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Evidence of Occurrence of Barley Crown Rust Caused by Puccinia coronata var. hordei and Sexual Reproduction of the Pathogen Under Field Conditions in China

Xiaxia Tian,[1] Qiang Yao,[2] Zedong Zhang,[1] Xiangrui Cheng,[1] Jianfeng Qin,[1] Zhensheng Kang,[1,†] and Jie Zhao,[1,†]

[1] Key State Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China
[2] Institute of Plant Protection, Qinghai Academy of Agriculture and Forestry Sciences, Xining 810016, China

Abstract

Crown rust of barley, caused by *Puccinia coronata* var. *hordei*, was first reported by Jin and Steffenson in 1992, and the fungus has been reported only in the United States and Hungary. In China, stripe, stem, and leaf rusts have been reported on barley, but not for crown rust. Recently, a sample (HZJ0004) of rust collected from barley in Qilian county in Qinghai, China, appeared different from the three rusts based on color, size, arrangements of uredinia and/or telia. Teliospores had crown-shaped appendages on the top. Based on the disease symptoms and morphology of urediniospores and teliospores, the fungus was identified as *P. coronata* var. *hordei*. Using the internal transcribed spacer sequences, the isolates HZJ0004 from barley and POR3 from buckthorn (*Rhamnus* sp.) were clustered in one clade with *P. coronata* var. *hordei* isolates from barley and *Elymus repens* but in a different clade from the isolate POC8 from wild oat and the varieties of *P. coronata* from oats and grasses. At the seedling stage, most of the tested cultivars of barley and rye were susceptible to *P. coronata* var. *hordei* isolates HZJ0004 and POR3, but the cultivars of oats, triticale, wheat, and most grasses of genera *Aegilops*, *Brachypodium*, *Bromus*, *Calamagrostis*, *Deschampsia*, *Elymus*, *Festuca*, and *Phleum* were resistant, indicating their host specialization on barley. To our knowledge, this is the first report of crown rust on barley in China.

Keywords: crown rust, *Hordeum vulgare*, life cycle

Barley (*Hordeum vulgare* L.), a member of the genus *Hordeum* in the family Gramineae (Lu 1996), is one of the most important small grain cereals, ranking fourth after wheat, corn, and rice (Food and Agriculture Organization of the United Nations 2013). Barley is grown on approximately 70 million hectares in the world (Akar et al. 2004). Hulless barley (*H. vulgare* var. *nudum*), called Qingke and cultivated mainly in the Qinghai-Tibetan Plateau and in other areas of northwestern and southwestern China, is a staple food grain for the people in Tibet and also as a beverage and animal feed.

Rust diseases of barley are important factors influencing barley production in many parts of the world (Dickson 1956; Griffey et al. 1994; Harder and Dunsmore 1991; Jin and Steffenson 1997, 1999; Jin et al. 1992; Stubbs 1985; Wellings 2011; Woldeab and Alemayhu 2001). They are caused by specific species of the genus *Puccinia*, including stripe rust caused by *P. striiformis* f. sp. *hordei* (Stubbs 1985), leaf rust by *P. hordei* (Jin and Steffenson 1997), stem rust by *P. graminis* f. sp. *tritici* (Luig 1985), and crown rust by *P. coronata* var. *hordei* (Jin and Steffenson 1999).

Crown rust, caused by *P. coronata sensu lato*, is an important disease infecting oat (Simons 1985) and can also infect barley, a minority of wheat cultivars (Jin et al. 1992; Niu et al. 2014), and some grasses (Jin and Steffenson 1999; Simons 1985). Barley crown rust was first found in 1991 in the United States and reported in 1992 by Jin et al. (1992), and in 1999, the causal pathogen of the disease was identified as a new variety of *P. coronata* and designated as *P. coronata* Corda var. *hordei* Jin & Steffenson based on morphological differences and host specificity of the fungus (Jin and Steffenson 1999). Based on molecular and morphological data, the scientific name of the pathogen attacking genera *Elymus* and *Hordeum* was changed to *P. coronati-hordei* (Liu and Hambleton 2013). Before the present study, barley crown rust had been reported in the United States and Hungary, but not in other parts of the world. Although crown rust does not cause significant yield loss, it is still considered potentially destructive in barley-producing regions (Jin and Steffenson 1999, 2002; Jin et al. 1992; Niks et al. 2013).

In China, stripe rust, leaf rust, and stem rust have been reported (Wang et al. 1988; Xu and Cai 1979; Zhuang 1985). In September 2013, a rust disease, different from the three rusts, was found in a barley field (cultivar unknown) in Binggou village (38°10.029′ N, 100°12.580′ E, elevation 2,861 m), Babao town, Qilian county in Qinghai Province of China. The disease incidence and severity were approximately 1 and 10%, respectively. The rust appeared like crown rust, but there was no report of crown rust on barley in China. Therefore, the objectives of this study were to identify the causal pathogen of this rust disease based on morphological observation, host specificity testing, and phylogenetic analysis of the rust fungus and to determine the life cycle.

Materials and Methods

Fungal isolates and plants. Leaf samples bearing rust pustules were collected from a barley field in Qinghai Province at the milk stage in September 2013 when the disease was found and from a buckthorn (*Rhamnus* sp.) plant in the same location in June 2019. Leaf samples of oat crown rust were collected from wild oat (*Avena fatua* L.) in Fengshui village, Luoyu town, Xihe county of Gansu Province in October 2018. After drying, the leaf samples were kept in paper envelopes and stored in a desiccator at 4°C until use.

Seeds of barley, oat, rye, wheat, and gramineous grasses, listed in Table 1, were used in this study. Five to 10 seeds of each cereal cultivar or grass were planted in a plastic pot (7 cm × 7 cm × 8 cm) filled with potting mix (Inner Mongolia Mengfei Biotech Co., Ltd., Hohhot, Inner Mongolia, China) and grown in a rust-free growth chamber. Seedlings of cereals and 1-month-old grass plants were used for inoculation.

†Corresponding authors: J. Zhao; jiezhao@nwsuaf.edu.cn, and Z. S. Kang; kangzs@nwsuaf.edu.cn

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Table 1. Evaluation of pathogenicity of *Puccinia coronata* var. *hordei* isolates (HZJ0004 and POR3) and a *P. coronata* var. *avenae* isolate (POC8) to barley, oat, rye, triticale, wheat, and gramineous grasses at seedling stage in the greenhouse

				Infection type[a]		
				Puccinia coronata var. *hordei*		*P. coronata* var. *avenae*
Cereal	**Entry**	**Cultivar**	**Origin**	**HZJ0004**	**POR3**	**POC8**
Barley	PN-001	Favorit	Gansu	;	4	;
	PN-002	Ganken 5	Gansu	4	4	0
	PN-003	Ganpi 2	Gansu	4	4	0, ;
	PN-004	Ganpi 3	Gansu	4	4	0, ;
	PN-005	Ganpi 4	Gansu	4	4	0, ;
	PN-006	Ganpi 5	Gansu	4	4	0
	PN-007	Ganpi 6	Gansu	4	4	0, ;
	PN-008	Ganpi 7	Gansu	;	4	0
	PN-009	Kenpi 6	Gansu	4	–[b]	0, ;
	PN-010	Kenpi 7	Gansu	4	4	0
	PN-011	Baiyingzi hulless barley	Qinghai	4	4	0
	PN-012	Beiqing 1	Qinghai	4	4	0
	PN-013	Chaiqing 1	Qinghai	2	1	0, ;
	PN-014	Changhei hulless barley	Qinghai	3	4	0
	PN-015	Datong zisileng hulless barley	Qinghai	4	4	0
	PN-016	Diqing 4	Qinghai	3	–	0
	PN-017	Duanbai hulless barley	Qinghai	3	4	0
	PN-018	Dulihuang	Qinghai	4	4	0
	PN-019	Erchang siduan hulless barley	Qinghai	X	4	0
	PN-020	Erdaomeizi hulless barley	Qinghai	3	4	0, ;
	PN-021	Ganqing 4	Qinghai	4	4	0
	PN-022	Hualong zisi hulless barley	Qinghai	3	4	0, ;
	PN-023	Huzhuhei hulless barley	Qinghai	3	4	0
	PN-024	II-I CK Guoluo	Qinghai	4	–	–
	PN-025	Kunlun 1	Qinghai	3	4	0, ;
	PN-026	Kunlun 10	Qinghai	3	4	0, ;
	PN-027	Kunlun 12	Qinghai	4	4	0, ;
	PN-028	Kunlun 14	Qinghai	4	4	0
	PN-029	Kunlun 15	Qinghai	2	4	0, ;
	PN-030	Kunlun 3	Qinghai	3	4	0, ;
	PN-031	Leduhei hulless barley	Qinghai	4	4	0, ;
	PN-032	Ludian hulless barley	Qinghai	4	4	0
	PN-033	Nixi zhengge	Qinghai	4	–	0
	PN-034	Qing 0048	Qinghai	4	4	0, ;
	PN-035	Qing 0050	Qinghai	4	4	0, ;
	PN-036	Qing 0083	Qinghai	4	–	0
	PN-037	Qing 0101	Qinghai	4	4	0
	PN-038	Qing 0109	Qinghai	4	3	0, ;
	PN-039	Qing 0121	Qinghai	4	4	–
	PN-040	Qing 0162	Qinghai	3	3	0
	PN-041	Qing 0165	Qinghai	4	4	0
	PN-042	Qing 0191	Qinghai	4	4	;
	PN-043	Qing 0230	Qinghai	4	4	0
	PN-044	Qing 0355	Qinghai	3	–	0, ;
	PN-045	Qing 444	Qinghai	4	4	;
	PN-046	Qingqing 97-9	Qinghai	2	2	–
	PN-047	Qingyong 119	Qinghai	4	4	0, ;
	PN-048	Qingyong 142	Qinghai	4	4	0, ;
	PN-049	Silenghei hulless barley	Qinghai	3	4	0
	PN-050	Tangdui Xiaochun hulless barley	Qinghai	4	3	0, ;
	PN-051	Unknown cultivar 1	Qinghai	3	3	0
	PN-052	Unknown cultivar 2	Qinghai	1	2	0
	PN-053	Xiaozhongdian Duanbai hulless barley	Qinghai	4	4	0
	PN-054	Xunhuazi hulless barley	Qinghai	4	4	0
	PN-055	Zang 62	Qinghai	0, ;	;	0
	PN-056	Zangqing 2000	Qinghai	4	3	0, ;
	PN-057	Xiyin 2	Shaanxi	4	–	–
	PN-058	Ganzi 813	Tibet	4	4	;
	PN-059	Guoluo	Tibet	4	4	0
	PN-060	Jiangdian e'chui	Tibet	4	–	0
	PN-061	Kangqing 1	Tibet	1	–	–

(*Continued on next page*)

[a] Infection type was scored according to the 0 to 4 scale described by Jin and Steffenson (1999), where 0 = immune, ; = necrotic flecking without sporulation, 4 = the most susceptible infection type, and 1, 2, and 3 indicate intermediate types based on uredinium size and degree of associated necrosis and chlorosis. "X" denotes mixed reactions on the same plant. Infection types 3 and 4 were susceptible, and infection types ;, 1, 2, and X were resistant.
[b] Unknown or missing data.
[c] Seed source: the Germplasm Bank of Wild Species in Southwest China (GBWS).
[d] Seeds were collected from the south campus of Northwest A&F University (NWAFU).

Table 1. (*Continued from previous page*)

Cereal	Entry	Cultivar	Origin	Infection type[a]		
				Puccinia coronata var. *hordei*		*P. coronata* var. *avenae*
				HZJ0004	POR3	POC8
	PN-062	Kangqing 2	Tibet	4	4	0
	PN-063	Kangqing 7	Tibet	1	–	–
	PN-064	Lianga Mubai hulless barley	Tibet	4	–	0, ;
	PN-065	Lianga Mubaichang hulless barley	Tibet	4	3	0, ;
	PN-066	Longzi Hei hulless barley	Tibet	3	3	;
	PN-067	Pengnai Gabu	Tibet	4	3	0, ;
	PN-068	QB 24	Tibet	4	4	;
	PN-069	QB 27	Tibet	4	3	0, ;
	PN-070	QB 28	Tibet	3	4	0, ;
	PN-071	QB 9	Tibet	4	4	;
	PN-072	Xinduqiao hulless barley	Tibet	3	2	0
	PN-073	Zangqing 14	Tibet	0, ;	;	0
	PN-074	Zangqing 23	Tibet	3	4	;
	PN-075	Zangqing 25	Tibet	4	4	0, ;
	PN-076	Zhigong hulless barley	Tibet	4	4	;
	PN-077	Zhikong Gaxia	Tibet	3	–	;
	PN-078	Zuzhuo hulless barley	Tibet	;	–	0
	PN-079	Bowman	–	X	–	–
	PN-080	Gold Promise	–	2	–	–
	PN-081	Morex	–	X	–	–
	PN-082	Steptoe	–	3	–	–
	PN-083	Topper	–	4	4	0
	PN-084	*Hordeum brevisubulatum*	–	3	2	;
Oat	PN-085	Baiyan 7	Qinghai	;	0	2
	PN-086	Qinghai sweet oat	Qinghai	;	0	4
	PN-087	Qingyan 1	Qinghai	;	0	4
	PN-088	*Avena fatua* L.	–	;	0	4
Rye	PN-089	*Secale cereal*	Qinghai	4	4	0
Triticale	PN-090	*Tritium* × *Secale*	Shaanxi	;	;	0
Wheat	PN-091	2015 Pingbi II	Gansu	;	0	0
	PN-092	2015 Pingbi III	Gansu	;	0	0
	PN-093	Lanhangxuan 121	Gansu	;	0	0
	PN-094	Lanhangxuan 122	Gansu	;	0	0
	PN-095	Qian 120402	Guizhou	;	0	0
	PN-096	Qian 140862	Guizhou	;	0	0
	PN-097	Qian 140774	Guizhou	;	0	0
	PN-098	Qian 140838	Guizhou	;	0	0
	PN-099	Qian 140908	Guizhou	0	0	0
	PN-100	Ningchun 4	Ningxia	;	0	;
	PN-101	Yong 1341	Ningxia	;	0	0
	PN-102	Abbondanza	Qinghai	;	0, ;	0
	PN-103	Gaoyuan 158	Qinghai	;	0	0
	PN-104	Gaoyuan 182	Qinghai	;	0	0
	PN-105	Gaoyuan 363	Qinghai	;	0	0
	PN-106	Gaoyuan 448	Qinghai	;	0	0
	PN-107	Gaoyuan 506	Qinghai	;	0	0
	PN-108	Huzhu 13	Qinghai	;	0	0
	PN-109	Huzhuhong	Qinghai	;	0	0
	PN-110	Lantian 15	Qinghai	;	0, ;	0
	PN-111	Moyin 2	Qinghai	;	0, ;	0
	PN-112	Qingchun 39	Qinghai	;	0	0, ;
	PN-113	Qingchun 533	Qinghai	;	0	0
	PN-114	Qingmai 4	Qinghai	;	0	0
	PN-115	Mingxian 169	Shaanxi	;	0	0
	PN-116	Pubing 201	Shaanxi	;	0	0
	PN-117	Pubing 297	Shaanxi	;	;	0
	PN-118	Pubing 326	Shaanxi	;	–	0
	PN-119	Pubing 49	Shaanxi	;	0	0
	PN-120	Xinong 109	Shaanxi	;	0	0
	PN-121	Xinong 136	Shaanxi	0, ;	0	0, ;
	PN-122	Xinong 9106	Shaanxi	;	0	0
	PN-123	Mianmai 1403	Xinjiang	;	0, ;	0
	PN-124	Mianmai 52	Xinjiang	;	0	0
	PN-125	Xindong 17	Xinjiang	;	0	0
	PN-126	Xindong 28	Xinjiang	;	0	0
	PN-127	Xindong 32	Xinjiang	;	0	0
	PN-128	Xindong 33	Xinjiang	;	0	0
	PN-129	Xindong 40	Xinjiang	;	0, ;	0
	PN-130	Zang 171	Tibet	;	0	0

(*Continued on next page*)

Table 1. (*Continued from previous page*)

Cereal	Entry	Cultivar	Origin	Infection type[a]		
				Puccinia coronata var. *hordei*		*P. coronata* var. *avenae*
				HZJ0004	POR3	POC8
	PN-131	Zang 173	Tibet	;	0	0
	PN-132	Zang 175	Tibet	;	0	0
	PN-133	Zang 177	Tibet	;	0	0, ;
	PN-134	Zang 178	Tibet	;	0	0
	PN-135	Zang 181	Tibet	;	0	0
	PN-136	Zang 184	Tibet	;	0	0
	PN-137	Zang 192	Tibet	;	0	0
	PN-138	Zang 194	Tibet	;	0	–
	PN-139	Zang 196	Tibet	;	0	0, ;
	PN-140	Zang 199	Tibet	;	–	0
	PN-141	Zang 200	Tibet	;	0	0, ;
	PN-142	Zang 202	Tibet	;	0	0, ;
	PN-143	Zang 204	Tibet	;	0	0
	PN-144	Zang 218	Tibet	;	0	0
	PN-145	Zang 219	Tibet	;	0, ;	0
	PN-146	Zang 222	Tibet	;	0	0
	PN-147	Zang 225	Tibet	;	0	;
	PN-148	Zang 235	Tibet	;	0	0
	PN-149	Zang 321	Tibet	;	0	0
	PN-150	Feimai	Tibet	;	0, ;	0, ;
	PN-151	Zangdong 20	Tibet	;	;	0
	PN-152	Zangdong 25	Tibet	;	0	0
	PN-153	Changdu 1 Hao	Tibet	;	0	0
Elymus	PN-154	*Elymus atratus*	GBWS[c]	0	4	0
	PN-155	*E. excelsus*	GBWS	4	0	0
	PN-156	*E. kamoji*	GBWS	4	2	0
	PN-157	*E. sibiricus*	GBWS	3	0	0
	PN-158	*E. nutans*	GBWS	4	0	0, ;
	PN-159	*Elymus* sp.	NWAFU[d]	3	0	0
Bromus	PN-160	*Bromus inermis*	GBWS	;	0	0
	PN-161	*B. himalaicus*	GBWS	4	1	;
	PN-162	*B. japonicus*	GBWS	;	0	;
Aegilops	PN-163	*Aegilops tauschii*	–	2	4	;
Festuca	PN-164	*Festuca arioides*	GBWS	0	0	0
	PN-165	*F. nitidula*	GBWS	;	0	0, ;
Calamagrostis	PN-166	*Calamagrostis epigeios*	GBWS	0	0	0, ;
	PN-167	*C. hedinii*	GBWS	0	0	X
Phleum	PN-168	*Phleum paniculatum*	GBWS	0	0	0, ;
Brachypodium	PN-169	*Brachypodium distachyon*	–	0	0	;
Deschampsia	PN-170	*Deschampsia littorali*	GBWS	0	0	0

Three isolates, HZJ0004 from urediniospores of the barley sample, POR3 from buckthorn, and POC8 from wild oat, were established through single uredinium or aecium isolation. The dried leaf samples of barley and wild oat were placed onto water-saturated filter papers (two to three layers) in plastic Petri dishes and kept at room temperature for 3 to 4 h. A single uredinium of the isolates was picked by using a scalpel to transfer onto a leaf of barley cultivar Zangqing 25 or oat cultivar Qingyan 1 for increasing urediniospores. For the buckthorn samples, an aecial cup (aecium) was cut from a leaf using a scalpel, transferred onto a glass slide, and broken for exposure of aeciospores. An aeciospore suspension was made by adding two drops of deionized water (50 µl) and then transferred onto the leaf surface of barley cultivar Guoluo. The inoculated plants were sprayed with deionized water to produce a water film instead of water droplets, covered with a plastic cylinder (<9 cm in diameter) to fit a lid of a plastic Petri dish (9 cm in diameter) on the top for avoiding cross-contamination and kept for 24 h at 20°C in a dew chamber in the dark. After incubation, the plants were transferred to a growth chamber for growing at 25°C during the daytime and 20°C at night, and a light period of 16 h of light/8 h of dark was used. Urediniospores were collected 14 to 20 days after inoculation by tapping infected leaves of barley into a glass test tube and put in a desiccator in a refrigerator (4°C) for drying until use. The infected plants were kept in a growth chamber with the same conditions as described above for telial production. After drying at room temperature, leaves bearing teliospores were collected and kept in a desiccator in the same conditions as described above.

Morphological observation. Urediniospores and teliospores of isolate HZJ0004 were observed morphologically and determined for size by measuring >100 fresh spores using a light microscope (DP22; Olympus, Japan).

DNA extraction. Approximately 5 mg of fresh urediniospores was added to a 1.5-ml microcentrifuge tube with approximately 5 mg of autoclaved quartz sand and ground to fine powder using a tissue grinding pestle (MGR 115; Sigma-Aldrich, MO) fixed in a hand-operated electronic drill. Genomic DNA was extracted using a DNA extraction kit (Biospin Fungus Genomic DNA Extraction Kit, catalog no. BSC14S1; BioFlux, Tokyo, Japan). DNA concentration was measured using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific Inc., DE).

PCR amplification and sequence alignment. PCR was conducted by amplification of the internal transcribed spacer (ITS) using primer pairs ITS1/ITS4 (White et al. 1990) for isolate HZJ0004 and ITS1RustF10d/ITS4 for isolates POR3 and POC8 (Barnes and Szabo 2007). PCR reaction consisted of 1× DNA *Taq* buffer, 0.2 µM of $MgCl_2$, 0.2 µM of dNTPs, 0.2 µM of each of the primers, 1.5 U/µl of *Taq* polymerase (5 U/µl), DNA (about 60 ng/µl), and autoclaved deionized water up to the total of 25 µl. Autoclaved deionized water, instead of DNA, was used as a negative control. PCR amplification was performed in a thermal cycler (ProFlex; Applied Biosystems, Waltham, MA) using program parameters as follows: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 40 s, 51°C for 40 s, 72°C for 90 s; and 72°C for 7 min for final extension. PCR products

were electrophoresed on 1% (vol/vol) agarose gel in 1 × TAE buffer at 3 to 5 v/cm for 40 to 60 min. The agarose gel was transferred into a 0.1-μg/ml ethidium bromide water solution for staining nucleic acid and analyzed using a gel imaging system (ChemiDox XRS; Bio-Rad, CA). The positive band was collected to elute DNA using a DNA gel extraction kit (BioTeke Corp., Beijing, China) according to the user's manual. The target band was cloned and sequenced by Beijing Aoke-Dingsheng Biotech Co., Ltd (Beijing, China). Comparison of sequence homology was performed at the NCBI website (http://www.ncbi.nlm.nih.gov) for identification of the crown rust pathogen.

Table 2. Representative isolates of *Puccinia* spp. and *Uromyces* sp. used for construction of phylogenetic tree based on internal transcribed spacer sequences

***Puccinia* species**[a]	**Host**	**GenBank accession**
P. coronata	*Avena ludoviciana*	AY956564
P. coronata	*Festuca rubra*	MF772824
P. coronata	*Holcus lanatus*	DQ355444
P. coronata	*Hordeum vulgare*	DQ355454
P. coronata	*Lolium perenne*	MF772856
P. coronata	*Poa paratensis*	MF772854
P. coronata* var. *avenae	*Avena fatua*	MT704989 (POC8)
P. coronata var. *avenae*	*Avena sativa*	HM131259
P. coronata* var. *hordei	*Hordeum vulgare* var. *nudum*	KU500626 (HZJ0004)
P. coronata* var. *hordei	*Rhamnus* sp.	MT704988 (POR3)
P. coronati-hordei	*Elymus repens*	HM131229
P. coronati-hordei	*Hordeum jubatum*	HM131231
P. graminis	*Berberis* sp.	JQ688946
P. graminis	*Hordeum vulgare*	MN385566
P. graminis	*Piptatherum exigum*[b]	HM131358
P. graminis	*Poa paratensis*	HQ317539
P. graminis	*Triticum aestivum*	AY874146
P. hordei	*Hordeum murinum*	HQ012449
P. hordei	*Hordeum vulgare*	DQ460717
P. striiformis	*Hordeum geniculatum*	AY956559
P. striiformis	*Leymus chinensis*	MK164276
P. striiformis	*Triticum aestivum*	HM057123
P. triticina	*Elytrigia repens*	DQ460721
P. triticina	*Hordeum vulgare*	KT982688
P. triticina	*Triticum turgidum* subsp. *durum*	DQ460724
Uromyces dactylidis[c]	*Dactylis glomerata*	HM057148

[a] Species in bold indicate samples from this study and the others from GenBank.
[b] *Piptatherum exigum* was previously published as *Oryzopsis exigua* (Liu and Hambleton 2013).
[c] Sequence of *Uromyce dactylidis* (GenBank accession HM057148) was used as the outgroup in this study.

Phylogenetic tree construction. To determine whether the isolates infecting barley and buckthorn were *P. coronata* var. *hordei*, sequence alignments of the ITS regions of isolates HZJ0004 and POR3 were performed online at the NCBI database (https://www.ncbi.nlm.nih.gov/). Sequences of a total of 23 rust fungal species, listed in Table 2, were downloaded from the NCBI database, and together with those of isolates HZJ0004, POR3, and POC8 were used for constructing a phylogenetic tree. Sequence comparison for the maximum length was edited using BioEdit software (Hall 1999), and the sequence format was converted using Clustal X software (Thomson et al. 1997). The phylogenetic tree was constructed based on the data matrices using maximum parsimony analysis in PAUP* version 4.0b10 (Swofford 2002) and edited using FigTree software, version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Testing *Rhamnus* species as alternate hosts for *P. coronata* var. *hordei*. Teliospores of isolate HZJ0004 on leaves of barley cultivar Zangqing 25 were treated at alternative temperatures of 4°C for 24 h and 16°C for 24 h three times to induce germination. Inoculations of three buckthorn (*Rhamnus*) species, *R. cathartica*, *R. davurica*, and *R. parvifolia*, obtained from the Germplasm Bank of Wild Species in Southwest China, Kunming, Yunnan, China, together with *R. utilis* from Tianshui, Gansu, were conducted with basidiospores from germinated teliospores according to the method described by Jin and Steffenson (1999). The aeciospores were inoculated onto barley cultivar Guoluo to produce urediniospores using the methods described above.

Evaluation of pathogenicity on cereals and grasses. To determine the pathogenicity, urediniospores of isolates HZJ0004, POR3, and POC8 were used to inoculate plants of barley, oat, rye (*Secale cereal*), triticale (*Triticum* × *Secale*), wheat, and gramineous species, listed in Table 1. Urediniospores were diluted in talc powder at a rate of 1:20 to 1:50 (vol/vol), mixed completely, and put into a 15-ml plastic tube. The tube was covered with two layers of gauze and reversed to tap over seedlings of the plants after spraying with 0.05% (vol/vol) Tween 20 solution on the plants along the four directions. The same conditions as described above were used for incubation in a dew chamber and cultivation after moving out of the chamber. Infection types (ITs) on the tested cereal cultivars were scored 14 to

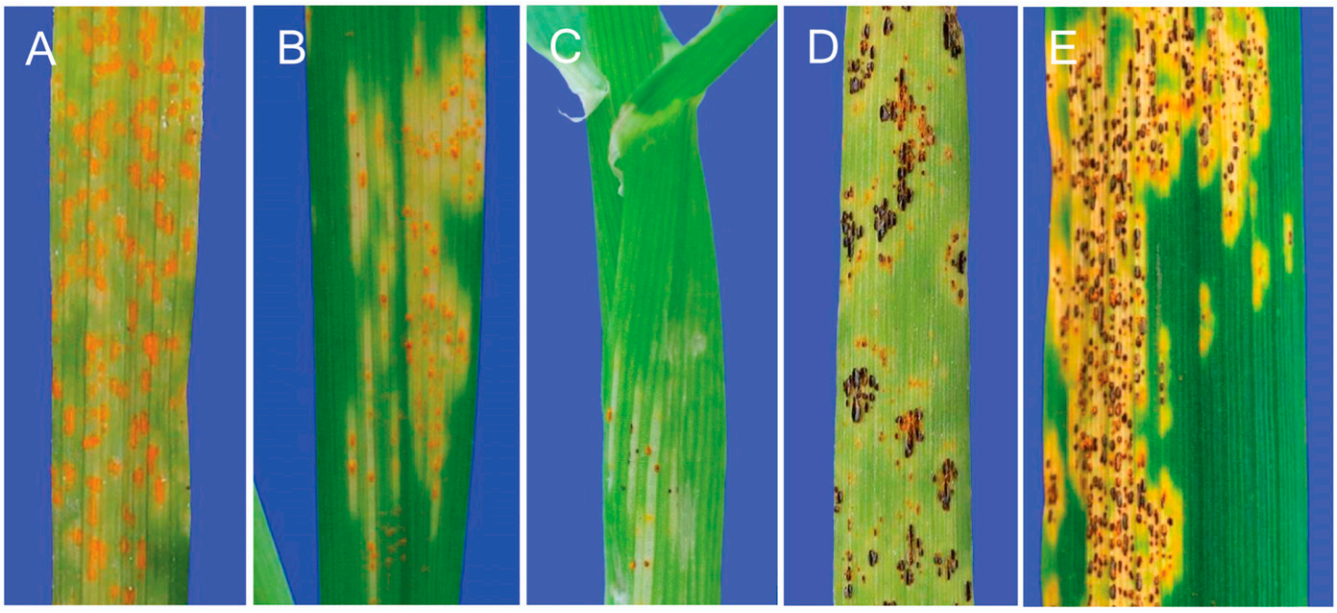

Fig. 1. Uredinial and telial production of *Puccinia coronata* var. *hordei* infecting barley (cultivar Zangqing 25). **A, B, and C,** Orange uredinia produced on the leaves and leaf sheaths of seedling and adult plants. **D and E,** Black telial production on leaves at the seedling and adult plant stages.

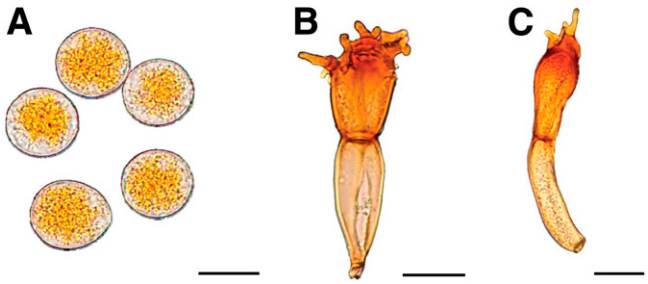

Fig. 2. Morphological observations on of *Puccinia coronata* var. *hordei* isolate HZJ0004 using a light microscope. **A,** Urediniospores. **B and C,** Teliospores with variable numbers of apical appendages. Bar = 20 μm.

20 days after inoculation based on the 0 to 4 scale described by Jin and Steffenson (1999). ITs 3 and 4 were considered susceptible and ITs ;, 1, 2, and X resistant.

Results

Morphological characterization. Bright orange uredinia were produced on leaves and leaf sheaths of barley (cultivar Zangqing 25) plants at the seedling and adult plant stages 14 days after inoculation with isolates HZJ0004 and POR3 (Fig. 1A, B, and C). Chlorosis (or yellow halo) appeared around uredinia on the infected tissues of the plants. Surrounding the infection sites, halos developed along leaf veins but remarkably remained between leaf veins and coalesced to form chlorotic patches (Fig. 1A, B, and C). Black telia formed on the sori of the uredinia 25 days after inoculation and were embedded

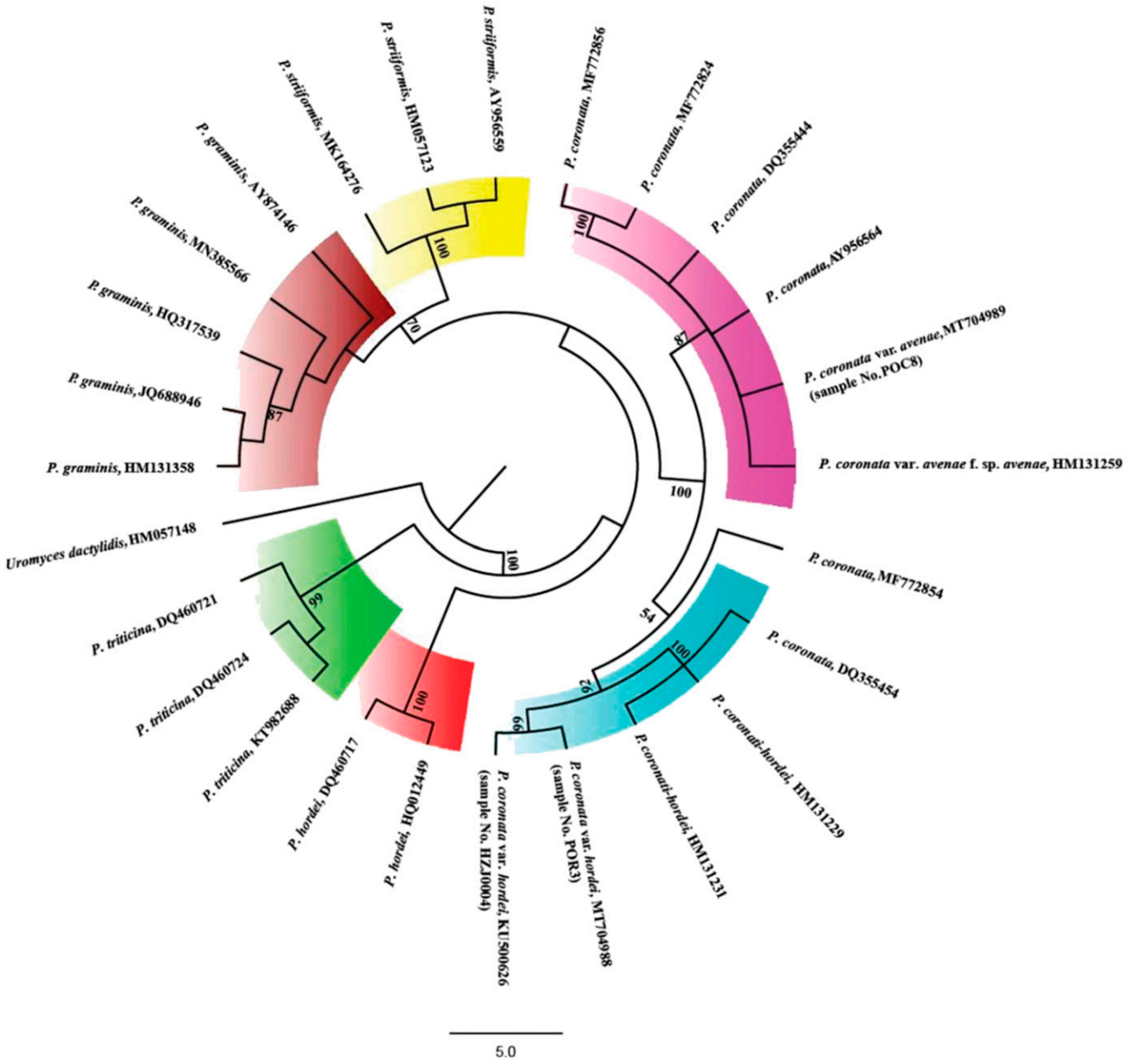

Fig. 3. Construction of the phylogenetic tree based on the neighbor-joining method with isolates HZJ0004 (GenBank No. KU500626) from barley, POR3 (MT704988) from buckthorn (*Rhamnus* species), and POC8 (MT704989) from wild oat, and the rust species listed in Table 1. Sequences were downloaded from NCBI GenBank. Bootstrap values indicated the percentage values for the branching in 1000 repetitions of the analysis. Different colored bars indicate different clades.

underneath the epidermis of the infected tissues at the seedling and adult plant stages (Fig. 1D and E).

Under a microscope, urediniospores were ovate in shape, light orange in color, and 29 (24 to 32) µm × 23 (21 to 25) µm in size (Fig. 2A). Teliospores were two-celled, dark brown, with a short, light-brown stalk at the base. The apical cell was darker than that of the basal cell. The apical cells of teliospores had appendages varying in number and length (Fig. 2B and C). Teliospores were 57.4 (43.4 to 73.3) µm × 15.5 (10.9 to 21.2) µm, and the length of appendages ranged from 4.3 (3.0 to 7.1) to 9.6 (5.2 to 14.6) µm (n = 105). Based on the barley host, rust symptoms and signs, and the morphology of urediniospores and teliospores, isolate HZJ004 was identified as *P. coronata* var. *hordei*.

Phylogenetic analysis. The amplified ITS fragment of isolate HZJ0004 (GenBank accession KU500626) was 672 bp and had the highest homology (93% identity, e-score = 0) with that of *P. coronata* var. *hordei* (GenBank accession DQ355454). The fragment of isolate POR3 (GenBank accession MT704988) was 640 bp, and the identity was 97% with that of isolate HZJ0004. Both HZJ0004 and POR3 were clustered to the same clade with those of *P. coronata* var. *hordei* isolates from *H. jubatum* (GenBank accession HM131231), *E. repens* (GenBank accession HM131229), and *H. vulgare* (GenBank accession DQ355454), but different from those of *P. coronata* from *A. ludoviciana* (GenBank accession AY956564), *Festuca rubra* (GenBank accession MF772824), *Holcus lanatus* (GenBank accession DQ355444), *Lolium perenne* (GenBank accession MF772856), *Poa paratensis* (GenBank accession MF772854), and *P. coronata* var. *avenae* on *A. sativa* (GenBank accession HM131259; Fig. 3). A bootstrap value of 92% was detected among the sequences of isolates HZJ0004 and POR3, and those of *P. coronata* from genus *Hordeum* (DQ355454) as well as *P. coronata* var. *hordei* from genera *Elymus* (HM131229) and *Hordeum* (HM131231). The sequence of POC8 had 100% identity (e-score = 0) with that of *P. coronata* var. *avenae* (GenBank accession HM131259), which were grouped into the same clade (Fig. 3). These results confirmed that isolates HZJ0004 and POR3 that were able to infect barley were *P. coronata* var. *hordei* and that isolate POC8 from wild oat was *P. coronata* var. *avenae*.

Pathogenicity on *Rhamnus* spp. After inoculation with basidiospores produced from germinated teliospores of isolate HZJ0004, the *R. davurica* and *R. utilis* plants produced pycnia 22 days postinoculation (Fig. 4A1 and B1), and aecia were observed 11 days after transferring pycniospores from one pycnium to another (Fig. 4A2, A3, and B2). The inoculated *R. cathartica* plants produced necrotic flecks without any pycnia (Supplementary Fig. S1). The inoculated *R. parvifolia* plants did not have any visible symptoms or signs. Fifteen days after inoculation of barley plants (cultivar Zangqing 25) with aeciospores produced on the *R. davurica* and *R. utilis* plants, uredinia were observed (Fig. 4A4 and B3). Thus, buckthorn was shown to be an alternate host for the *P. coronata* var. *hordei* isolate HZJ0004 from barley.

Pathogenicity on cereals and grasses. We evaluated 153 cultivars of cereal crops, including barley, oats, rye, triticale, and wheat at the seedling stage with isolates HZJ0004, POR3, and POC8. The *P. coronata* var. *avenae* isolate POC8 was highly virulent to all tested oat cultivars but did not produce any susceptible reaction on tested barley cultivars (Fig. 5; Table 1). In contrast, the *P. coronata* var. *hordei* isolates (HZJ0004 and POR3) were virulent to most barley cultivars but did not produce any susceptible reaction on all tested oat cultivars. Of the 84 barley entries tested with HZJ0004, 69 were susceptible (ITs 3 or 4) and 15 were resistant (ITs 0, ;, 1, 2, or X). Similarly, isolate POR3 from buckthorn produced virulent reactions (ITs 3 and 4) on 60 barley entries and avirulent reactions (ITs ;, 1, and 2) on five barley entries (11 entries, no data). The rye genotype was susceptible (IT 4) to both *P. coronata* var. *hordei* isolates HZJ0004 and POR3 but immune (IT 0) to the *P. coronata* var. *avenae* isolate POC8. The triticale genotype was immune (IT 0) to the *P. coronata* var. *avenae* isolate and near-immune (IT ;) to the *P. coronata* var. *hordei* isolates. All wheat entries were immune or near-immune to all *P. coronata* var. *avenae* and *P. coronata* var. *hordei* isolates (Fig. 5).

We also evaluated 17 gramineous grass species of eight genera, including *Elymus*, *Bromus*, *Aegilops*, *Festuca*, *Calamagrostis*, *Phleum*, *Brachypodium*, and *Deschampsia* with HZJ0004, POR3, and POC8. Isolate HZJ0004 was virulent to five of the six *Elymus* species (*E. excelsus*, *E. kamoji*, *E. sibiricus*, *E. nutans*, and *Elymus* sp. [PN-160]) and

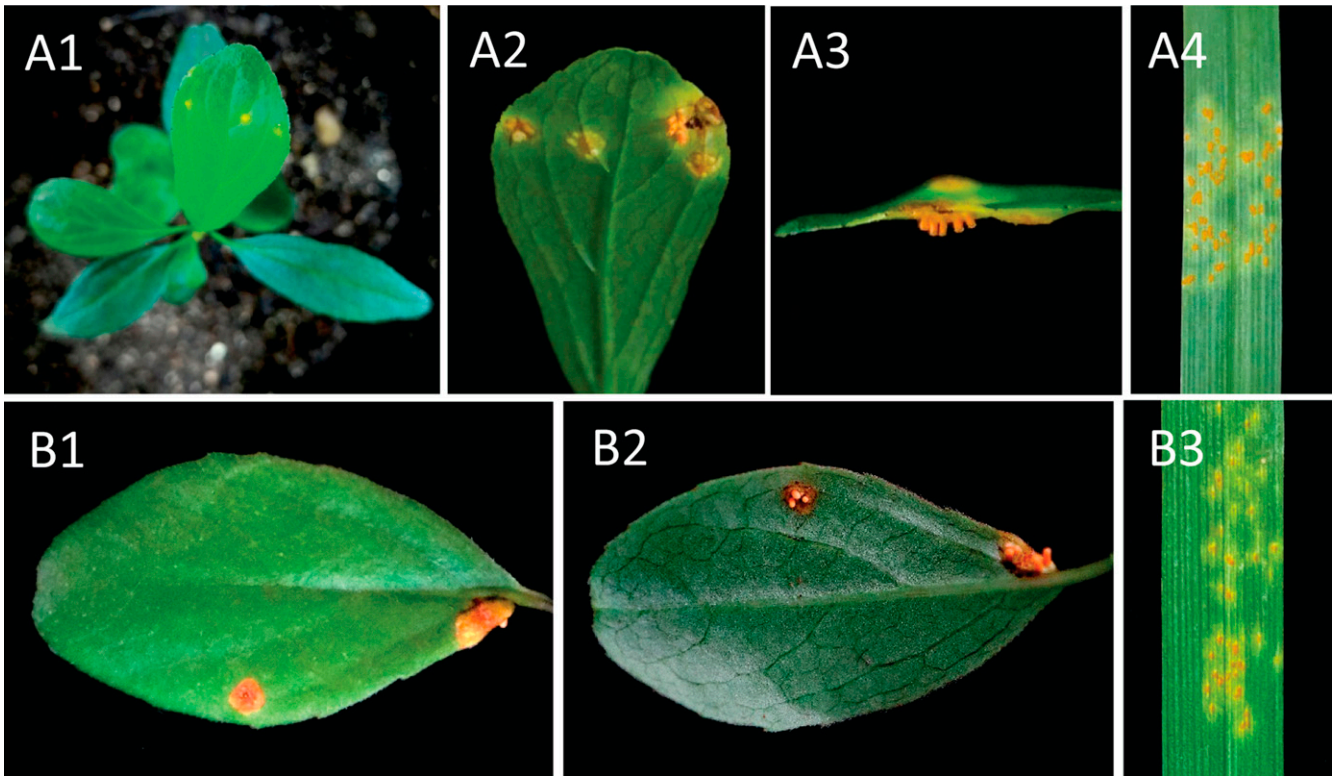

Fig. 4. Identification of seedlings of buckthorn (*Rhamnus davurica* and *R. utilis*) as alternate hosts for *Puccinia coronata* var. *hordei*, the causal pathogen of barley crown rust, using basidiospores produced from germinated teliospores under controlled conditions. **A1 and B1,** Pycnial stage. **A2, A3, and B2,** Aecial stage. A3, side view of the aecium shown in A2. **A4 and B3,** Uredinial production on leaves of barley (cultivar Guoluo) after inoculation with aeciospores from A2 and B2.

Bromus himalaicus, but avirulent to *E. atratus*, and *B. inermis*, *B. japonicas*, *A. tauschii*, and other tested species of genera *Festuca*, *Calamagrostis*, *Phleum*, *Brachypodium*, and *Deschampsia*. All tested grass species were immune to resistant reactions (ITs 0, ;, 1, or 2) when tested with *P. coronata* var. *avenae* isolate POC8. These results further support that isolates HZJ0004 and POR3 are *P. coronata* var. *hordei* and different from the *P. coronata* var. *avenae* isolate POC8, and they indicate that most barley cultivars are susceptible to the *P. coronata* var. *hordei* isolates.

Discussion

To our knowledge, this is the first report of crown rust on barley in China. The disease is a new threat to barley production because most tested barley cultivars are susceptible to the *P. coronata* var. *hordei* isolates. Previous studies reported that *P. coronata* var. *hordei* has quite a wide host range, including many wild gramineous grass species, rye, and some cultivated wheat accessions. These hosts were artificially or naturally infected by *P. coronata* var. *hordei* (Jin and Steffenson 1993, 1999; Jin et al. 1993). Therefore, *P. coronata* var. *hordei* is a potentially damaging pathogen of cereals (barley, wheat, and rye), and some important forage grasses (Jin and Steffenson 1999). In the present study, *P. coronata* var. *hordei* was pathogenic to rye but failed to infect any of 63 wheat cultivars from seven provinces in China (Table 1). Although all 63 wheat cultivars tested in the present study were immune or near-immune to the two *P. coronata* var. *hordei* isolates, more wheat varieties should be tested with more *P. coronata* var. *hordei* isolates to determine whether any wheat cultivars are susceptible. More importantly, more barley cultivars need to be tested with more *P. coronata* var. *hordei* isolates to estimate the potential damage and identify resistant germplasm for breeding new resistant cultivars.

The phylogenetic analysis showed that HZJ0004 isolated from barley and POR3 isolated from *Rhamnus* sp. were clustered together with isolates of *P. coronata* var. *hordei* from *H. vulgare*, *H. jubatum*, and *E. repens* into the same clade, supporting the identification of the two isolates as *P. coronata* var. *hordei*. The differences in ITS sequence among these isolates indicate that genetic variation exits among the variety of *P. coronata* var. *hordei*. More *P. coronata* var. *hordei*

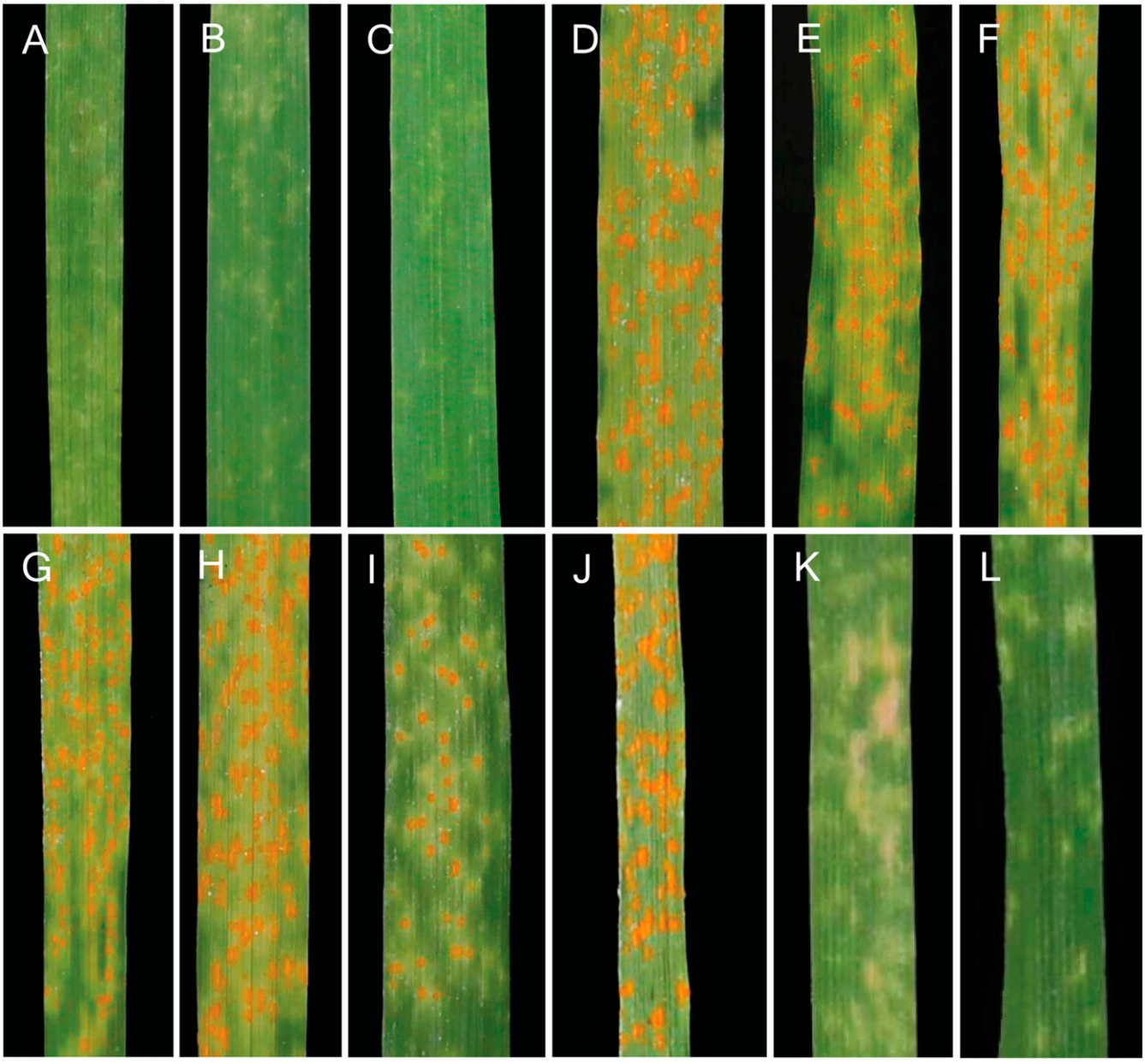

Fig. 5. Responses of cereal cultivars to *Puccinia coronata* var. *hordei* isolate HZJ0004 after 19 days postinoculation. Oat: **A,** Baiyan 7; **B,** Qingyan 1; and **C,** Qinghai sweet oat. Barley: **D,** II-I CK Guoluo; **E,** Steptoe; **F,** Zangqing 25; **G,** Zangqing 2000; **H,** unknown cultivar 1 (PN-051); and **I,** unknown cultivar 2 (PN-052). Rye: **J,** *Secale cereale*. Wheat: **K,** Mingxian 169. Triticale: **L,** *Triticum* × *Secale*.

isolates from barley, rye, grasses, and *Rhamnus* spp. are needed to determine the genetic variation in the *P. coronata* var. *hordei* population in China.

Barley can be infected by the *P. coronata* complex (Lutey and Covey 1959; Peturson 1954; Sampson and Watson 1985; Schwinghamer 1955). Peturson (1954) reported that *P. coronata* var. *secalis* (syn. *P. coronata* f. sp. *secalis*) collected from *R. cathartica* (alternate host for *P. coronata*) in Canada was pathogenic to many varieties of barley and rye, but not on oats. Schwinghamer (1955) reported that *P. coronata* f. sp. *agropyri* collected from quack grass (*E. repens*, syn. *Agropyron repens*) in North Dakota in the United States was virulent to species in the genera *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, and *Secale* of tribe Hordeae but avirulent to species in tribes of Agrostideae, Avenae, and Festuceae and the genus *Triticum.* Subsequently, Sampson and Watson (1985) reported that *P. coronata* collected from *E. repens* (syn. *Agropyron repens*) in western Canada was pathogenic to all tested species in the genera *Hordeum* and *Elymus*, as well as some cultivars of triticale and rye, but not to *Avena* and *Triticum aestivum*. Lutey and Covey (1959) observed natural infections of crown rust on barley by *P. coronata* f. sp. *secalis*. This rust fungus was highly pathogenic on rye, moderately pathogenic to tested barley cultivars, but nonpathogenic to oats under artificial inoculation. Jin and Steffenson (1999) reported that serious infection of crown rust was observed on winter barley in Nebraska in the United States in 1991 and that the causal pathogen of the disease on barley, caused by a new variety of *P. coronata*, was formally designated as *P. coronata* var. *hordei.* In their study, all tested species in *Hordeum* and some in *Secale cereale* were susceptible to *P. coronata* var. *hordei*, but all tested species in *Avena* were resistant to the pathogen. Based on morphology, pathogenicity on *Hordeum* and *Bromus*, and ITS sequences, Niks et al. (2013) considered an isolate of crown rust fungus collected from wild couch grass (*E. repens*, syn. *Agropyronrepens*) as a representative European specimen of *P. coronata* var. *hordei*. In the present study, the tested oat varieties were not infected by the *P. coronata* var. *hordei* isolates, indicating that the isolates collected from barley and buckthorn infecting barley were not *P. coronata* var. *avenae* but should be *P. coronata* var. *hordei*.

P. rangiferina, causing crown rust on *Calamagrostis* spp., was reported in Japan (Ito 1909) and China (Cummins 1951). The species was later renamed *P. coronata* var. *rangiferina* (Cummins 1971). Jin and Steffenson (1999) reported that teliospores of *P. coronata* var. *rangiferina* isolates were similar in morphology to those of their *P. coronata* var. *hordei* isolates, but they did not determine whether they were the same species because of a the lack of sufficient experimental evidence. We think that *P. coronata* var. *rangiferina* reported previously in China could be the same as *P. coronata* var. *hordei* based on similar morphological characteristics. However, crown rust collections from *Calamagrostis* spp. are needed to test this hypothesis.

Intraspecific classification of *P. coronata* has been inconsistent because of the lack of established criteria in previous studies, resulting in confusing intraspecific taxa. Jin and Steffenson (1999) suggested the use of variety as an intraspecific taxon under species *P. coronata* based on morphological differences in combination with aecial and telial host specificity. In China, the subspecific classification of *P. coronata* has been changed several times. In the 1970s, *P. coronata* was divided into 10 varieties, including *P. coronata* var. *agropyri*, var. *agrostiss*, var. *alopecuri*, var. *avenae*, var. *calmagrostis*, var. *festucae*, var. *glyceriae*, var. *holci*, var. *lolii*, and var. *phalaridis*, based on the results from cross-inoculation of plants using urediniospores (Wei 1979). Later, these varieties were rearranged into three varieties: *P. coronata* var. *coronata*, var. *avenae*, and var. *himalensis* (Liu et al. 2003; Wang and Zhuang 1998). The present study showed that teliospore morphology, especially the length of apical appendages and numbers of dichotomous branches, of the isolate from barley was distinct from those of *P. coronata* var. *avenae* on *Avena sativa* (Jin and Steffenson 1999), *P. coronata* var. *coronata* on *Poa* spp. (Liu et al. 2003), and *P. coronata* var. *himalensis* (Department of Crop Sciences 1987) but similar to those of *P. coronata* var. *hordei* reported by Jin and Steffenson (1999).

In the present study, the *P. coronata* var. *hordei* isolates infected most barley cultivars, and thus, crown rust is considered a new threat to barley production. Barley cultivar Xiyin 2 (original name: Qianjianmai) was introduced from Nagano, Japan, in 1980 to China and grown widely for feeding animals or used as an important source of disease resistance for barley breeding (Gao et al. 1985; Lin et al. 1988; Meng et al. 2006). Natural infections of Xiyin 2 by *P. striiformis*, the causal agent of stripe rust of wheat and barley, were observed in fields with high severity in 1985 (Lin et al. 1988). No other rusts have been reported to infect this cultivar in China. In the present study, we found that this cultivar is highly susceptible to barley crown rust.

In the present study, of the 10 malting barley cultivars tested with *P. coronata* var. *hordei* isolate HZJ0004, five belonging to the Ganpi series were highly susceptible. There was only one exception; cultivar Ganpi 7 was resistant. When tested with isolate POR3, all malting barley cultivars were highly susceptible. Similarly, Jin and Steffenson (1997) reported that most malting barley cultivars that were native or introduced to the northern Great Plains of the United States were susceptible to *P. coronata* var. *hordei*. Therefore, crown rust could be a serious problem for the malting barley industry, and barley breeders should consider developing barley cultivars resistant to this disease.

Barley is an important small grain cereal and grown throughout China. More than 500 barley cultivars are currently grown in various production regions, especially in the middle and lower reaches of Yangtze River and the Qinghai-Tibetan Plateau (Lu 1996). However, the planting area of barley has decreased from about 8 million hectares in the 1910s to 0.26 million hectares in the 2019 to 2020 crop season in China (Lu 1996; U.S. Department of Agriculture 2020). Even so, nationwide investigations on barley crown rust to determine the disease distribution and potential risk are needed. More barley germplasm should be collected to evaluate for resistance to crown rust. This will provide a basis for developing integrated management strategies for controlling crown rust of barley.

Literature Cited

Akar, T., Avci, M., and Dusunceli, F. 2004. Barley: Post-harvest operation. Central Research Institute for Field Crops. http://www.fao.org/fileadmin/user_upload/inpho/docs/Post_Harvest_Compendium_-_BARLEY.pdf

Barnes, C. W., and Szabo, L. J. 2007. Detection and identification of four common rust pathogens of cereals and grasses using real-time polymerase chain reaction. Phytopathology 97:717-727.

Cummins, G. B. 1951. Uredinales of continental China collected by S. Y. Cheo. II. Mycologia 43:78–98.

Cummins, G. B. 1971. The rust fungi of cereals, grasses and bamboos. Springer, New York, NY.

Dickson, J. G. 1956. Diseases of field crops, 2nd ed. McGraw-Hill Book Co. Inc., New York, NY.

Food and Agriculture Organization of the United Nations. 2013. FAOSTAT. Archived from the original in 2013. http://www.fao.org/statistics/en/

Gao, R. S., Wang, G. X., and Li, W. R. 1985. Characteristics and cultivated technique of Xiyin 2 barley. Shaanxi J. Agric. Sci. 3:1–3.

Griffey, C. A., Das, M. K., Baldwin, R. E., and Waldenmaier, C. M. 1994. Yield losses in winter barley resulting from a new race of *Puccinia hordei* in North America. Plant Dis. 78:256–260.

Hall, T. A. 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Harder, D. E., and Dunsmore, K. M. 1991. Incidence and virulence of *Puccinia graminis* f. sp. *tritici* on wheat and barley in Canada in 1990. Can. J. Plant Pathol. 13:361–364.

Department of Crop Sciences. 1987. Rusts of turfgrasses, report on plant disease, RPD No. 412. University of Illinois at Urbana-Champaign. http://ipm.illinois.edu/diseases/rpds/412.pdf

Ito, S. 1909. On the Uredineae parasitic on the Japanese Gramineae. J. Coll. Agric. Tohoku Imp. Univ. 3:180–262.

Jin, Y., and Steffenson, B. J. 1993. Natural occurrence of barley crown rust on forage grasses in North Dakota and its pathogenicity on gramineous species (Abstr.). Phytopathology 83:884.

Jin, Y., and Steffenson, B. J. 1997. Crown rust. Pages 41, 42, and 78 in: Compendium of barley diseases, 2nd ed. D. E. Mathre, ed. American Phytopathological Society, St. Paul, MN.

Jin, Y., and Steffenson, B. J. 1999. *Puccinia coronata* var. *hordei* var. nov.: Morphology and pathogenicity. Mycologia 91:877–884.

Jin, Y., and Steffenson, B. J. 2002. Sources and genetics of crown rust resistance in barley. Phytopathology 92:1064–1067.

Jin, Y., Steffenson, B. J., Oberthur, L. E., and Baenziger, P. S. 1992. *Puccinia coronata* on barley. Plant Dis. 76:1283.

Jin, Y., Steffenson, B. J., Wesenberg, D. M., and Bockelman, H. E. 1993. Reaction of *Triticum* species and related genera to barley crown rust (Abstr.). Phytopathology 83:1387.

Lin, X. M., Li, Z. Q., Shang, H. S., and Kang, Z. S. 1988. A brief report on the stripe rust of barley. J. Northwest A&F Univ. (Nat. Sci. Ed.) 16:91–92.

Liu, M., and Hambleton, S. 2010. Taxonomic study of stripe rust, *Puccinia striiformis sensu lato*, based on molecular and morphological evidence. Fungal Biol. 114:881–899.

Liu, M., and Hambleton, S. 2013. Laying the foundation for a taxonomic review of *Puccinia coronata* s. l. in a phylogenetic context. Mycol. Prog. 12:63–89.

Liu, S. Y., Xu, B., Sun, Y. H., Zhang, J. H., and Liu, X. M. 2003. A new disease caused by *Puccinia coronata* Corda var. *coronata* on *Poa* sp. in Jilin Province. J. Jilin Agric. Univ. 25:365–366.

Lu, L. S. 1996. Barley sciences in China. China Agriculture Press, Beijing, China.

Luig, N. H. 1985. Epidemiology in Australia and New Zealand. Pages 301–328 in: The cereal rusts. Vol. II. Disease, distribution, epidemiology, and control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Orlando, FL.

Lutey, R. W., and Covey, R. P. 1959. Crown rust on barley in Minnesota. Plant Dis. Rep. 43:1287.

Meng, F. L., Zhao, Y. B., Qiang, X. L., and Hu, Y. G. 2006. Comparison on agronomic traits of barley from different regions and improvement of Tibet naked barley. J. Triticeae Crops 26:175–178.

Niks, R. E., Heyzen, S. V., Szabo, L. J., and Alemu, S. K. 2013. Host status of barley to *Puccinia coronata* from couch grass and *P. striiformis* from wheat and brome. Eur. J. Plant Pathol. 136:393–405.

Niu, Z. X., Puri, K. D., Chao, S. M., Jin, Y., Sun, Y. L., Steffenson, B. J., Maan, S. S., Xu, S., and Zhong, S. B. 2014. Genetics analysis and molecular mapping of crown rust resistance in common wheat. Theor. Appl. Genet. 127:609–619.

Peturson, B. 1954. The relative prevalence of specialized forms of *Puccinia coronata* that occur on *Rhamnus cathartica* in Canada. Can. J. Bot. 32: 40–47.

Sampson, M. G., and Watson, A. K. 1985. Host range of *Puccinia coronata*, *Puccinia graminis*, and *Puccinia recondita* isolates from *Agropyron repens.* Can. J. Plant Pathol. 7:417–420.

Schwinghamer, E. A. 1955. A form of crown rust occurring on *Agropyron* spp. in North Dakota. Plant Dis. Rep. 39:322–324.

Simons, M. D. 1985. Crown rust. Pages 131–172 in: The cereal rusts. Vol. II. Diseases, distribution, epidemiology, and control. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, Orlando, FL.

Stubbs, R. W. 1985. Stripe rust. Pages 61–101 in: The cereal rusts. Vol. II. A. P. Roelfs and W. R. Bushnell, eds. Academic Press, New York, NY.

Swofford, D. L. 2002. PAUP: phylogenetic analysis using parsimony and other methods, version 4.0b10.

Thomson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.

U.S. Department of Agriculture. World Agricultural Production. Circular Series, 16 April 2020. USDA Foreign Agricultural Service, Washington, DC. https://downloads.usda.library.cornell.edu/usda-esmis/files/5q47rn72z/ft849d88n/q811m8874/production.pdf

Wang, Y. Z., and Zhuang, J. Y. 1998. Uredinales (I). Pages 1–66 in: Flora Fungorum Sinicorum, Vol. 10, J. Y. Zhuang, S. X. Wei, and Y. Z. Wang, eds. Science Press, Beijing, China.

Wang, Z. H., Kong, C. X., and He, T. 1988. Sampling and identification of plant diseases in Tibet. Tibet J. Agric. Sci. 1:33–35.

Wei, J. C. 1979. Manual on taxonomic outline of fungi. Shanghai Science and Technology Press, Shanghai, China.

Wellings, C. R. 2011. Global status of stripe rust: A review of historical and current threats. Euphytica 179:129–141.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in: PCR protocols: A guide to methods and applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press Inc., San Diego, CA.

Woldeab, G., and Alemayhu, F. 2001. On farm yield loss due to leaf rust (*Puccinia hordei* Otth) on barley. Pest Manage. J. Ethiop. 5:29–35.

Xu, Y. G., and Cai, Z. F. 1979. Crop diseases in Tibet. Tibet J. Agric. Sci. 3:29–49.

Zhuang, J. Y. 1985. The rust diseases of important economic plants in East Himalayan mountainous region. Mountain Res. 3:311–316.