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ORIGINAL ARTICLE

Basidiospores of *Puccinia striiformis* f. sp. *tritici* succeed to infect barberry, while Urediniospores are blocked by non-host resistance

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Abstract Stripe rust (Yellow rust) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is a major disease of wheat worldwide. The use of resistant cultivars to control *Pst* has been very effective, low-cost, and ecologically sound. However, virulence patterns of *Pst* can quickly change, which may render resistant cultivars susceptible. The discovery of infection of *Berberis spp.* by basidiospores of *Pst* in 2010 raised important concerns about the evolution of new virulent races of the pathogen. Little is known about the infection process of *Berberis spp.* by basidiospores of *Pst* and the interaction between *Berberis spp.* and asexual urediniospores. In this study, the interaction between *Pst* urediniospores and *Berberis spp.* was investigated at histological and cytological levels. Our results indicate that *Berberis spp.* expresses a continuum of layered defenses comprised of structural and chemical changes in the cell wall as well as post-haustorial hypersensitive responses to urediniospore infection. Our study also re-examines in detail the infection process of *Pst* basidiospores on *Berberis spp.* and provides useful information for further research on the molecular mechanisms governing the interaction between *Berberis spp.* and *Pst*.

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Keywords Basidiospore · Barberry · Host resistance · Non-host resistance · *Puccinia striiformis* · Urediniospore · Wheat

Introduction

Puccinia striiformis f. sp. *tritici* (*Pst*) is one of the "Top 10" fungal pathogens based on its economic importance and potential utility in molecular plant pathology (Dean et al. 2012). It is an obligate biotrophic pathogen, with a specific lifestyle that slowly damages infected tissue of susceptible host plants. Asexual urediniospores are responsible for the most damaging disease "stripe rust (yellow rust) of wheat" (Bux et al. 2012; Chen 2005). After urediniospores adhere to the surface of a leaf and encounter permissive temperatures and humidity, a germ tube is produced which grows towards a stoma. After the germ tube produces an appressorium, the plant is invaded through the stomate and the fungus differentiates a series of infection structures, the substomatal vesicle (SV), primary infection hypha (PIH), haustorial mother cell (HMC), and finally a haustorium (H). Intercellular hyphae can grow throughout the leaf and form a network of mycelium within the mesophyll tissue. These hyphae form nutrient-absorbing haustoria, which are localized between the host cell wall and the plasma membrane. Taking up nutrients from its host through haustoria, the fungus forms sporogenic tissue, the uredinium, near the surface of the leaf and produces urediniospores, thus completing the asexual life cycle (Kang et al. 2002; Voegele et al. 2001; Wang et al. 2010; Zhang et al. 2012).

P. striiformis is a heteroecious fungus with five spore stages: pycniospores, aecidiospores, urediniospores, teliospores, and basidiospores. Prior to 2010, *P. striiformis* was known as a strictly asexual fungus causing epidemics. However, in 2010, the sexual stage was first observed on

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barberry plants (*Berberis* spp.) in Minnesota, USA, under greenhouse conditions (Jin 2011). Wang and Chen (2014) found that Oregon grape (*Mahonia aquifolium*), a species closely related to *Berberis spp.*, may also serve as an alternate host for *Pst*. Zhao et al. (2011, 2013) confirmed that some *Berberis* species, including *B. shensiana* Ahrendt used in this study, could be infected by *Pst* basidiospore under controlled greenhouse conditions. Wang et al. (2014) reported that *Berberis spp.* is essential for stem rust epidemics but apparently not for stripe rust under natural conditions in the US Pacific Northwest. The investigation of barberry plants as alternate host for *Pst* is just beginning, and many aspects of this interaction have not been revealed.

Non-host resistance (NHR) confers immunity of an entire plant species against all genotypes of a pathogen and is a common strategy for plants (Jones and Dangl 2006; Lipka et al. 2008; Thordal-Christensen 2003). Constitutive barriers form a first line of defense against a pathogen before penetration, including preformed physical and chemical barriers, such as cell wall and plant surface antimicrobial enzymes and secondary metabolites (Thordal-Christensen 2003). Post invasion, plants employ inducible defense mechanisms against further colonization by invading pathogens, for instance, formation of papillae beneath the penetration site. The oxidative burst, production of reactive oxygen species (ROS), primarily superoxide and H_2O_2, at the attempted penetration site, is one of the most rapid and effective defense reactions to pathogen attack (Apostol et al. 1989).

The specificity of reaction of different spore types and infection structures of *Pst* on different host plants has not been thoroughly investigated. In this study, the infection process of basidiospores on *Berberis spp.* and the interaction between urediniospores and *Berberis spp.* were examined by systematic histological and cytological analyses. Basidiospores were found to be capable of infecting detached leaves of *Berberis spp.* Urediniospores attempted infection of *Berberis spp.*, but this process was counteracted by structural and chemical modifications of the cell wall and by a post-haustorial hypersensitive response. Abnormal substomatal vesicles, primary infection hyphae, and haustorial mother cells developed, and few haustoria were formed inside *Berberis spp.* cells. This work provides a histological and cytological basis for future research on the molecular mechanisms of the interaction between *Berberis spp.* and *Pst*.

Material and methods

Plants, pathogens, and inoculation

Plants of *B. shensiana* were grown in a greenhouse. Wheat cultivar Mingxian169 (MX169) and Chinese *Pst* race CYR32 were used in this study. MX169 is susceptible to CYR32. Wheat plants were grown in a growth chamber at 20 °C with 60% relative humidity and a 16-h photoperiod, and 7- to 9-day-old wheat plants were used for *Pst* inoculation.

The adaxial surface of leaves of *Berberis spp.* were inoculated with basidiospores following methods described by Jin (2011), while detached leaves were placed on cotton wool containing distilled water in a petri dish (10 cm diameter). Petri dishes were placed in a growth chamber (20 °C with 60% relative humidity and 16/8 h day/night photoperiod). For inoculation with urediniospores, fresh *Pst* urediniospores suspended in water were applied with a fine paintbrush to the adaxial side of wheat leaves and the abaxial side of *Berberis spp.* Leaves were placed on cotton wool containing distilled water in a petri dish (10 cm diameter). The petri dishes were placed in a dark humidity chamber for 24 h at 12 °C and then moved to a growth chamber (20 °C with 60% relative humidity and 16 h/8 h day/night photoperiod).

Light microscopy

Infected barberry (1.5 × 1.5 cm = 2.25 cm^2) and wheat leaf pieces (0.5 × 2 cm = 1 cm^2) were harvested at pre-determined time points after inoculation, fixed and decolorized in ethanol/tri-chloromethane (3/1 *v*/*v*) containing 0.15% (*w*/*v*) trichloroacetic acid for 3 days, and then cleared in saturated chloral hydrate. For microscopic observations, leaf segments were stored in 50% glycerol and observed by differential interference contrast (DIC) optics.

To visualize pathogen structures, samples were stained with Calcofluor (Sigma-Aldrich, St. Louis, MO, USA) and wheat germ agglutinin (WGA) conjugated to Alexa 488 (Invitrogen, Carlsbad, CA, USA) (Ayliffe et al. 2011). Fluorescence-stained tissue was examined with a fluorescence microscope.

For each sample, at least 50 infection units from at least eight leaf segments were examined. An Olympus BX-53 microscope (Olympus Corporation, Tokyo, Japan) was used for all microscopic examinations.

Ultrastructural examination (TEM)

At 10 days post inoculation (dpi) (when chlorotic spots appeared on *Berberis spp.*) and 15 dpi (when pycnia appeared on *Berberis spp.*), leaves were removed for cytological analysis. Three leaves were harvested and prepared for TEM examination according to procedures previously described (Kang et al. 2002). Samples were cut into small pieces and fixed with 4% (*v*/*v*) glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) for 3–6 h at 4 °C. Samples were rinsed thoroughly with the same buffer, post-fixed with 1% (*w*/*v*) osmium tetroxide for 2 h at 4 °C, dehydrated in a graded alcohol series, embedded in gelatin capsules filled with LR White resin (Sigma-Aldrich), and polymerized at

60 °C for 48 h. For TEM observations, ultra-thin sections were cut with a diamond knife and collected on 200-mesh copper grids. After contrast staining with uranyl acetate and lead citrate, the tissue was examined with a JEM-1230 TEM (JEOL Co. Ltd., Tokyo, Japan) at 80 kV.

Histochemical analyses

H_2O_2 production in plant tissue was detected by staining with 3-3′ diaminobenzidine (DAB) (Amresco, Solon, OH, USA). Specimen preparations and microscopic observations were performed following procedures previously described (Thordal-Christensen et al. 1997; Wang et al. 2007). Callose deposition was visualized under UV light after modified aniline blue staining as described (Hood and Shew 1996). Leaf segments were fixed and cleared, washed twice with 50% (*v*/*v*) ethanol for 15 min, rinsed with water, incubated in 0.067 M K_2HPO_4 (pH 9.0) for 30 min, and stained overnight with 0.05% (*w*/*v*) aniline blue. Specimens were examined with an Olympus BX-53 microscope (Olympus Corporation). Autofluorescence of infected mesophyll cells was observed with an epifluorescence microscope (excitation filter, 485 nm; dichromic mirror, 510 nm; barrier filter, 520 nm).

Data analyses

DAB area and numbers of each location type at different times were collected from three biological replicates of DAB-stained tissue, each containing at least 30 infection units on three leaves and analyzed by SPSS version 6.0 (SPSS, Chicago, IL, USA). Data are presented as mean ± SE. Different letters in figures indicate significant difference ($P < 0.05$) as determined by Tukey's test.

Results

Development of basidiospores of *Pst* on the alternate host *Berberis*

Several hours after basidiospores of *Pst* were deposited on the surface of a *Berberis spp.* leaf, a germ tube emerged from each spore opposite the apiculus (Fig. 1a) and differentiated an appressorium at its apex (Fig. 1b). However, some germ tubes failed to directly form appressoria but continued elongating to a length several times the length of the spore. Germ tubes of basidiospores penetrated the epidermal layer directly. At the time of appressorium formation, disc-shaped papillae often formed inside the outer epidermal cell wall at points directly beneath the appressoria (Fig. 1a). However, growth of the fungus was not arrested and it further penetrated the host plasmalemma and formed an ovate intra-epidermal vesicle (Fig. 1c). Infection hyphae developed inside infected epidermal and neighboring cells (Fig. 1c) and also could be observed in the underlying palisade parenchyma cells and intercellular spaces (Fig. 1d). About four dai, several branches of primary hyphae developed and were separated from each other by septa (Fig. 1e). At six dai, secondary hyphae grew downward into the leaf tissue and differentiated secondary haustorial mother cells and haustoria (Fig. 1f). Two days later, a large radial network of hyphae was visible (Fig. 1g). Macroscopically, approximately 10 dai, clearly visible chlorotic spots appeared on the leave of *Berberis spp.* (Fig. 1h). At 13 dai, orange to yellow pycnia developed in clusters in varying numbers on the adaxial side of the leaf (Fig. 1i). Two days later, pycnia also appeared on the abaxial side of the leaf (Fig. 1j) and first aecidia were observed on the abaxial leaf side of *Berberis spp.* leaves.

Viewed with a TEM, mononucleate infection hyphae were observed in the intercellular space (Fig. 2a). Penetrated pegs were formed by intercellular hyphae (Fig. 2b). Haustorial mother cells and haustorial necks were closely associated with palisade parenchyma cells (Fig. 2c), and mononucleate haustoria were developed (Fig. 2d). After masses of hyphae gathered beneath the leaf surface (Fig. 2e), pycnia were formed, with pycniospores and paraphyses protruding through the leaf surface (Fig. 2f) and pyciniosporophores (Ps) arranged in parallel and new-born and mature pycniospores (Pa) separated by annular scar from the pyciniosporophores (Fig. 2h) in the pycnium (Fig. 2g).

Phenotypic reactions of wheat cultivar Mingxian169 and *Berberis spp.* in response to Urediniospores of *Pst* race CYR32

Phenotypes of *Pst* race CYR32 infection on *Berberis spp.* and wheat were clearly different. Wheat cultivar MX169 was susceptible to CYR32. Massive numbers of uredinia were produced on the wheat leaf surfaces of MX169 at 15 dpi (Fig. 3a). However, no uredinia were observed on *B. shensiana* leaves at 15 dpi (Fig. 3c).

Histological differences of host reactions and development of *Pst* in susceptible wheat and alternate host

On wheat, most urediniospores of *Pst* germinated and produced germ tubes 6 h post-inoculation (hpi). Some germ tubes grew towards stomata and penetrated them. Substomatal vesicle (Fig. 4a), the hallmark of successful invasion of the host, developed gradually up to 12 hpi. At 18 hpi, one to three primary infection hyphae and primary haustorial mother cells developed. The first infection structure contacting host mesophyll cells formed by 24 hpi (Fig. 4b). In further biotrophic development of *Pst*, haustoria took up nutrients from the invaded cells and then the pathogen produced numerous

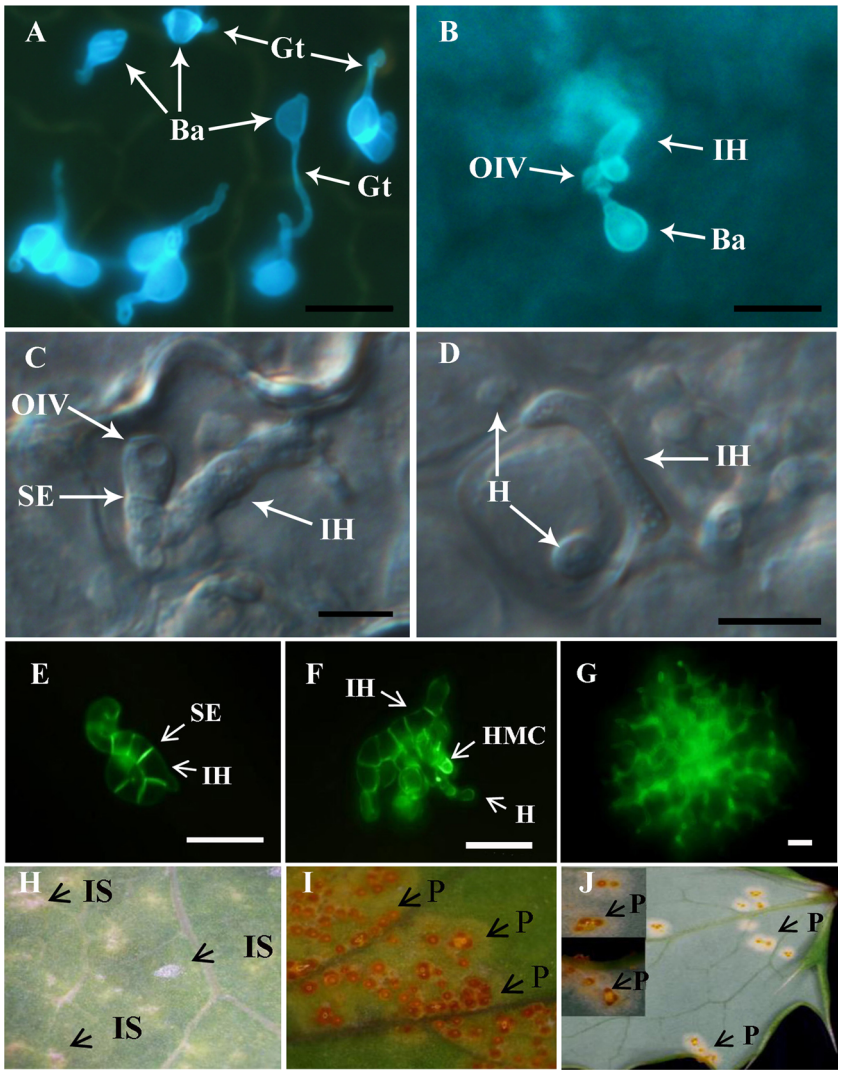

Fig. 1 Infection process of basidiospores of *Puccinia striiformis* f. sp. *tritici* on *Berberis spp.* **a** Each basidiospore produces a germ tube from the end opposite the apiculus. **b**, **c** After penetration, an ovate intra-epidermal vesicle (OIV) is formed in the host plasmalemma and primary infection hyphae (IH) develop. A septum (SE) is visible in **C** (**b** Calcofluor stain; **c** differential interference contrast). **d** Secondary infection hyphae (IH) with haustorial mother cells develop in the intercellular space, and haustoria (H) form in underlying palisade parenchyma cells (differential interference contrast). **e** At 4 dai, infection hyphae grow extensively, and septa (SE) are clearly visible (WGA stain). **f** At 6 dai, secondary hyphae have formed, growing further into the leaf tissue and differentiate secondary haustorial mother cells (HMC) and haustoria (WGA stain). **g** At 8 dai, a large radial hyphal network has developed (WGA stain). **h** At 10 dai, chlorotic spots are evident at the infection sites (IS). **i** At 13 dai, orange to yellow pycnia (P) accumulate in clusters on the abaxial side of the leaf. **j** At 15 dai, pycnia appear on the adaxial side of the leaf. Bars: 20 μm

secondary infection hyphae, haustorial mother cells, and haustoria by 4 dpi (Fig. 4c). Afterwards, the fungus developed an extensive network of hyphae and sporogenous tissue producing new urediniospores.

After adhesion and hydration, the majority of urediniospores produced germ tubes on barberry leaf surfaces. Germ tubes grew in random directions on the leaf surface, and only a small percentage formed appressoria over stomata (Fig. 4e, g). After an infection peg was produced penetrating into a stomate, a substomatal vesicle was formed within the substomatal cavity (Fig. 4f), from which infection hyphae developed (Fig. 4d). Substomatal vesicles were abnormal and incompletely filled (Fig. 4h). At almost all infection sites, development of *Pst* was blocked at the stage of haustorial mother cell formation. At very few sites, a haustorial mother cell formed at the tip of an infection hypha when it contacted a mesophyll cell at 18–24 hpi (Fig. 4d). A red, unidentified substance appeared in epidermal cells surrounding the invaded stoma (Fig. 4d–f).

Histochemical evaluation of *Berberis spp.* NHR response to *Pst*

To study the responses of *Berberis spp.* to *Pst*, DAB was used to detect the production of H_2O_2. Aniline blue was used to detect callose (1-3-β-D-glucose). The combined staining with WGA and DAB showed that invasion of *Pst* induced an H_2O_2 burst (Fig. 5d–f). Callose was be detected in epidermal cell walls at 96 hpi (Fig. 4g). After germination of urediniospores, germ tubes grew on the leaf surface and some encountered stomata and attempted to penetrate (Fig. 4e). After invasion, *Pst* formed substomatal vesicles within the stomatal cavity, some of which had an abnormal shape (Fig. 5i, j). Fungal development varied to some extent; some hyphae produced branches, but most of them were poorly developed and did not produce haustorial mother cells. In a few instances, substomatal vesicles formed short branches at the apex (Fig. 5i), and HMCs seemed to be formed directly at these branches (Fig. 5j). No development of haustoria was observed.

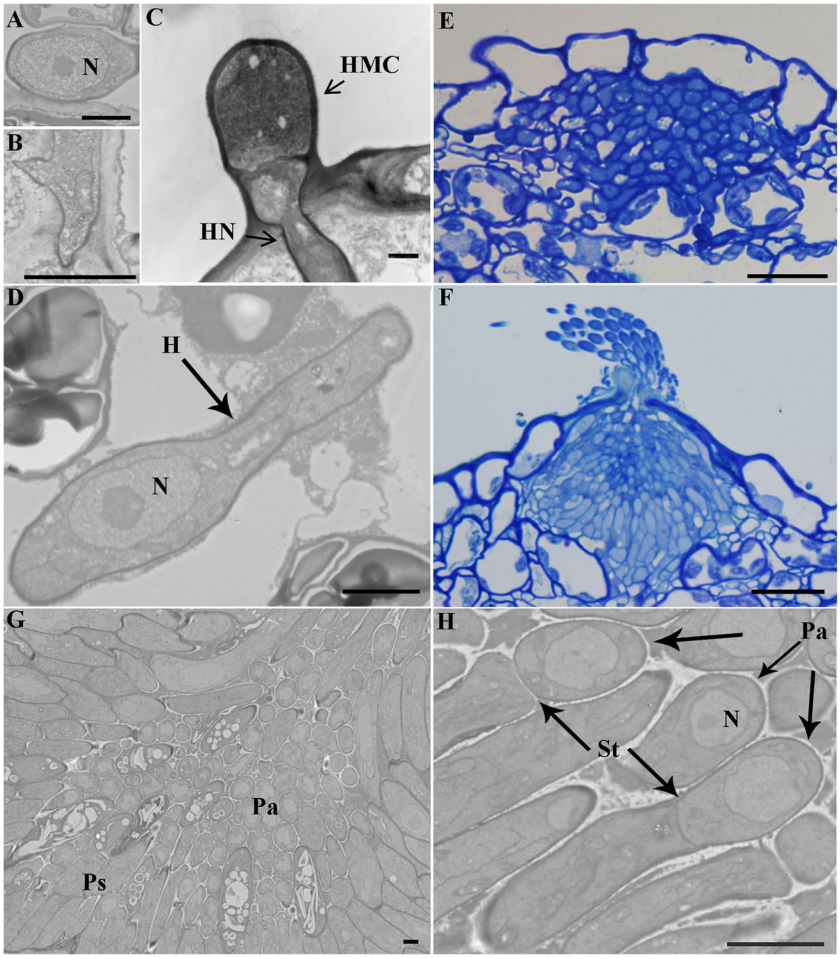

Fig. 2 Cytological observation of infection structures of basidiospores of *Puccinia striiformis* f. sp. *tritici* on *Berberis spp.* **a** Mononucleate infection hyphae can be observed in the intercellular space. **b** Penetration peg in papollar shape generated from intercellular hyphae. **c** Haustorial mother cell with haustorial neck closely associated with a palisade parenchyma cell. **d** Mononucleate haustorium inside a palisade parenchyma cell. **e** Mass intercellular hyphae clustered beneath the leaf surface and were about to form pycnium. **f** Mature pycnium formed with pycniospores and paraphyses protruding through the upper leaf surface. **g** Pyciniosporophores (Ps) arranged in parallel with newly formed and mature pycniospores (Pa) in the pycnium. **h** Mature pycniospores were separated from the pyciniosporophores by the annular scar (St). Bars: 2 μm

Based on the microscopic observations in the interaction of *Berberis spp.* and *Pst*, three types of H_2O_2 localization were revealed by DAB staining: type I: H_2O_2 was located only inside fungal cells (Fig. 5d); type II: H_2O_2 was detected in both fungal and plant cells (Fig. 5e); and type III: slight H_2O_2 was observed (Fig. 5f). The frequency of different types of H_2O_2 localization at certain times after inoculation was calculated (Fig. 6a). Obviously, interaction type II prevailed among the three types, comprising 73.2%, at 18 hpi; 67.1% at 24 hpi; and 43.6% at 48 hpi, respectively. Type III increased from 11.8% at 18 hpi to

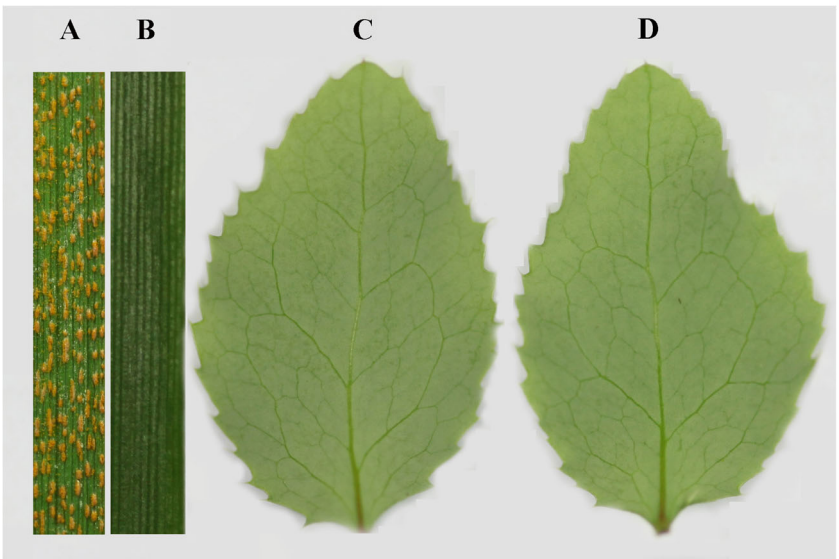

Fig. 3 Variable phenotypic reactions in different types of interactions at 15 dpi. **a** Orange uredinia on wheat cultivar MX169 in the interaction with urediniospores of *Puccinia striiformis* f. sp. *tritici* race CYR32. **b** Mock inoculated control. **c** Absence of visible symptoms on *Berberis shensiana* in the interaction with urediniospores of *Pst* race CYR32. **d** Mock inoculated control

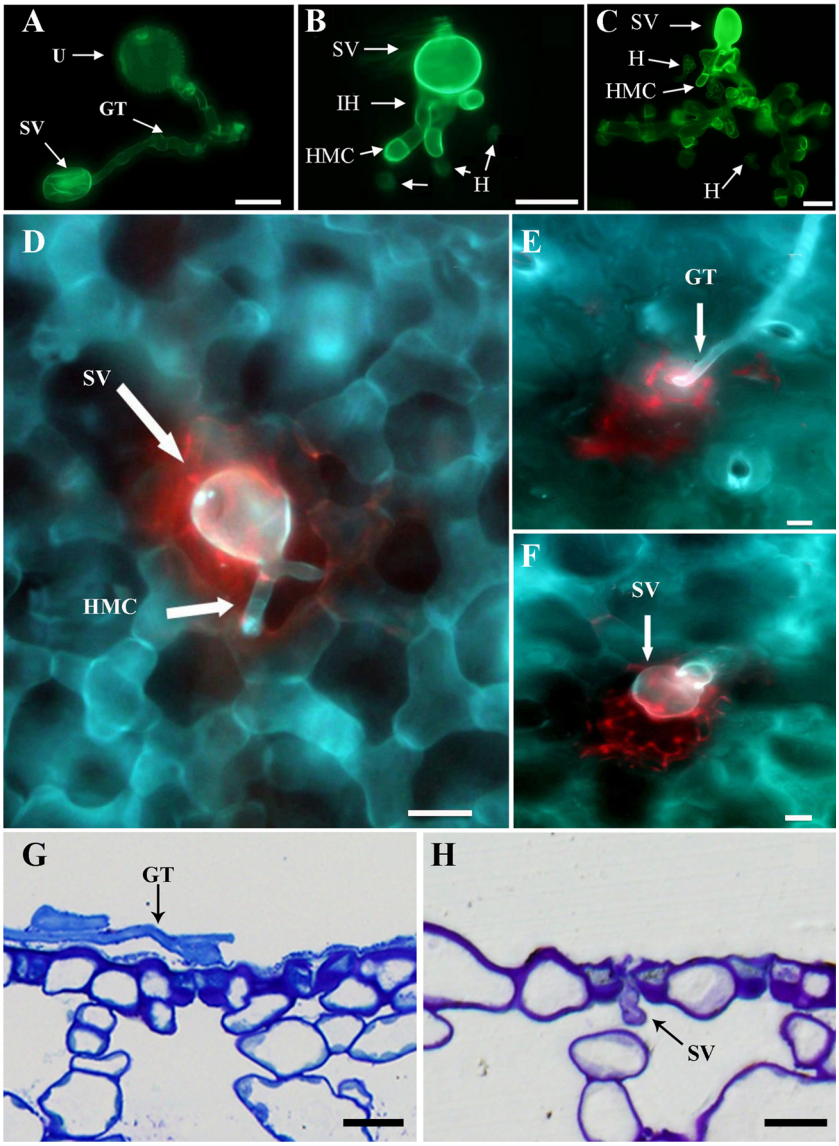

Fig. 4 Different responses of wheat cultivar MX169 (**a**–**c**) and *Berberis shensiana* (**d**–**h**) after inoculation with urediniospores (U) of *Puccinia striiformis* f. sp. *tritici* race CYR32 observed by light microscopy. **a** CYR32 forms a substomatal vesicle (SV) on wheat at 12 hpi. **b** Primary infection hyphae (IH) develop from the SV, and haustorial mother cells (HMCs) and haustoria (H) are formed by 24 hpi. **c** CYR32 extends from the infection site and forms secondary IH, HMC, and H, resulting in a large hyphal network by 4 dpi. **d** CYR32 forms infection structures, such as SV, IH, HMC, but does not form H. A reddish, unidentified material is visible at the attacked and neighboring epidermal cells at 24 hpi. **e** A germ tube grows over a stomatal opening on *Berberis spp.* and attempts to penetrate. **f** A SV forms in the substomatal cavity at 24 hpi. **g** A germ tube produced by a urediniospore grows towards a stomate and forms an appressorium. **h** Abnormal SV formed in the substomatal chamber. Specimens in **a** and **b** were stained with WGA and observed under a fluorescence microscope. Specimens in **c**–**f** were stained with Calcofluor and observed under a fluorescence microscope. **g** and **h** were observed under a light microscope. Bars: 20 μm

25.5% at 24 hpi but did not increase further at 48 hpi (27.7%). Type I decreased from 15.0% at 18 hpi to 7.5% at 24 hpi, then increased again to 28.7% at 48 hpi. Areas stained by DAB (Fig. 6b) gradually increased from 30,504.26 pixel^2 (18 hpi) to 45,411.75 pixel^2 (24 hpi) and finally 79,708.11 pixel^2 (48 hpi).

Discussion

After the sexual stage of *Pst* was first found on barberry plants (*Berberis* spp.) in Minnesota, USA, in 2010 (Jin 2011), further studies confirmed that Oregon grape (*M. aquifolium*) also can serve as an alternate host for *Pst* (Wang and Chen 2014) and some *Berberis* species in China may also be infected by *Pst* (2011; 2013). All those experiments were performed under controlled greenhouse conditions, and whole plants were inoculated. However, this increases the difficulty of microscopic observation of infection sites. In this study, we inoculated detached leaves, thus providing better control of conditions, shorter time, and more precise observations and reproducible results.

Furthermore, the specificity of different types of spores of *Pst* for different host plant species has not been thoroughly investigated. In this study, the infection process of basidiospores on *Berberis spp.* and the interaction between urediniospores and *Berberis spp.* were subjected to histological and cytological analyses.

B. shensiana is susceptible to basidiospores of *Pst* CYR32

The infection process of *Pst* basidiospores on *Berberis spp.* is similar to the infection process of basidiospores of *P. graminis* f. sp. *tritici* (Allen et al. 1991), *Uromyces appendiculatus* (Gold and Mendgen 1984), and *Gymnosporangium juniperi-virginianae* (Nusbaum 1935). After penetration of the cell wall,

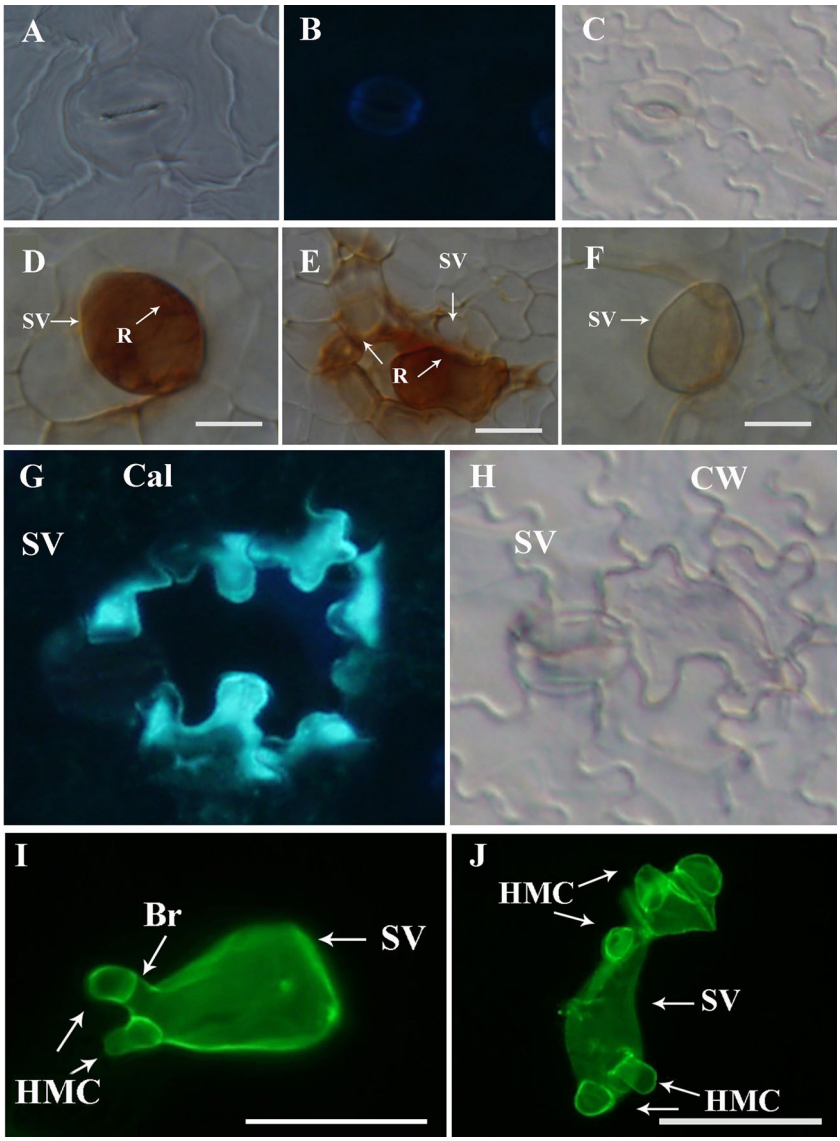

Fig. 5 Response of *Berberis shensiana* to attempted infection by *Puccinia striiformis* f. sp. *tritici*. (**a**–**c**) Non-inoculated *Berberis spp.* leaves were stained with DAB (**a**) and aniline blue (**b**–**c**). **a** No H_2O_2 burst were detected near the stoma. **b, c** No callose deposition was detected in epidermal cell. **d**–**f** Inoculated *Berberis spp.* leaves were stained with DAB at 18 hpi. Three different types of H_2O_2 localization. **d** Solely inside an invaded cell. **e** Inside an invaded cell and in neighboring cells. **f** No H_2O_2 production inside the invaded cell but minimal production in neighboring cells. **g**, **h** Callose (Cal) deposition detected in epidermal cell walls (CW) adjacent to the infected stoma at 96 hpi. **i, j** Multiple shapes of infection structures stained with WGA at 24 hpi. **i** HMCs at the apex of extremely short and thin branches. **j** SV and HMCs without branches. Bars: 20 μm

primary hyphae develop in epidermal cells. Each primary hypha gives rise to a branch producing secondary intercellular hyphae that grow into the sub-epidermal tissue. However, about nine more days are required for pycnia to appear, considerably longer than 4 days required for *P. graminis* f. sp. *tritici* (Allen et al. 1991). When pycniospores of one mating type are transferred to a pycnium of the opposite mating type, small granules appear in the paraphyses. Aecidia are produced with several aecidiospores inside the cluster cup (Allen et al. 1991). In our study, relatively few aecidia were produced. The nutritional condition of detached leaves may be a critical consideration for *Pst* development.

In this study, we found that *Pst* basidiospores germinate apically and laterally. In contrast, *U. appendiculatus* basidiospores germinate mainly apically (Gold and Mendgen 1984), whereas *G. juniperi-virginianae* basidiospores germinate laterally (Mims 1977). Some other species do not exhibit a specific location of germ tube emergence (Gray et al. 1983; Metzler 1982). Our results indicate that single, unbranched germ tubes usually are produced on the leaf surface, similar to previous reports for *U. appendiculatus* (Gold and Mendgen 1984), *G. asisticum* (Kohno et al. 1977), *G. fuscum* (Metzler 1982), and *Cronartium quercuum* f. sp. *fusiforme* (Gray et al. 1983; Jacobi et al. 1982). However, some spores with two or more branched germ tubes were observed on water-agar medium (data not shown). Appressoria are formed at the tip of the short germ tubes.

B. shensiana expresses "non-host" resistance to urediniospores of *Pst*

The leaves of *Berberis spp.* and wheat display different surface features, such as the frequency and size of stomata, and wax crystals (Koch et al. 2009). These surface features all affect

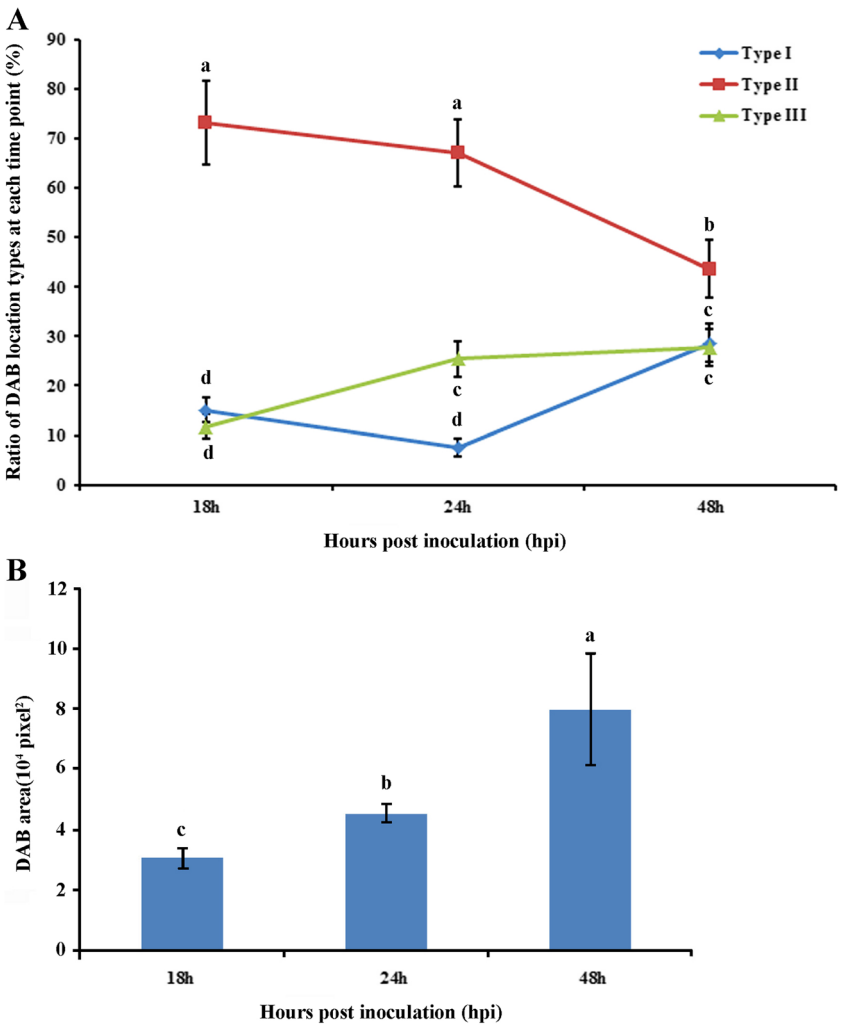

Fig. 6 H_2O_2 levels in the interaction of *Berberis spp.* and *Pst* urediniospores. **a** The graph shows the frequency of three types H_2O_2 localization at 18, 24, and 48 hpi. Type I shows DAB staining inside the invaded cell alone. Type II shows DAB staining inside the invaded as well as in neighboring cells. Type III shows slight H_2O_2 production inside the invaded cell and only slight production in neighboring cells. **b** Area of H_2O_2 production at 18, 24, and 48 hpi. Different *letters* indicate significant difference ($P < 0.05$) as determined by Tukey's test

fungal development (Allen et al. 1991). This may explain our finding of higher frequencies of infection by urediniospores on the abaxial surface compared to that on adaxial surfaces. Although no significant decrease was observed in the frequency of penetration of *Berberis spp.* (abaxial surface) compared with that in wheat, penetration frequencies of urediniospores of *Pst* on other non-host plants are very low (Cheng et al. 2013, 2012). Therefore, control of pre-invasion entry does not appear to be a major component of NHR in the interaction of *Berberis spp.* and *Pst* urediniospores.

The term "early abortion" has been used to indicate arrested growth just after the formation of the first infection hyphae and few haustorial mother cells (Niks 1982). Typical shapes of early aborted colonies are sparsely branched infection hyphae and one to three HMCs (Niks 1983b). Colonization after infection by urediniospores was halted before the haustorium formation stage in *Berberis spp.*, which is similar to previous studies of other non-host interactions of rust fungi (Heath 1974; Heath1979; Hoogkamp et al. 1998; Niks 1983a, b). Several strong defense responses in *Berberis spp.* were induced, including callose deposition and ROS accumulation. These defense responses are common in other non-host interactions of rust fungi (Heath 1974). Thus, these responses may be responsible for limiting further growth of unadapted fungal pathogens. The phenomenon of deformed infection structures indicates that, after penetration, the development of *Pst* within the substomatal chamber is severely challenged by the plant defense system. No visible symptoms on *Berberis spp.* leaves appeared, and these were therefore classified as type I NHR (Schulze-Lefert and Panstruga 2011). However, under microscopic examination, a few areas of HR and cell death appeared. Some differences in ROS responses were evident between the interaction of *Pst* with the alternate-host plant (*Berberis spp.*) and *Pst* with the host plant and non-host plants. Nearly 89% of total infection units produced an H_2O_2 burst at 18 hpi (Fig. 5). This is remarkably higher than in the interaction of *Pst* with a susceptible host (Wang et al. 2007). Furthermore, H_2O_2 bursts appear much earlier in the interaction of *Pst* and the alternate-host *Berberis spp.* H_2O_2 was detected when the substomatal vesicle was formed. Heath (1979) stated that "non-host responses typically occur during the earliest stages of infection." The intensity,

duration, and localization of ROS reflect different ROS signals of the plant cell (Mittler et al. 2004). The location of H_2O_2 is also interesting. The H_2O_2 burst was often observed in the fungus itself, but in the interaction of *Pst* with the host plant wheat, and non-host plants, such as rice (Yang et al. 2014), broad bean (Cheng et al. 2012), and *Arabidopsis thaliana* (Cheng et al. 2013), the H_2O_2 burst was not detected in *Pst* itself. The attempted infection by urediniospores on *Berberis spp.* induced the production of ROS, callose deposition, and HR. The hyphae that developed were blocked at the post-haustorial stage, similar to the NHR Type I. However, little difference in ROS responses was observed in *B. shensiana* compared with that in other non-host plants, such as broad bean, rice, and Arabidopsis. Further work is required to determine why different spore stages of the same rust pathogen behave differently on two hosts.

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Authors' contributions JuG and ZK designed experiments; MJ performed the experiments; CT and LW analyzed the data; HZ and JiG joined the discussion and gave the original ideas; and MJ, JuG, and ZK wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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