Pathotypes of *Colletotrichum capsici*, the Causal Agent of Chili Anthracnose, in Thailand

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ABSTRACT

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Eleven isolates of *Colletotrichum capsici* were screened on nine chili genotypes derived from four cultivated species of *Capsicum*: *Capsicum annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens*. Host reactions were assessed 9 days after inoculation by microinjection of spores into the pericarp of red fruit. A set of disease scales, with 0 to 9 scores, were developed for anthracnose infection of each *Capsicum* sp. based on percent lesion size in relation to fruit size, appearance of necrotic or water-soaked tissue, and presence of acervuli. Three pathotypes, PCc1, PCc2, and PCc3, were identified according to differential qualitative infection of fruit of *C. chinense* genotypes PBC932 and C04714. PCc1 was the most virulent pathotype, infecting all genotypes of *C. annuum*, *C. chinense*, and *C. frutescens*, whereas PCc3 was the least virulent pathotype, infecting only the genotypes *C. annuum* and *C. frutescens*. Quantitative infection occurred in all chili genotypes except for genotypes of *C. baccatum*, where no infection occurred, demonstrating various levels of aggressiveness of isolates within pathotypes.

Chili (*Capsicum* spp.) is the fourth most important vegetable in the world and the first in Asia, with world production in 2006 at approximately 25.9 million t for fresh chili and 2.8 million t for dry chili (1). Thailand produces 420,000 t of chili fruit from 72,000 ha of chili plants with an approximate value of US\$136 million (4). Anthracnose, caused by *Colletotrichum* spp., is a serious problem for chili production in the tropics and subtropics worldwide. In Thailand, severe epidemics resulted in yield losses of up to 80% (7). At least three species of *Colletotrichum*, *Colletotrichum capsici*, *C. gloeosporioides*, and *C. acutatum*, have been identified as important pathogens causing chili anthracnose in Asia. In Thailand, *C. capsici* and *C. acutatum* have been reported to be the most important pathogens causing anthracnose of chili, although *C. acutatum* was mostly restricted to the north and north east of Thailand (10).

Management of chili anthracnose mainly has been through the use of fungicides. However, fungicide applications are only partially effective under environmental conditions that are favorable for pathogen infection. Furthermore, fungi-

cides are not sustainable, especially in small-holder farming systems in Thailand, due to the high cost and risks to the environment. Breeding for resistance has mainly focused on introgression of resistance from *Capsicum chinense* and *C. baccatum* into elite susceptible genotypes of *C. annuum* (5). For breeding programs to successfully develop resistant genotypes, knowledge of the variability and pathotype structure within the pathogen population is important.

Sharma et al. (8) reported the existence of 15 pathotypes of *Colletotrichum capsici* from the Himachal Pradesh area of northern India based on quantitative differences in lesion development on inoculated fruit of *Capsicum annuum* genotypes. Than et al. (10) showed pathotype differences within *Colletotrichum acutatum* isolates from infected strawberry and chili fruit. Isolates from chili were able to infect inoculated fruit of the resistant *Capsicum*

chinense genotype PBC932, whereas isolates from strawberry were unable to infect this genotype. Both isolates were able to infect the susceptible *C. annuum* chili genotype Bangchang. Pathotype differences in chili were identified based on the size of lesions that developed on inoculated fruit (10).

Fruit size and shape of genotypes from different *Capsicum* spp. vary considerably; therefore, to standardize host reactions between *Capsicum* spp., Kanchana-udomkan et al. (2) and Pakdeevaporn et al. (5) measured host reactions based on lesion size as a proportion of fruit size. The development of disease scales for measuring host reactions that incorporated fruit size and severity of infection (lesion size, presence of acervuli, and necrosis or water-soaked tissue) would be appropriate for assisting in screening many isolates across a range of genotypes from different *Capsicum* spp.

The aims of this study were to develop a set of disease scales for efficient, accurate measurement of the severity of infection in a range of *Capsicum* spp. and to identify pathotypes of *Colletotrichum capsici* based on qualitative differences of infection in differential genotypes of *Capsicum* spp.

MATERIALS AND METHODS

***C. capsici* collection.** Ten *C. capsici* (Syd.) E.J. Butler & Bisby isolates were collected from chili fruit infected with anthracnose in western Thailand (Kanchanaburi, Nakhon Pathom, Suphan Buri) and one from northern Thailand (Chiang Mai) (Table 1). Isolations and establishment of single-spore cultures were carried out according to the procedures of Photita et al. (6) and Than et al. (10).

Table 1. Eleven single-spore isolates of *Colletotrichum capsici* used in the chili pathotype study

No.	<i>Colletotrichum</i> isolate	Origin
1	F4-2C	Kanchanaburi, western Thailand
2	F4-5A	Kanchanaburi, western Thailand
3	F7-3A	Nakhon Pathom, western Thailand
4	F8-1A	Suphan Buri, western Thailand
5	F8-2A	Suphan Buri, western Thailand
6	F8-3A	Suphan Buri, western Thailand
7	F8-3B	Suphan Buri, western Thailand
8	F8-4C	Suphan Buri, western Thailand
9	F8-5A	Suphan Buri, western Thailand
10	F8-5B	Suphan Buri, western Thailand
11	SKP16	Chiangmai, northern Thailand

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Colletotrichum cultures and inoculum preparation. A conidial suspension of each *C. capsici* isolate was prepared from conidia that were harvested from 7-day-old cultures grown on potato dextrose agar (PDA; Difco, Becton, Dickinson and Company, Sparks, MD) under 12 h of darkness and 12 h of light at 28 to 30°C. The *C. capsici* culture plates were flooded with sterilized water and the conidia gently scraped from the culture plate using a sterilized loop. The conidial suspension was adjusted to a concentration of approximately 10⁶ conidia/ml.

Chili genotypes. Nine chili genotypes of *Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., and *C. frutescens* L. were screened for reaction to the *Colletotrichum capsici* isolates. Of the nine genotypes, Bangchang, Jinda, and Kheenoo Suan were Thai commercial cultivars and the rest originated from AVRDC (The World Vegetable Center, Tainan, Taiwan).

Pathogenicity study. Five fruit at red-ripe stage (40 to 50 days after flowering, depending on genotype) harvested from each chili genotype were inoculated with each *C. capsici* isolate using an injection method. After the calyxes were removed, the fruit were surface sterilized with 1% (wt/vol) sodium hypochlorite for 5 min and then rinsed twice with distilled water. Approximately 1,000 spores in 1 µl of suspension were injected once into the chili fruit pericarp in the middle of each fruit using a microinjector consisting of a Micro Syringe model 1705 TLL and a dispenser PB600-1 (Hamilton, Switzer-

land) to ensure equal inoculum volume in each injection. The needle of the microinjector was 1 mm in diameter and was fixed at 1 mm long, ensuring uniform wound size on all fruit. The inoculated fruit were placed in plastic boxes (20 by 30 by 10 cm) on a tray with 500 ml of water in the bottom and incubated at 28°C, 12 h of darkness and 12 h of light, and 100% relative humidity. After 3 days, lids were removed from the boxes to maintain 70 to 80% relative humidity.

Anthrachnose symptoms at the inoculation site were evaluated at 9 days after inoculation on the basis of the percent lesion size relative to the overall size of the fruit. The size of the lesions was visually estimated following the method of Pakdevaraporn et al. (5). Data for all isolates and genotypes, except for *Capsicum baccatum* genotypes, which did not become infected, were included in analysis of variance using factorial (11 isolate by 6 genotype) in a completely randomized design (SPSS software version 13-0, SPSS Inc.; 3) to test for main treatments and interaction effects. Duncan's multiple range and least significant difference tests were used for means separations ($P = 0.05$). Each result was arcsin square root transformed before analysis and the means were back transformed for presentation. All experiments were repeated and gave identical results.

Disease scales were developed for each *Capsicum* sp., in which disease severity was scored on a 0-to-9 scale, where 0 was no infection and 9 was infection greater than 25%.

Pathotypes were identified where qualitative differences in infection (0 versus 1-to-9 scores) occurred for isolates on a specific chili genotype. Differences in lesion size were an indication of the levels of aggressiveness of each isolate.

RESULTS

Differential reactions of chili genotypes to anthracnose infection. The genotypes of *Capsicum* spp. used in the bioassay exhibited a range of host reactions to infection by the 11 isolates of *Colletotrichum capsici* (Table 2). *Capsicum frutescens* and *C. annuum* were highly susceptible, with mean percent lesion size ranging from 27% for *C. annuum* Bangchang to 98% for *C. frutescens* Kheenoo Suan, with the latter being significantly more susceptible to infection than the three *C. annuum* genotypes. Among the *C. annuum* genotypes, there was a range of disease severity, with 83-168 (89%) being significantly more susceptible to all isolates than Bangchang (35%) and Jinda (27%). The *C. chinense* genotypes C04714 and PBC932 were susceptible (19%) to moderately susceptible (12%), respectively. However, within each of the two *C. chinense* genotypes, there was a range of disease severity reactions from 0 to 59% lesion size. *C. baccatum* genotypes were the most resistant with no infection.

Aggressiveness of *Colletotrichum capsici* isolates. Differences among pathogen isolates in quantitative level of infection occurred in all genotypes of *Capsicum* spp. except for *Capsicum baccatum*, where no

Table 2. Mean percent lesion size of 9 chili genotypes at 9 days after inoculation with 11 *Colletotrichum capsici* isolates^x

Isolates	<i>Capsicum annuum</i>			<i>C. baccatum</i>			<i>C. chinense</i>		<i>C. frutescens</i>	
	Bangchang	83-168	Jinda	PBC 80	PBC 81	PBC 1422	PBC932	C04714	Kheenoo Suan	Mean ^y
F4-2C	11 st	100 a	27 klmno	0	0	0	0 u	11 t	100 a	44 F
F4-5A	32 jklm	100 a	35 hijkl	0	0	0	0 u	30 klm	100 a	54 D
F7-3A	36 hijk	62 def	19 opqr	0	0	0	0 u	0 u	95 b	28 G
F8-1A	66 d	100 a	27 klmno	0	0	0	53 gf	55 f	100 a	75 A
F8-2A	29 klmn	100 a	25 lmnp	0	0	0	22 mnopq	23 mnop	81 b	51 DE
F8-3A	27 klmno	100 a	25 lmnp	0	0	0	30 klm	21 nopq	100 a	58 C
F8-3B	56 ef	100 a	35 hijkl	0	0	0	18 pqrs	27 klmnop	100 a	65 B
F8-4C	19 opqr	100 a	41 hij	0	0	0	59 def	25 lmnp	100 a	66 B
F8-5A	68 d	100 a	29 klmn	0	0	0	0 u	34 ijkl	100 a	60 C
F8-5B	35 hijkl	42 hi	25 lmnp	0	0	0	33 ijkl	27 klmnop	100 a	48 E
SKP16	12 rst	65 de	15qrst	0	0	0	0 u	0 u	45 gh	15 H
Mean ^z	35 C	89 B	27 D	0	0	0	12 F	19 E	98 A	...

^x Values for pathogen isolate–host genotype combinations with the same letter are not significantly different ($P = 0.05$) based on Duncan's multiple range test.

^y Means for isolates exclude reactions of *C. baccatum* genotypes. Values in the column with the same letter are not significantly different ($P = 0.05$) based on Duncan's multiple range test.

^z Values in the row with the same letter are not significantly different ($P = 0.05$) based on Duncan's multiple range test.

Table 3. Anthracnose severity scores on chili fruits, chili resistance levels, and symptom description

Score	Resistance level	Symptom details
0	HR, highly resistant	No infection
1	R, resistant	1–2% of the fruit area shows necrotic lesion or a larger water-soaked lesion surrounding the infection site
3	MR, moderately resistant	>2–5% of the fruit area shows necrotic lesion, acervuli may be present, or water-soaked lesion up to 5% of the fruit surface
5	MS, moderately susceptible	>5–15% of the fruit area shows necrotic lesion, acervuli present, or water-soaked lesion up to 25% of the fruit surface
7	S, susceptible	>15–25% of the fruit area shows necrotic lesion with acervuli
9	HS, highly susceptible	>25% of the fruit area shows necrosis, lesion often encircling the fruit; abundant acervuli

infection occurred (Table 2). There were significant differences in the level of aggressiveness of the isolates, with F8-1A being the most aggressive, resulting in 75% mean lesion size on all host genotypes (excluding the *C. baccatum* genotypes), followed by F8-4C (66%) and F8-3B (65%). The least aggressive isolates were SKP16 (15%) and F7-3A (28%), which did not infect any of the *C. chinense* and *C. baccatum* genotypes.

Disease scales for *Colletotrichum capsici* infection of *Capsicum* spp. A set of disease ratings of 0 to 9 was established based on the percent lesion size relative to the overall size of the fruit, appearance of infected tissue (necrotic or water soaked), and the presence of acervuli. A rating of 0 was no infection and 9 was infection greater than 25% (Table 3). A set of disease scales with disease severity based on the 0-to-9 scale was developed for fruit types of *Capsicum annuum*, *C. baccatum*, *C. frutescens*, and *C. chinense* (Fig. 1). These scales compensated for the different size of fruit and size of lesion. *C. chinense* genotypes consisted of small circular fruit while *C. baccatum* genotypes were large, elongated, and wide fruit.

Using the scales, *C. frutescens* and *C. annuum* genotypes were found to be highly susceptible, a rating of 9, to most or all pathogen isolates (Table 4). Both *C. chinense* genotypes C04714 and PBC932 reacted with ratings from 0 to 9 depending on the pathogen isolate. *C. baccatum* genotypes were the most resistant, with no infection (rating 0) by any isolate.

Pathotype identification of *Colletotrichum capsici*. Based on the qualitative differential reactions (Table 4), three pathotypes of *Colletotrichum capsici* (PCc) were identified, PCc1, PCc2, and PCc3. The PCc1 group contained isolates F8-1A, F8-2A, F8-3A, F8-3B, F8-4C, and F8-5B. These were able to infect all genotypes of *Capsicum annuum*, *C. frutescens*, and *C. chinense*, with disease scores ranging from 5 to 9, but did not infect *C. baccatum*. The PCc2 group of isolates (F4-2C, F4-5A, and F8-5A) was able to infect all genotypes of *C. annuum*, *C. frutescens*, and *C. chinense* C04714 but not *C. chinense* PBC932 or any genotype of *C. baccatum*. The PCc3 isolates (F7-3A and SKP16) infected only genotypes of *C. annuum* and *C. frutescens* but did not infect genotypes of *C. baccatum* and *C. chinense*.

DISCUSSION

Three pathotypes (PCc1, PCc2, and PCc3) of *Colletotrichum capsici* were identified from 11 isolates collected from the western and northern districts of Thailand, based on qualitative differences in infection on *Capsicum chinense* genotypes PBC932 and C04714. Sharma et al. (8) reported the existence of 15 pathotypes of *Colletotrichum capsici* from 35 isolates

collected in the Himachal Pradesh area of northern India. In contrast, these isolates were screened on nine genotypes of *Capsicum annuum* where "pathotype" differences were based on quantitative differences in the level of infection between cultivars. However, according to Taylor and Ford (9), a pathotype is defined as a group of isolates distinguished from others of the same species by a qualitative difference in disease severity. A quantitative difference in infection reflects the natural

distribution of aggressiveness within a population, ranging from low to high. Therefore, it is most likely that Sharma et al. (8) only observed differences in aggressiveness between isolates and did not identify true pathotype differences of *Colletotrichum capsici*. Moreover, only *Capsicum annuum* genotypes were used to screen for differences in infection. *C. annuum* genotypes are known to be very susceptible to anthracnose (11). The *C. annuum* genotype Bangchang used in the current study

Fig. 1. Disease diagrams representing degrees of infection ranging from scores of 0 to 9 (from left to right in each row) in various chili fruit types: **A**, large and elongate type as *Capsicum annuum* Bangchang; **B**, large Kheenoo type as *C. annuum* Jinda and 83-168; **C**, small Kheenoo type as *C. frutescens* Kheenoo Suan; **D**, *C. baccatum* type; **E**, conical type as *C. chinense* C04714; and **F**, round type as *C. chinense* PBC932.

Table 4. Anthracnose severity evaluated as disease scores on a 0-to-9 scale and pathotypes of the 11 *Colletotrichum capsici* isolates^z

Isolates	<i>Capsicum annuum</i>			<i>C. baccatum</i>			<i>C. chinense</i>		<i>C. frutescens</i>	Pathotype
	Bangchang	83-168	Jinda	PBC80	PC81	PBC1422	PBC932	C04714	Kheenoo Suan	
F8-1A	9	9	9	0	0	0	9	9	9	PCc 1
F8-2A	9	9	9	0	0	0	7	7	9	PCc 1
F8-3A	9	9	9	0	0	0	9	7	9	PCc 1
F8-3B	9	9	9	0	0	0	7	9	9	PCc 1
F8-4C	7	9	9	0	0	0	9	9	9	PCc 1
F8-5B	9	9	9	0	0	0	9	9	9	PCc 1
F4-2C	5	9	9	0	0	0	0	5	9	PCc 2
F4-5A	9	9	9	0	0	0	0	9	9	PCc 2
F8-5A	9	9	9	0	0	0	0	9	9	PCc 2
F7-3A	9	9	9	0	0	0	0	0	9	PCc 3
SKP16	5	9	5	0	0	0	0	0	9	PCc 3

^z Disease scores are as described in Table 3.

showed susceptible scores from 5 to 9 depending on the aggressiveness of the isolate.

Infection of fruit that results in blemishes and lesions diminishes the marketability of the fruit. If more than 5% infection of the fruit surface was infected, then this was rated as a susceptible reaction (disease score of 5 to 9). Infection levels greater than 25% were assigned the highest rating because such levels lead to severe impact on the survivability of the fruit, with high levels of inoculum being produced from acervuli. Although resistant (score 1) and moderately resistant (score 3) host fruit reactions were not observed for *Colletotrichum capsici* infection, these host reactions have been observed with low-aggressive isolates of *C. capsici* in Australia and were more common in genotypes infected with *C. acutatum* (*unpublished data*). In some genotypes, particularly of *Capsicum baccatum* infected by *Colletotrichum acutatum* (*unpublished data*), a resistant reaction (scores 1 and 3) on the fruit was observed as water-soaked lesions surrounding the infection site, with a notable absence of acervuli.

Knowledge of both existence of pathotypes and level of aggressiveness of the pathogen isolates is extremely important

when choosing appropriate isolates to screen germplasm and lines for resistance in plant breeding programs. Further work should include screening of more isolates, particularly from other chili-growing regions of Thailand, on the differential genotypes *Capsicum chinense* PBC932 and C04714.

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