Host Range of *Plasmodiophora brassicae* on Cruciferous Crops and Weeds in China

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Abstract

Ren, L., Xu, L., Liu, F., Chen, K., Sun, C., Li, J., and Fang, X. 2016. Host range of *Plasmodiophora brassicae* on cruciferous crops and weeds in China. Plant Dis. 100:933-939.

Clubroot caused by *Plasmodiophora brassicae* is an increasingly important soilborne disease in China. The host range of *P. brassicae* was investigated with 30 cruciferous plants, including 16 crop species, 9 ornamentals, and 5 weeds in field and pot-cultured conditions. In the field, 17 species from five genera produced visible galls, and these included radish, *Capsella bursa-pastoris*, *Orychophragmus violaceus*, *Sinapis alba*, and 13 *Brassica* crops. In pot-cultured conditions, an additional 13 plant species (11 genera) were determined to be hosts of *P. brassicae*. Five common weeds were found to be hosts of *P. brassicae*, including *C. bursa-pastoris*, *Lepidium apetalum*, *Descurainia sophia*, *S. alba*, and *Thellungiella salsuginea*. The infection of these plants

was confirmed via polymerase chain reaction (PCR) with primers specific to *P. brassicae*. No galls were found on *Matthiola incana* roots in the field or in pots and no resting spores of *P. brassicae* were observed in *M. incana* roots, although *P. brassicae* was detected in *M. incana* roots via PCR. Microscopic examination revealed infection only in the root hairs of *M. incana* roots. These results suggested that *M. incana* was highly resistant to *P. brassicae* in China and could be developed as a bait crop. In total, 297 accessions of oilseed rape were tested in the field, and 3 accessions of *Brassica napus* and 1 accession of *B. juncea* were found to be highly resistant to clubroot disease. These resistant resources provide options for managing clubroot in *P. brassicae*-infested fields.

Clubroot of cruciferous plants caused by *Plasmodiophora brassicae* is an economically important soilborne disease. The disease has been reported in more than 60 countries and can result in total yield loss in heavily infested fields (Dixon 2009; Hwang et al. 2012). In China, clubroot is distributed throughout the country and causes an average yield loss of 20 to 30% (Chai et al. 2014; Wang et al. 2011). Chinese cabbage (*Brassica rapa pekinensis*) and oilseed rape (*B. napus*) are the most common cruciferous crops in China and clubroot is a major constraint in production of these crops.

Knowledge regarding clubroot hosts in China is fragmentary. Theoretically, all species of the family Brassicaceae are potential hosts for *P. brassicae*. Karling (1968) described more than 300 clubroot hosts; however, isolates of the pathogen have different capacities to infect specific host genotypes (Honig 1931). Few infection studies have been performed outside the genera *Brassica*, *Raphanus*, and *Arabidopsis* (Dixon 2009; Howard et al. 2010). To the best of our knowledge, natural infections were reported in genera *Brassica*, *Raphanus*, *Arabidopsis*, *Eruca* (Paz Lima et al. 2004), *Capsella* (Kim et al. 2011), *Cardamine* (Tanaka et al. 1993), *Matthiola* (Liu 2012), and *Isatis* (Xiong et al. 2012); among these, *Brassica*, *Raphanus* (Zhang and Zhao 2012), *Isatis*, *Matthiola*, and *Capsella* (Ren et al. 2014) were reported in China. Some noncruciferous plants are also listed as hosts of *P. brassicae*, including nasturtium (*Tropaeolum majus*), papaya (*Carica papaya*), corn poppy (*Papaver rhoeas*), and clover (*Trifolium repens*) (Ludwig-Müller et al. 1999). The root hair (primary) infection in these species was observed with artificial inoculation under ideal environmental conditions.

Cruciferous weeds may play a role in clubroot epidemics because the disease has been reported in at least five species of cruciferous

weeds (Kim et al. 2011; Ren et al. 2014; Tanaka et al. 1993). In Japan, clubroot on the cruciferous weed *Cardamine flexuosa* was distributed in 43 prefectures. Thus, this plant was considered to be the most suitable plant species to investigate the distribution of clubroot (Tanaka et al. 2006). In Britain, Buczacki and Ockendon (1979) observed clubroot infection on several common cruciferous weeds under controlled conditions, including wild cabbage (*Brassica oleracea*), shepherd's purse (*Capsella bursa-pastoris*), wall rocket (*Diplotaxis muralis*), treacle mustard (*Erysimum cheiranthoides*), white mustard (*Sinapis alba*), charlock (*S. arvensis*), hedge mustard (*Sisymbrium officinale*), and field penny cress (*Thlaspi arvense*). However, few studies have been conducted on the susceptibility of weeds to clubroot in China.

C. bursa-pastoris, *Sinapis alba*, herb sophia (*Descurainia sophia*), and pepperweed (*Lepidium apetalum*) are widespread cruciferous weeds in China. Saltwater cress (*Thellungiella salsuginea*) is also a common weed in saline fields in northern China. Knowledge of the susceptibility of these weeds to *Plasmodiophora brassicae* is limited but extremely important for management of clubroot cultivated on cruciferous crops.

In this article, a field survey of natural infection and assays in pot-cultured plants were conducted to gain a better understanding of the host range of *P. brassicae* in China.

Materials and Methods

Pathogen isolates and plant species. *P. brassicae* isolates collected from field-grown oilseed rape plants in Hubei and Sichuan Provinces, previously determined to be pathotype 4 based on the Williams system (Ji et al. 2013), were used in this study. The cruciferous plants used in this study are listed in Tables 1 and 2. In total, 30 species in 17 genera were tested. The selected genotypes of crops and ornamentals were obtained from Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences or purchased from commercial seed companies. Seed of cruciferous weeds were collected from plants in agricultural fields or roadside verges (Table 1); seed of *C. bursa-pastoris* were collected in fields planted with oilseed rape or Chinese cabbage in which crops showed clubroot symptoms. Seed were stored in sealed bags at 4°C and surface disinfected with 5% sodium hypochlorite for 5 min before use.

Field surveys. Field surveys for clubroot diseases of cruciferous plants were conducted in 13 provinces from the northeast (Heilongjiang Province) to the southwest (Yunnan Province) of China from

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This research was supported by a grant from the Natural Science Foundation of China grants (31401720, 31501617 and 31101124) and by the Special Fund for the Agro-scientific Research in the Public Interest (201003029).

Accepted for publication 20 November 2015.

2010 to 2014. The investigation was conducted based on visible galls on roots. Additionally, susceptibility of selected plants (Tables 1 and 2) to *P. brassicae* was tested in fields with a history of clubroot. Seed were directly sown in *P. brassicae*-infested fields in Hubei Province. The experiment was established as a randomized complete block design with two replications. The plot size was 2 by 1.2 m (four rows) with 100 seedlings. After 2 months, the plants were uprooted and examined for gall formation, and disease incidence and disease index (DI) were calculated. Root symptoms were evaluated on a 0-to-3 scale and DI was calculated as previously described (Kuginuki et al. 1999; Xue et al. 2008), where 0 = no galls, 1 = a few small galls on less than one-third of the roots, 2 = moderate galling (small to medium galls on one-third to two-thirds of the roots), and 3 = severe galling (medium to large galls on more than two-thirds of the roots). Results are expressed as means of two replicates.

Pot trials. Root galls (50 g) collected from fields were homogenized in a household blender (HR2003; Philips Electronics Hong Kong Ltd.) with 200 ml of distilled water. The slurry was filtered through eight layers of cheesecloth and the filtrate was centrifuged at $2,000 \times g$ for 5 min (4°C). The pellets were suspended in 200 ml of distilled water, and the resting spore concentration was quantified using a hemocytometer under a microscope. The pH of the spore suspension was adjusted to 6.0 with 5% nitric acid. The freshly prepared resting spore suspension was used for inoculation. The inocula were mixed with a sterilized soil matrix (vermiculite and peat soil at 3:1 ratio) in plastic pots to a final concentration of 1×10^7 spores/g of soil. Soil treated with the same volume of sterile water was used as a control. Seed of each plant species were pregerminated on moist filter paper for 2 to 5 days (20°C) and then seeded into the pots, with 6 seeds/pot and five pots for each plant species.

Table 1. Cruciferous plants surveyed or tested in pots for susceptibility to clubroot

Common name	Botanical name	N ^b	Source	Clubroot ^a		
				Field	Pot	PCR
Oilseed rape	<i>Brassica napus</i>	>10	Hubei	+	+	+
Chinese cabbage	<i>B. rapa pekinensis</i>	3	Jingyan Yinong, Beijing	+	+	+
Brown mustard	<i>B. juncea</i>	>10	Hubei	+	+	+
Chinese kale	<i>B. alboglabra</i>	3	LuHeng Seeds, Beijing	+	+	+
Cabbage	<i>B. oleracea</i>	6	LuHeng Seeds, Beijing	+	+	+
Kale	<i>B. oleracea</i> var. <i>acephala</i>	1	Bejo Seeds, The Netherlands	+	+	+
Radish	<i>Raphanus sativus</i>	8	Syngenta and Tézier	+	+	+
Kohlrabi	<i>B. oleracea</i> var. <i>gongylodes</i>	2	Dayu Seeds, Hebei	+	ND	ND
Tuber mustard	<i>B. juncea</i> var. <i>tumida</i>	2	Chengdu, Sichuan	+	ND	ND
Sprouting broccoli	<i>B. oleracea</i> var. <i>italica</i>	2	Xinye, Henan	+	ND	ND
Watercress	<i>Nasturtium officinale</i>	1	Luhe Seeds, Jinan, Shandong	ND	+	+
Arugula	<i>Eruca sativa</i>	1	Lujinlan Seeds, Beijing	ND	+	+
Woad	<i>Isatis tinctoria</i>	2	Yunnan	ND	+	+
Sweet alyssum	<i>Lobularia maritima</i>	2	Takii Seed, Kyoto, Japan	ND	+	+
English wallflower	<i>Cheiranthus cheiri</i>	1	Takii Seed, Kyoto, Japan	ND	+	+
Siberian wallflower	<i>C. allionii</i>	1	Guomei Horticulture Co., Ltd., Zhejiang	ND	+	+
Wallflower	<i>C. maritimus</i>	1	Guomei Horticulture Co., Ltd., Zhejiang	ND	+	+
Dames rocket	<i>Hesperis matronalis</i>	1	Guomei Horticulture Co., Ltd., Zhejiang	ND	+	+
Globe candytuft	<i>Iberis umbellata</i>	1	Takii Seed, Kyoto, Japan	ND	+	+
Violet orychofragmus	<i>Orychophragmus violaceus</i>	2	Guomei Horticulture Co., Ltd., Zhejiang	+	+	+
Virginia stock	<i>Malcolmia maritime</i>	1	Qinghai	–	+	+
Stock	<i>Matthiola incana</i>	8	Xinnongfeng Seed and PanAmerican Seed	–	–	+
White mustard	<i>Sinapis alba</i>	3	Qinghai	+	+	+
Shepherd's purse	<i>Capsella bursa-pastoris</i>	3	Sichuan	+	+	+
Herb sophia	<i>Descurainia sophia</i>	1	Qinghai	–	+	+
Pepperweed	<i>Lepidium apetalum</i>	1	Qinghai	–	+	+
Saltwater cress	<i>Thellungiella salsuginea</i>	1	Qinghai	–	+	+

^a Symbols: + = visible clubs on the roots or specific fragment present via polymerase chain reaction (PCR), – = no visible clubs on the roots, and ND = not determined.

^b Number of varieties.

Table 2. Disease incidences and disease indices (DI) of clubroot on different species of *Brassica* in the field^a

Common name	Species Botanical name	N ^c	Disease incidence (%)		DI		DI ranges (N) ^b		
			Range	Average	Range	Average	<30	30–50	>50
Oilseed rape	<i>Brassica napus</i>	100	26.1–100	81.9	20.3–96.3	61.2	3	24	63
	<i>B. rapa</i>	100	52.8–100	81.0	40.5–96.5	64.4	0	19	81
	<i>B. juncea</i>	97	45.5–100	85.1	25.8–100	71.5	1	10	86
Turnip	<i>B. rapa</i> var. <i>rapa</i>	2	83.8–88.0	85.9	59.5–85.7	68.2	...	...	...
Flowering stalk	<i>B. rapa</i> ssp. <i>Chinensis</i> var. <i>utiliz</i>	2	39.6–77.3	58.5	44.7–72.7	54.0	...	...	...
Chinese kale	<i>B. alboglabra</i>	2	55.0–56.5	55.8	36.2–41.7	39.9	...	...	...
Tuber mustard	<i>B. juncea</i> var. <i>tumida</i>	2	39.3–66.7	53.0	27.8–55.6	37.0	...	...	...
Potherb mustard	<i>B. juncea</i> var. <i>crispifolia</i>	2	23.6–58.3	41.0	16.7–38.9	24.1	...	...	...
Sprouting broccoli	<i>B. oleracea</i> var. <i>italica</i>	2	28.0–47.1	37.5	22.7–23.5	22.9	...	...	...
Kohlrabi	<i>B. oleracea</i> var. <i>gongylodes</i>	2	9.1–38.1	23.6	6.1–28.6	13.6	...	...	...

^a Trials were laid out in a randomized complete block design with two replications. Results were means of two experiments.

^b Number of accessions with DI ranges shown.

^c Number of accessions.

To ensure the reliability of the results, one seed of Chinese cabbage 'Beijing New No. 3', highly susceptible to *P. brassicae*, was also sown in two of the five pots for each species. The pots were then placed in a chamber at 15 to 25°C under daily cycles of 14 h of light and 10 h of darkness and watered weekly with Hoagland nutrient solution (Luo et al. 2014) adjusted to pH 6.0. Four weeks after planting, plants in each pot were harvested to assess disease incidence and DI. Plants of stock (*Matthiola incana*), *D. sophia*, and *L. apetalum* were incubated for more than 2 months until flowering or podding. The roots of each plant were washed for 3 min under tap water for rating clubroot and for detecting *P. brassicae* with polymerase chain reaction (PCR). Pot trials were independently performed three times and results were expressed as means of three experiments.

PCR amplification. Because primary infection by *P. brassicae* caused no visible symptoms on plants, PCR was performed to confirm infection. One plant of each variety was randomly selected for extraction of genomic DNA using a cetyltrimethylammonium bromide protocol (Feng et al. 2013). PCR amplifications were conducted with primers Zgzb3-F (5'-GTATGGTAACAACGGACAAGGA-3') and Zgzb3-R (5'-AACGAACAGAAACCGACACTC-3'), which have been successfully used to characterize *P. brassicae* (Ren et al. 2012). All amplifications were conducted in a 25- μ l volume containing 2.5 μ l of 10 $\times$ PCR buffer, 2.0 μ l of 2.5 mM dNTP, 0.5 μ l of each forward and reverse primer, 1.0 μ l (1 U) of Taq DNA polymerase (Takara Co.), and 1.0 μ l (50 ng) of DNA from roots. A negative control consisting of DNA template of each plant species from noninfested soils was included in each PCR assay to ensure that the primers were *P. brassicae* specific. The amplification program consisted of an initial heat denaturation step at 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products (5 μ l) were analyzed on a 1% agarose gel in Tris-acetate-EDTA buffer, stained with ethidium bromide, and visualized on a Gel Imaging System (G:BOX XL1.4; Syngene, USA).

Infection of *M. incana* by *P. brassicae*. In preliminary studies, no galls were observed on the roots of *M. incana* in the field or the greenhouse. To determine whether *P. brassicae* structures were present in *M. incana*, roots were harvested to observe root hairs via microscopy and resting spores in cells via scanning electron microscopy (SEM) and for further PCR evaluation. One *M. incana* variety ('Vintage Mix'; PanAmerican Seed) was selected, and the seed were pregerminated on wet filter paper at 20°C for 5 days, transferred to *P. brassicae*-infected Hoagland solutions (Luo et al. 2014), and incubated in a plant incubator for 45 days. At least 30 plants were incubated in liquid. Fresh nutrient solutions (pH 6.0) were added to the tubes as required. Four or five *M. incana* plants were harvested at 2, 3, 5, and 7 days after inoculation (dai) and rinsed with tap water. Roots of three plants were cut from the hypocotyl and stained with Phloxine B to facilitate observation under microscopy (Donald and Porter 2004). The infected root hairs were counted to calculate the infection rate. One (for 5 and 7 dai) or two (for 2 and 3 dai) plants were used for DNA isolation. DNA from the galled roots of Chinese cabbage was used as a positive control in PCR, and DNA from *M. incana* incubated for 7 days in noninfected solution was used as a negative control. Meanwhile, to ensure that the spores of *P. brassicae* that adhered to the roots had been washed off under the tap water, an *M. incana* plant from *P. brassicae*-free solution was harvested and immersed in a resting spore suspension for 10 min and rinsed for DNA isolation. This specimen was labeled as a rinsed control.

SEM. Infected and noninfected roots of 45-day-old *M. incana* seedlings were harvested and washed with sterile distilled water. Meanwhile, galls of Chinese cabbage and globe candytuft (*Iberis umbellata*) were also harvested as positive controls for SEM. Sections measuring 1 to 2 mm were cut and fixed in 2.5% glutaraldehyde (4°C, 4 h) and 1% osmium tetroxide (4°C, 2 h) in turn. The sections were dehydrated; rinsed with alcohol at successive concentrations of 50, 70, 80, 90, and 100%; and subsequently dried with a Hitachi HCP-2 critical-point dryer. The slice was then placed on the sample stage and sputtered for 5 min with gold using a Hitachi E102 ion sputter. Examinations were performed with a Hitachi SU8010

scanning electron microscope from the Wuhan Institute of Virology, Chinese Academy of Sciences.

Statistical analysis. Analysis of variance (SAS version 8.01; SAS Institute Inc.) was used to analyze the disease incidences and DI of each variety and root infection rates of *M. incana* and Chinese cabbage. The percentage data were arcsine transformed to ensure homogeneity of variance prior to analysis. Means separation was conducted based on Fisher's least significant difference test at $P = 0.05$.

Results

Natural infection. During 2010 to 2014, more than 200 fields in 13 provinces (municipalities) were surveyed for clubroot. The disease was observed on radish (*Raphanus sativus*), *C. bursa-pastoris*, and six *Brassica* spp., including oilseed rape, cabbage (*B. oleracea*), Chinese cabbage, flowering stalk (*B. rapa* subsp. *chinensis* var. *utiliz*), tuber mustard (*B. juncea* var. *tumida*), and sprouting broccoli (*B. oleracea* var. *italica*). Clubroot of the common weed *C. bursa-pastoris* was observed in Sichuan Province in a field in which rapeseed was heavily infected with *P. brassicae*. The disease was observed in all of the 13 provinces (municipalities) investigated, including Heilongjiang, Jilin, Liaoning, Shandong, Anhui, Henan, Shannxi, Hubei, Hunan, Chongqing, Sichuan, Yunnan, and Guizhou. Disease incidence for these plants ranged from 6.2 to 100%.

Seven species of *Brassica* were found to be hosts of clubroot in replicated field trials, including brown mustard (*B. juncea*), field mustard (*B. rapa*), potherb mustard (*B. juncea* var. *crispifolia*), turnip (*B. rapa* var. *rapa*), kale (*B. oleracea* var. *acephala*), Chinese kale (*B. alboglabra*), and kohlrabi (*B. oleracea* var. *gongylodes*). Characteristic galls were also observed on the roots of *S. alba* and violet orychofragmus (*Orychophragmus violaceus*). PCR with *P. brassicae*-specific primers yielded the expected DNA fragment of 576 bp. The sequence was identical to the published sequence of *P. brassicae*, confirming the presence of the pathogen in these plants. No amplicons were for negative controls with symptomless plant DNA as templates.

Susceptibility of *Brassica* spp. All species of *Brassica* were infected by *P. brassicae* with different disease incidences and DI, and most were highly susceptible to clubroot (Table 2). Potherb mustard, sprouting broccoli, and kohlrabi were more resistant to clubroot than turnip.

Three types of oilseed rape (*B. juncea*, *B. napus*, and *B. rapa*) also showed different levels of susceptibility to clubroot. Approximately 100 accessions of each type of oilseed rape were directly sown in the field using a random complete block design with two replicates. Various levels of resistance to *P. brassicae* were observed among 297 genotypes of oilseed rape in the field. *B. juncea* was the most susceptible to clubroot and *B. napus* was the most resistant (Table 2). More resistant genotypes (with DI < 50) were found in *B. napus*, in which 24% of the accessions had DI of 30 to 50. In particular, accessions Y031, Y059, and Y178 were highly resistant with DI < 30. No highly resistant (HR) genotypes (DI < 30) were identified in *B. rapa* and only one HR genotype (AY122) was found in *B. juncea*.

Pot trials. In all, 24 species were selected to investigate the host range of clubroot under pot-cultured conditions. In addition to the hosts confirmed in field trials, 13 species from 11 genera showed typical galls symptoms in *P. brassicae*-infested soils, including watercress (*Nasturtium officinale*), arugula (*Eruca sativa*), sweet alyssum (*Lobularia maritima*), English wallflower (*Cheiranthus cheiri*), Siberian wallflower (*C. allionii*), wallflower (*C. maritimus*), dames rocket (*Hesperis matronalis*), *I. umbellata*, woad (*Isatis tinctoria*), and four weed species (Table 1). Most plants were highly susceptible to clubroot and formed obvious and large galls on their roots, with the exception of kale and *N. officinale*, which showed slight root hypertrophy.

Infection of cruciferous weeds by *P. brassicae*. Five common weeds, including *T. salsuginea*, *S. alba*, *Capsella bursa-pastoris*, *Lepidium apetalum*, and *D. sophia*, were tested for their susceptibility to clubroot in the field and in pots. In every test, the susceptible Chinese cabbage Beijing New No. 3 showed 100% disease

incidence, thereby demonstrating the suitable conditions in the pot trials for clubroot development.

All five weed species produced macroscopic galls on the main roots in inoculated pots. The galls on the weed roots were much smaller than those on the Chinese cabbage roots. The weed roots were small and thin and the galls were correspondingly small. *T. sal-suginea* and *S. alba* exhibited the greatest clubroot incidences among the tested weeds, with an incidence of 100%. *D. sophia* exhibited the lowest incidence of 33.3%. The disease was less severe in the field than in the pots and the difference was significant ($P = 0.01$) for all tested weeds. For example, the roots of *L. apetalum* and *D. sophia* did not develop visible galls in the field, whereas the clubroot incidence ratings in pots were 84.6% for *L. apetalum* and 33.3% for *D. sophia*. For the inoculated *S. alba* and *C. bursa-pastoris*, the clubroot incidence ratings in pots were 100 and 62.5%, respectively, whereas the incidence ratings in field conditions were 72.2 and 6.2%, respectively.

Infection of *M. incana* by *P. brassicae*. In both the pot and field tests, the roots of *M. incana* inoculated with *P. brassicae* showed root reductions. However, no hypertrophy was observed, despite the fact that the indicator plant, Chinese cabbage, formed galls in the same pot that was planted with *M. incana* (Fig. 1A). SEM studies of infected Chinese cabbage roots revealed the presence of resting spores of *P. brassicae*, which were distributed throughout the host tissues (Fig. 1B). The spores were nearly circular, with spikes on the surfaces; the average spore diameter was 2.9 μm (range 2.7 to 3.2). Resting spores resembling those found in Chinese cabbage roots were also observed in the roots of *Iberis umbellata* (Fig. 1C); however, no spores were found in the roots of *M. incana* collected from the soil infested with *P. brassicae* (Fig. 1E).

Despite this, a 576-bp PCR amplicon was obtained from the DNA of *M. incana* roots. No fragment was obtained from the DNA of non-infected *M. incana* roots, suggesting that the amplified DNA band was from *P. brassicae*. No fragment was detected from DNA of

Fig. 1. Phenotypes of *Matthiola incana*, Chinese cabbage, and globe candytuft (*Iberis umbellata*) after infection with *Plasmodiophora brassicae* for 45 days and scanning electron micrographs of roots. **A**, Phenotypes of healthy *M. incana* from *P. brassicae*-free soils (Hst), diseased Chinese cabbage from *P. brassicae*-infested soils (Dcb), diseased *M. incana* (Dst), diseased *Iberis umbellata* (Dgc), and healthy *I. umbellata* (Hgc). **B**, Resting spores (rs) distributed throughout the root of Chinese cabbage after growth in *P. brassicae*-infested soil. **C**, Resting spores in the roots of *I. umbellata* after growth in *P. brassicae*-infested soil. **D**, Root of *M. incana* from sterile soil. **E**, No resting spores present in root of *M. incana* grown in *P. brassicae*-infested soil.

the rinsed control, which demonstrated that rinsing was effective for removing resting spores from root surfaces. Thus, the PCR band was not from resting spores adhering to the root surface. To confirm that the *M. incana* roots were infected by *P. brassicae*, a solution culture method was used to observe root hair infection. *P. brassicae* was

detected via PCR in both Chinese cabbage and *M. incana* as early as 2 dai (Fig. 2F). Root hair observation via microscopy indicated that no apparent pathogen structures were observed in the root hairs at 2 and 3 dai in the *M. incana* roots. Primary plasmodia were first observed in a few root hairs of the *M. incana* at 5 dai, which was

Fig. 2. Infection of *Matthiola incana* and Chinese cabbage by *Plasmodiophora brassicae* in liquid culture. **A**, Healthy root of *M. incana* incubated in Hoagland solution for 7 days (control). **B**, Root of *M. incana* with numerous infected root hairs. **C**, Primary plasmodia in the root hairs of *M. incana*. **D**, Noninfected root hairs of Chinese cabbage. **E**, Infected root hairs of Chinese cabbage. **F**, Molecular detection of *P. brassicae* via polymerase chain reaction. Lane M = marker; lanes 1 to 4 = *M. incana* at 2, 3, 5, and 7 days after inoculation (dai), respectively; lanes 5 to 8 = Chinese cabbage at 2, 3, 5, and 7 dai, respectively; lane 9 = positive control of galls from Chinese cabbage roots; lane 10 = negative control of *M. incana* roots from noninfected solution; and lane 11 = negative control of noninfected *M. incana* roots from noninfected solution. **G**, Seedlings of *M. incana* and Chinese cabbage after incubation for 45 days; H = healthy plants from control solutions and D = diseased plants from *P. brassicae*-infested solutions.

slower than in Chinese cabbage, where primary plasmodia could be seen 2 dai. More infected root hairs in *M. incana* and Chinese cabbage were detected at 7 dai, with average infection rates of 32.7 and 67.9%, respectively (Fig. 2B and E). Zoosporangia were also detected in a few root hairs (Fig. 2C). Compared with Chinese cabbage, the infection rate of *M. incana* root hairs was significantly lower ($P < 0.05$) on each observation day following inoculation (Fig. 2B, E, and F). Obvious galls developed on the roots of Chinese cabbage at 45 dai, indicating that the disease developed to the cortical infection stage (Fig. 2G). However, at the end of incubation, no galls were found on the roots of the *M. incana*.

Discussion

All members of the family Brassicaceae are potential hosts of *P. brassicae* (Dixon 2009); however, systematic studies on this subject are lacking. This study demonstrated that *P. brassicae* can infect and sporulate in the roots of 16 genera of crops, ornamentals, and weeds (Table 1); however, *M. incana* was resistant to the pathogen. Two lines of evidence support this conclusion. First, we randomly selected eight varieties (five from the United States and three from China) of *M. incana* to test resistance. None of these varieties produced galls when grown in naturally infested soil in the field or in inoculated pots; however, the Chinese cabbage plants were severely infected under similar conditions (Table 1). Second, resting spores were observed in the roots of *P. brassicae*-infected Chinese cabbage and *I. umbellata* via SEM; however, no similar structures were found in the roots of *M. incana* plants (Fig. 1). Despite the fact that specific PCR could detect *P. brassicae* DNA in *M. incana* roots and primary infections were observed in the root hairs, no clubroot symptoms developed on *M. incana* roots in all test conditions.

The clubroot disease cycle consists of two phases, a primary phase in root hairs and a secondary phase in cortical cells which leads to gall formation and yield loss (Kageyama and Asano 2009). Root hair infection occurs prior to the development of visible galls. In this study, *M. incana* did not produce galls; however, root hair infections were observed microscopically. Root hair infection has been reported in resistant (Hwang et al. 2011) and even in nonhost plants, such as papaya and clover (Ludwig-Müller et al. 1999). Differences were reported by Hwang and others (2011) in resistant and susceptible canola, which indicated that the incidence of root hair infection was two- to threefold higher in susceptible than in resistant cultivars. Similar results were obtained in this study. Primary infection in *M. incana* occurred later and the infection rate was significantly lower than in Chinese cabbage. The incidence of root hair infection in *M. incana* roots at 7 dai was 32.7%, compared with 67.9% for Chinese cabbage.

Cortex infection is the period during which susceptibility or resistance becomes apparent. The secondary phase is reduced or delayed in resistant plants (Diederichsen et al. 2009; Donald et al. 2008; Gludovacz et al. 2014). Because root hair infection causes no yield loss, a crop that has only root hair infection or stimulates resting spore germination could be planted to reduce resting spore populations in heavily infested fields. These plants can serve as bait crops. Various plants such as leafy daikon (*R. sativus*), red clover (*Trifolium pratense*), bentgrass (*Agrotis palustris*), and perennial ryegrass (*Lolium perenne*), which had similar responses to clubroot (root hair infection only) as *M. incana*, are known to reduce clubroot severity (Ahmed et al. 2011; Murakami et al. 2000). In the current study, no cortical infection was observed in *M. incana* roots, suggesting that *M. incana* can function as a potential bait crop for controlling clubroot. However, more work is required before *M. incana* can be used as a management strategy.

Previously, Liu (2012) reported the occurrence of *M. incana* clubroot in 2006 in Yunnan Province of China. Such contradictory results could be due to the differences in pathotypes of *P. brassicae* and the different *M. incana* varieties used. Physiological races have long been known to occur in *P. brassicae*. Numerous sets of differential hosts have been proposed to identify races of *P. brassicae* (Buczacki et al. 1975; Williams 1966), with the Williams differential system being the most commonly used in China. In total, eight

races were identified in 12 provinces and municipalities of China using the Williams differentiation system (Ji et al. 2013; Peng et al. 2013; Shen et al. 2009). Race 4 represents 78.5% of the population characterized in China thus far. Race 4 was also thought to be predominant on cruciferous plants and in the process of spreading the disease throughout China (Chai et al. 2014). Other races, such as 1, 2, 7, 9, 10, 11, and 13, have also been identified with limited occurrence. The physiological race of *P. brassicae* in our sampling sites was pathotype 4, as determined using the Williams differentiation system (Ji et al. 2013); however, the pathotype of *P. brassicae* in Liu's experiments was not known. Moreover, several pathotypes may occur in one population (Manzanares-Dauleux et al. 2001). Although pathotype 4 was identified in the sampling locations in 2013, the pathotype composition of *P. brassicae* populations may change in response to the selection pressure provided by the cultivars used (Diederichsen et al. 2009). Therefore, in order to determine the host range of a specific pathotype, further studies must use single-spore isolates.

Resistance is the most effective strategy for clubroot management. Over the past decades, many efforts have been made to screen for clubroot resistance, which has been reported in some crops, including Chinese cabbage, field mustard, kale, radish, and oilseed rape (Crisp et al. 1989; Peng et al. 2014; Zhang et al. 2015). Among the 297 accessions of oilseed rape of three *Brassica* spp., three accessions of *B. napus* and one accession of *B. juncea* were highly resistant to clubroot and these resistant resources provide options for disease management. *M. incana* was found to be nearly immune to clubroot. Accordingly, the eight *M. incana* varieties used in the current study are good sources of resistance to the predominant *P. brassicae* pathotype in China.

In our study, many cultivars and weeds were highly susceptible to clubroot. Effective management of susceptible cruciferous weeds is important in clubroot management. Susceptible weeds may play an important role in providing *P. brassicae* primary inoculum within the cropping season. *C. bursa-pastoris*, *S. alba*, *Thellungiella salsuginea*, *D. sophia*, and *Lepidium apetalum* are common weeds in China that showed obvious root hypertrophy after inoculation with *P. brassicae*. Naturally clubroot-infected *C. bursa-pastoris* and *S. alba* were also found in the field. These cruciferous weeds may serve as alternative hosts for *P. brassicae* in the absence of susceptible crops and may contribute to the persistence of the pathogen.

The current study confirmed the universal hosts of *P. brassicae* (including *Brassica* cultivars and radish) and identified an additional 16 host species, including 3 crops (*N. officinale*, *E. sativa* and *Isatis tinctoria*), 8 ornamentals (*Lobularia maritima*, *Cheiranthus cheiri*, *C. allionii*, *C. maritimus*, *H. matronalis*, *Iberis umbellata*, *O. violaceus*, and *Malcolmia maritima*), and 5 weeds (*Capsella bursa-pastoris*, *D. sophia*, *Lepidium apetalum*, *T. salsuginea*, and *S. alba*). The current study also showed that the cruciferous ornamental *Matthiola incana* was highly resistant to *P. brassicae*. Primary infection of root hairs occurred in *M. incana* but secondary infection in cortical cells did not occur, resulting in no gall formation. Consequently, *M. incana* has the potential to be developed as a bait crop. This study also highlights the importance of controlling cruciferous weeds for effective management of clubroot.

Acknowledgments

We thank W. Chen of United States Department of Agriculture–Agricultural Research Service, Washington State University, for language editing.

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