonomic study of stripe rust, *Puccinia striiformis* sensu lato, based on molecular and morphological evidence. *Fungal Biol.* 2010;114(10): 881–99.
- Liu TG, Peng YL, Chen WQ, Zhang ZY. First detection of virulence in *Puccinia striiformis* f. sp. *tritici* in China to resistance genes *Yr24* (=Yr26) present in wheat cv. Chuanmai 42. *Plant Dis.* 2010;94(9):1163.
- Manners JG. *Puccinia striiformis* Westend. var. *dactylidis* var. nov. *Trans Br Mycol Soc.* 1960;43(1):65–8.
- Murray GM, Ellison PJ, Watson A, Cullis BR. The relationship between wheat yield and stripe rust as affected by length of epidemic and temperature at the grain development stage of crop growth. *Plant Pathol.* 1994;43(2):397–405.
- Niu YC, Li ZQ, Shang HS. *Puccinia striiformis* West. f. sp. *leymi* and f. sp. *elymi*, two new formae speciales. *J Northwest A&F Univ (Nat Sci Ed).* 1991;19:58–62 (in Chinese).
- Rapilly F. Yellow rust epidemiology. *Annu Rev Phytopathol.* 1979;17:59–73.
- Stakman EC, Levin MN, Cotter RU. Origin of physiologic forms of *Puccinia graminis* through hybridization and mutation. *Sci Agric.* 1930;10(11):707–20.
- Stubbs RW. Stripe rust. In: Bushnell WR, Roelfs AP, editors. *The cereal rusts*, vol. II, diseases, distribution, epidemiology, and control. New York: Academic Press; 1985.
- Tian Y, Zhan GM, Chen XM, Tungruentragoon A, Lu X, Zhao J, et al. Virulence and simple sequence repeat marker segregation in a *Puccinia striiformis* f. sp. *tritici* population produced by selfing a Chinese isolate on *Berberis shensiana*. *Phytopathology.* 2016;106(2):185–91.
- Tollenaar H. A comparison of *Puccinia striiformis* f. sp. *poae* on bluegrass with *P. striiformis* f. sp. *tritici* and f. sp. *dactylidis*. *Phytopathology.* 1967;57(4):418–20.
- Wan AM, Chen XM, He ZH. Wheat stripe rust in China. *Aust J Agric Res.* 2007; 58(6):605–19.
- Wang L, Zhen D, Zuo SX, Chen XM, Zhuang H, Huang LL, et al. Inheritance and linkage of virulence genes in Chinese predominant race CYR32 of the wheat stripe rust pathogen *Puccinia striiformis* f. sp. *tritici*. *Front Plant Sci.* 2018;9:120.
- Wellings CR. *Puccinia striiformis* in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period 1979–2006. *Aust J Agric Res.* 2007; 58(6):567–75.
- Wellings CR, Burdon JJ, McIntosh RA, Wallwork H, Raman H, Murray GM. A new variant of *Puccinia striiformis* causing stripe rust on barley and wild *Hordeum* species in Australia. *Plant Pathol.* 2000;49(6):803.
- White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press; 1990.
- Xia CJ, Wang M, Yin C, Cornejo OE, Hulber SH, Chen X. Genomic insights into host adaptation between the wheat stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) and the barley stripe rust pathogen (*Puccinia striiformis* f. sp. *hordei*). *BMC Genomics.* 2018;19:664.
- Zadoks JC. Yellow rust on wheat studies in epidemiology and physiologic specialization. *Tijdschrift Over Plantenziekten.* 1961;67(3):69–256 <https://doi.org/10.1007/BF01984044>.
- Zadoks JC, van den Bosch F. On the spread of plant disease: a theory on foci. *Annu Rev Phytopathol.* 1994;32:503–21.
- Zhao J, Wang L, Wang ZY, Chen XM, Zhang HC, Yao JN, et al. Identification of eighteen *Berberis* species as alternate hosts of *Puccinia striiformis* f. sp. *tritici* and virulence variation in the pathogen isolates from natural infection of barberry plants in China. *Phytopathology.* 2013;103(9):927–34.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

