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RESEARCH ARTICLE

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Isolation and identification of *Phytophthora capsici* in Guangdong Province and measurement of their pathogenicity and physiological race differentiation

Abstract Five isolates collected from different pepper-cropping regions in Guangdong Province, China were determined. Based on their morphological characteristics and symptoms after being re-inoculated to pepper, these isolates were identified as *Phytophthora capsici* Leonian. The sporangia induced on carrot medium (CA) were morphologically similar and most of their shapes were ovate or elliptic, and obviously papillate. The mean size of the sporangium was 40.8−45.9 (*l*) μm × 23.2–30.9 (*b*) μm, with *l*/*b* ratio 1.4–1.8. There were evident differences in mycelial growth rate, productivity of sporangia and pathogenicity to pepper among the isolates. A test of physiological characteristics showed that one isolate was determined as Race 1, and the other four isolates belonged to Race 3. It is concluded that Race 3 is most likely to be the predominent race in Guangdong Province, China.

Keywords pepper phytophthora blight, *Phytophthora capsici*, pathogenicity, physiological race

1 Introduction

Phytophthora blight, caused by *Phytophthora capsici* Leonian is one of the most destructive diseases in pepper production worldwide. *P. capsici* has highly infectious capacity and mainly spreads in soil and /or rain, and therefore its outbreak may occur in a short time. Up to the present, this disease has been reported in many areas of China, with large economic losses brought and ever increasing in severity in recent years (Lu et al., 1995; Yang et al., 2004; Peng et al., 2005). During the last decade, pepper was commercially cultivated as a vegetable for northern markets in Guangdong Province, China and its cropping area reached 166.7 thousand hectares. As a result of increasing in area and continuous cropping of pepper, and also due to less resistant varieties available and limited chemical control, this disease has been becoming more and more serious, particularly in Guangdong where the hot and wet conditions are conducive to this pathogen, which can commonly cause 20%–30% losses of pepper in yield, with the highest figure of up to 80%. Concerning the isolation and identification of *P. capsici* in Guangdong, China, Wang et al. (2001) reported three isolates of *P. capsici*, finding that there was a distinct difference in pathogenicity to pepper. In order to further characterize isolates of *P. capsici* in Guangdong Province, China and provide the basic data for the breeding of *P. capsici* resistant cultivars, several isolates were isolated and identified from different pepper-cropping regions in Guangdong Province, China and their physiological race differentiation was also determined.

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2 Methods

2.1 Isolation of pathogen

Isolation of pathogen was conducted according to the method of Zheng (1997). Infected peppers or stems with representative symptoms of *P. capsici* were collected from diseased fields in five areas of Guangdong, China. After being rinsed with tap water for more than 12 h, the tissues between infected and health parts of stems or peppers were cut into two to three pieces and then plated on a medicated carrot medium (CA) (Penicillin 50 mg, derosal 5 mg, guintozene 50 mg, rifampin 100 mg, carrot juice 200 g, agar 20 g/L), a half-selective culture medium for *P. capsici.* Following inoculation for two days at 25°C–28°C, the isolates were purified by picking out the mycelia at the edge of colony and transferring them to the CA medium without medicament, followed by incubating for three days under the same conditions. After purification, mycelial plugs were cut with a scalpel and inoculated back to

stems of pepper plants, the isolates that caused symptoms similar to those of plants in fields were used for identification of pathogen. The isolation origin and the infected tissues of isolates used in this study are shown in Table 1.

2.2 Identification of pathogen

For identification of pathogen, the purified isolates were transferred to the center of petri plates of CA medium and cultivated at 25°C–28°C for three days, after which the mycelial plugs with 4 mm in diameter, cut from the edge of the colony, were moved onto a new CA medium and then incubated for another three to five days at an invariable temperature of 28°C in darkness. Subsequently, the characteristics of colony and mycelium were observed. To induce sporangia, the cultures were incubated for five to seven days at 28°C with continuous lighting. Thereafter, some mycelial plugs with induced sporangia were picked out and transferred into a test tube with 1 mL distilled water added into it. Sporangia were disengaged by smacking the test tube with hand. The morphology and size of sporangia, as well as the height of papilla and length of sporangiophore were observed or measured using microscopy.

2.3 Mycelial growth rate and ability of producing sporangia

Mycelial growth rate and the ability of producing sporangia of the isolates were measured on OMA (oat 60 g, agar 12.5 g/L), TA (tomato juice 200 mL, $CaCO_3$ 4 g, agar 20 g/L), PSA (potato 200 g, sucrose 20 g, agar 20 g/L) and CA media. Mycelial plugs of purified isolates were transferred to the center of 9-cm petri plates containing the before-mentioned media, with three petri dishes each isolate, and semidiameter of colony was measured at four different directions after being incubated for three days at 25°C in darkness. After measurement, the cultures were continuously cultivated for seven days at the same temperature with continuous lighting to induce sporangia. The number of sporangia in four randomly selected viewing fields was recorded under a microscope.

2.4 Pathogenicity of isolates

2.4.1 Pepper varieties and seedling raising

Eight varieties (lines) of pepper, namely "Perennial", "Guangjiao No. 2", "Gaofengyangjiao", "Luzaosheng", "97031", "Maojiao No. 7", "198-1-2"and "Qiemen", were used to exam the pathogenicity of the isolates. Seedlings were raised in separated plastic flats with 100 cells (hxφ = 7 cm × 5 cm). Seeds of these varieties were sown in a mixed medium containing sterilized peat and vermiculite (3 : 1, v/v). Before sowing, pregermination of seeds was performed at 25°C–28°C for four to five days. One or two of germinated seeds were sown in each cell on August 6 and 15 in 2005, respectively. The seedlings were thinned to one plant for each cell at the cotyledon stage and inoculated at six- and seven-leave stages.

2.4.2 Inoculation and disease rating

Sporangia production was induced on the CA medium (9-cm petri) as described in the 2.4.1 section. After adding 20 mL of sterilized water into each petri dish, we transferred them to a refrigerator to incubate them for one hour at 4°C, and then onto a laboratory bench at the room temperature to stimulate zoospore release. The number of zoospores was counted with a hemacytometer and the concentration of zoospore suspension adjusted to 2×10^4 zoospore/mL as the wanted inoculum. Prior to inoculation, seedlings were sufficiently irrigated and inoculated by injecting 3 mL of the zoospore suspensions into the medium at a distance of 1–2 cm from the seedling stem with an injector. The inoculated seedlings were then kept moist at least for 12 h; 30–33 plants for each variety were inoculated and one susceptible plant was also injected with 3 mL of distilled water as a control. After inoculation, the plants were fully watered, usually twice daily, to maintain a high media-moisture. During the experiment, the average minimum and maximum temperatures were 25.1°C and 37.0°C, respectively. Disease severity of each individual plant was rated three days after inoculation based on the standard scale described by Yi et al. (2003), and the disease index of each variety was calculated thereafter.

2.5 Determination of physiological race

2.5.1 Characterization of hosts

Four hosts for testing physiological race of *P. capsici* were "Early calwonder", "CNPH703", "PBC602" and "PI201234", which were provided by Dr. Wang of Asian Vegetable Research and Development Center (AVRDC). "Early calwonder" is highly susceptible to *P. capsici*, and thus considered as a host that does not bring any resistant gene. Other three hosts with different resistant genes can be combined

Table 1 Origin, host and infected tissue of isolates

Isolate	Code	Origin	Host/variety	Infected tissue	Date of isolation (year/month/day)
Pc.1	ZLT0566	Zhongluotan, Guangzhou	Pepper/Guangjiao No. 2	Stem	05/05/06
Pc.2	HZ05628	Huicheng, Huizhou	Pepper/Dongfangshenjian	Pepper	05/06/28
Pc.3	ZH0575	Doumen, Zhuhai	Pepper/Huaye No. 39	Pepper	05/07/05
Pc.4	LZ0579	Baiyunzhuang, Liangzhou	Pepper/Maojiao No. 4	Stem	05/07/09
Pc.5	YD05720	Xihutang, Yinde	Pepper/Unknown	Pepper	05/07/20

each other to distinguish Races 1, 2 and 3 of *P. capsici* (Table 2).

Table 2 Physiological race characterization of *Phytophthora capsici*

Host	Physiological race		
	Race 1	Race 2	Race 3
Early calwonder	S	S	S
CNPH703	R	S	S
PBC602	R	R	S
PI201234	R	R	R

Note: S = susceptible type and R = resistant type.

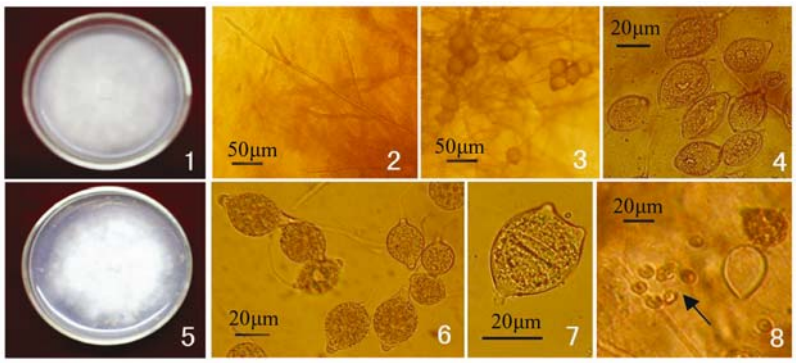
2.5.2 Identification of physiological race

Seedling raising of hosts, sporulation induction of isolates and inoculation were conducted in the same ways as described above. The concentration of inoculum was 1×10^5 zoospore/mL. When seedlings grew up with six to seven leaves, a set of hosts was inoculated with each testing isolate and 30–33 plants were used for each host. Disease on the base part of each plant was individually scored weekly and the percentage of diseased plants 21 days after inoculation was used to determine the physiological race of the isolates. The susceptible or resistant reaction type of the hosts was categorized as follows: susceptible type = disease rate > 20%, and resistant type = disease rate ⩽ 20%.

3 Results

3.1 Morphological characteristics of isolates

When the purified isolates collected from five regions in Guangdong Province were cultivated on the CA medium for three days, all of them formed white colonies, except that the colony of the Pc.3 showed a dense cottony shape, the other isolates produced a petal like colony with sparse mycelium. The growing hypha of all the isolates on the CA medium was hyaline, and branching irregularly with no septum (Plate I). The sporangia of five isolates were all similar in shape with light brown color, and most of them were ovoid or ellipsoidal, usually with an evident single papilla, occasionally double papillae could be seen. For all isolates, no chlamydospores produced, and pairing isolates with each other did not produce oospores, indicating a same mating type. With all five isolates, the sporangial size, length of sporangiophore and height of papilla varied depending on different growth stages, but no significant differences could be found among five isolates in the average range of 40.8–45.9 μm in length, 23.2–30.9 μm in width, and 1.4–1.8 *l*/*b* ratio for sporangia, and 32.4–35.6 μm length for sporangiophore, and 5.1–5.9 μm height for papilla (Table 3). Based on the morphology of hypha, the sporangial characteristics and its size, as well as the symptom as inoculated to pepper plants, all the five isolates could be considered as *P. capsici* Leonian.



Note: 1 and 2 stand for colony of Pc.1 and mycelium of Pc.1, respectively; 3 and 4 for sporangia of Pc.1; and 5, 6, 7, and 8 for colony of Pc.3, sporangia of Pc.3, double papilla of Pc.1 and released zoospore of Pc.3, respectively.

Plate I Morphology of colony and sporangia of the isolates Pc.1 and Pc.3

Table 3 Morphological characteristics of sporangia of isolates

Isolate	Sporangia[a)]			Height of papilla /μm	Length of sporangiophore /μm
	Length /μm	Width /μm	*l*/*b* ratio		
Pc.1	25.5–60.5(45.9)	20.5–40.8(30.9)	0.7–2.7(1.5)	4.0–8.6(5.9)	18.2–55.8(35.6)
Pc.2	20.8–60.4(43.3)	19.4–66.2(28.1)	1.0–2.2(1.6)	3.2–7.2(5.3)	20.0–58.2(35.2)
Pc.3	30.2–60.2(44.4)	20.0–38.2(27.7)	1.1–2.5(1.6)	3.5–8.0(5.4)	15.8–65.2(35.1)
Pc.4	21.6–58.2(40.8)	20.4–36.2(28.2)	1.0–2.4(1.4)	3.2–7.3(5.4)	15.2–58.1(32.4)
Pc.5	31.6–66.4(42.4)	17.5–27.8(23.2)	1.5–2.4(1.8)	3.2–9.2(5.1)	16.5–65.2(35.3)

Note: [a)] represents sporangia produced on the CA medium, and the data in bracket are average values.

3.2 Growth rate of mycelium

The mycelial growth rates of the five *P. capsici* isolates measured on different media are shown in Table 4. It was found that these five isolates had an evidently different rate of mycelium on varied media. All the purified isolates exhibited the fastest growth of mycelium on OMA medium, but with an extremely sparse hypha, followed by those on CA and PSA media where they formed a plimmed candour colony with clear boundaries. On TA medium, however, all of the five isolates grew most slowly and their colonies had irregular appearance. The differences in the growth rate of mycelium among the five isolates were almost the same on the four media. Based on the mean value of mycelial growth rates on the five media, Pc.1, Pc.4 and Pc.5 had no significant difference in the rate, but grew faster than Pc.2 or Pc.3, followed by Pc.2 and Pc.3, respectively.

Table 4 Growth rate on different media of *Phytophthora capsici* isolates from Guangdong

Isolate	Growth rate at linear direction /(mm·d^{-1})				
	OMA	TA	PSA	CA	Mean
Pc.1	15.6A	9.3A	10.7A	10.9A	11.6
Pc.2	15.0B	7.4BC	9.6BC	10.6A	10.6
Pc.3	11.7C	5.7C	9.0C	8.8B	8.8
Pc.4	15.5AB	9.5A	10.7A	10.9A	11.6
Pc.5	15.1AB	8.9AB	10.3AB	10.4A	11.2
Mean	14.6	8.1	10.0	10.3	

Note: Data with same letters indicate no significant difference by Duncan's test at $P < 0.01$ ($n = 4$).

3.3 Capability of producing sporangia

The capability of producing sporangia of five isolates was measured at 25°C. Figure 1 shows that the sporulation ability of the five isolates varied depending on the cultural media. The isolates Pc.1, Pc.4 and Pc.5 produced the most amounts of sporangia on CA medium, with the less on OMA and TA media, and the least on PSA medium. However, Pc.2 and Pc.3 isolates could produce a large amount of sporangia on CA, OMA and TA media, where no significant difference existed among them, and also least on PSA medium. As for the difference in sporulation ability between isolates, Pc.1, Pc.4 and Pc.5 had greatest capability for producing sporangia followed by Pc.2, and Pc.3 was the worst.

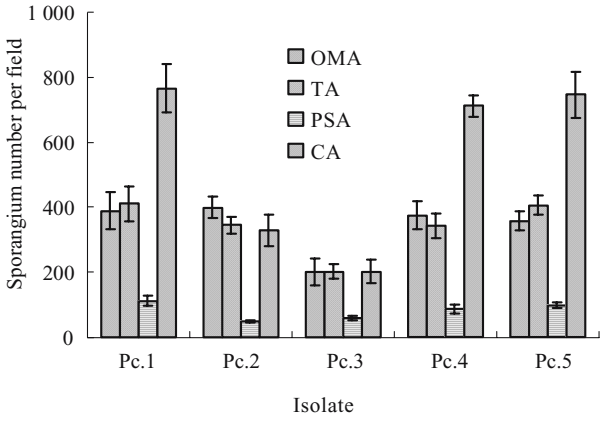


Fig. 1 Ability of sporangia production on different media of five *Phytophthora capsici* isolates from Guangdong, China

3.4 Difference of pathogenicity

Eight hot (bell) pepper varieties were used to exam the pathogenicity of the isolates from different regions of Guangdong by using a root-irrigating inoculation method. The disease index, three days after inoculation, showed that there was a significant difference in pathogenicity among the five isolates (Table 5). Based on the average data of eight varieties, the pathogenicity to pepper was Pc.1 > Pc.5 > Pc.4 > Pc.2 > Pc.3 , namely, the Pc.1 isolate collected from Baiyun (Guangzhou) had the strongest infectivity to pepper, which was three times as strong as Pc.3 with the least virulence, and the second strongest was the two isolates from Yingder and Liangzhou, and the virulence of the isolate obtained from Huizhou was comparatively lower.

3.5 Identification of physiological race

The five isolates of *P. capsici* from Guangdong were determined for physiological race by using the host identification method provided by Dr. Wang of AVRDC. The result shown in Table 6 indicated that Pc.1, Pc.2, Pc.4 and Pc.5 belonged to Race 3, and Pc.3 to Race 1, and none from them was found belonging to Race 2, thus Race 3 accounted for 80% in the tested isolates.

4 Discussion

In the present study, the five isolates from different areas in Guangdong Province have been identified. Based on the findings that the morphology and average size of their sporangia produced on the CA medium have no significant differences, and contradistinguishing with the classification standard for *Phytophthora* detailed by Zheng (1997) and Lu et al. (1984), and these five isolates can be confirmed as *P. capsici* Leonian.

Recent studies suggest that various isolates of *P. capsici* have different pathogenicity and physiological race differentiation exists within *P. capsici* (Polach and Webster, 1972; Black, 1999; Oelke and Bosland, 2003). Oelke and Bosland (2003) investigated ten isolates originated from New Mexico, Italy, Turkey and Korea by using 18 pepper hosts with significant differences in resistance or susceptibility to *P. capsici*, and identified nine isolates as different physiological races for phytophthora root rot, and four as different races for phytophthora foliar blight.

Table 5 Pathogenicity of *Phytophthora capsici* isolates from Guangdong

Isolate	Location	Index of disease three days after inoculation								
		Perennial	Guangjiao No. 2	Gaofeng yangjiao	Luzaosheng	97031	Maojiao No. 7	198-1-2	Qiemen	Mean
Pc.1	Baiyun	3.0HR	33.9MR	50.3S	55.8S	62.4S	58.2S	61.2S	60.6S	48.2
Pc.2	Huizhou	2.4HR	14.5R	25.5R	57.0S	57.0S	50.3S	34.5MR	51.5S	36.6
Pc.3	Zhuhai	0.0 I	6.1HR	2.0HR	18.2R	15.8R	17.6R	27.9MR	40.0MR	16.0
Pc.4	Lianzhou	0.0 I	4.8HR	33.3MR	35.2MR	55.2S	49.1MR	61.3S	68.5S	38.4
Pc.5	Yingde	1.3HR	29.7R	41.2MR	48.5MR	56.4S	52.7S	57.3S	56.0S	42.9

Note: I stands for immunity ($DI = 0$), HR for highly resistant ($0 < DI \leqslant 10$), R for resistant ($10 < DI \leqslant 30$), MR for moderately resistant ($30 < DI \leqslant 50$), and S for susceptible ($DI > 50$).

Table 6 Identification of the physiological races of *Phytophthora capsici* isolates from Guangdong

Isolate	Responses to hosts				Result
	Early calwonder (0)	CNPH703 (Race 1)	PBC602 (Races 1, 2)	PI201232 (Races 1, 2, 3)	
Pc.1	S(100.0)	S(100.0)	S(90.9)	R(6.1)	Race 3
Pc.2	S(100.0)	S(100.0)	S(93.9)	R(3.0)	Race 3
Pc.3	S(100.0)	R(12.3)	R(6.1)	R(0.0)	Race 1
Pc.4	S(100.0)	S(100.0)	S(87.6)	R(3.3)	Race 3
Pc.5	S(100.0)	S(100.0)	S(93.9)	R(6.7)	Race 3

Note: Data in bracket are the morbidity (%) 21days after inoculation.

Our study showed that the five isolates of *P. capsici* also differed in colony morphology, growth rate of mycelium and pathogenicity, among which the Pc.3 isolated from Zhuhai had a denser floss colony, compared with the other four isolates. Pc.3 not only exhibited a slower growth of mycelium on OMA, TA, PSA and CA media, but also had the lowest capability of producing sporangia and pathogenicity to pepper. Identification with a set of differential hosts provided by AVRDC revealed that Pc.3 belonged to Race 1 and the other five isolates to Race 3, thus Race 3 was the dominant among the three races. It seems that Race 3 of *P. capsici* is the superior race in Guangdong Province. The reason may be its stronger pathogenicity and sporulation ability. To confirm the fact, further studies are needed with more isolates from the whole province.

In addition, the studies conducted by AVRDC on physiological race differentiation within *P. capsici* of Taiwan showed that Race 3 has become the predominating race in Taiwan in recent years. In their results, it can also be found that the resistance to the three races appears to be additive among resistant sources, i.e. Race-1-resistant lines may be resistant only to Race 1, but Race-2-resistant lines carry equal or greater resistance to Race 1, and Race-3-resistant lines have equal or greater resistance to Races 1 and 2. In our study, although Race 2 could not be found in the isolates determined, the resistances to Races 3 and 1 of the tested varieties have shown a similar tendency, which makes it possible to breed three races-resistant pepper varieties in Guangdong by using a Race 3 isolate for identification of resistance and screening for the disease resistant sources.

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