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Four (4) Downy mildew pathotypes are present on cucumbers in the northern region of Thailand

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Abstract

Downy mildew is caused by the oomycete fungus *Pseudoperonospora cubensis* (Berk.& Curtis.,Rostovzev), one of the most prevalent pathogens affecting cucumber production worldwide. *P. cubensis* is an obligate biotroph parasite that infects a specific host range within the cucurbitaceae family. Plant disease management requires a successful disease resistance breeding program to develop host resistance that can adequately control cucumber downy mildew without the use of fungicides. To identify downy mildew resistance in cucumbers, the physiological specialization of the pathogen was studied. Pathotypes research is helpful in screening germplasm of cucumbers against *P. cubensis* to develop effective and lasting downy mildew resistance. The specificity on differential hosts of the seventeen *P. cubensis* isolates collected during 2005-2008 from cucumbers cultivated in northern Thailand were determined using artificial inoculation under greenhouse conditions. Downy mildew reactions were assessed within 7 days after inoculation. These isolates were classified into pathotypes 1, 2, 3 and 4 and it was found that they had 1, 3, 11 and 2 cucumber downy mildew isolates, respectively. Because of the effectiveness of this procedure, as shown in this study, the four pathotypes of *P. cubensis* present in the cultivated areas could be used to identify downy mildew rather than by their morphological character. The host specificity of *P. cubensis* deserves further study to identify strategies for durable resistance and management.

Keywords: cucumber, Downy Mildew, disease resistance, plant disease management

Introduction

Downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & Curtis., Rostovzev) is one of the most important diseases of cucumber (*Cucumis sativus* L.) in humid production areas of the

world (Thomas, 1986). The host range of *P. cubensis* is reported to include 40 species in approximately 20 genera of the family Cucurbitaceae. Palti (1974) reported that the differences in response to the pathogen were likely due to the physiological races in various countries. Five pathotypes of *P. cubensis* have been analyzed based on their compatibility with specific hosts (Thomas *et al*., 1987). Cohen *et al*. (2003) studied host range pathogenicity of a *P. cubensis* isolate that occurred on pumpkin (*Cucurbita moschata)* and summer squash (*Cucurbita pepo* subsp. *pepo)* in Israel and designated this new isolate as pathotype 6. Host resistance is an important tool in plant disease management. Successful disease resistance provides adequate control of cucumber downy mildew without the use of fungicides. To improve the base population of cucumber for downy mildew resistance, we studied seventeen *P. cubensis* isolates collected from cucumber cultivated areas in the northern region of Thailand. The study aimed to determine the host specificities of these *P. cubensis* isolates that would be useful for further characterization and identification of the isolates. These isolates should be tested with cucumber lines to screen for downy mildew resistant varieties in breeding programs.

Materials and Methods

Plant materials

The methods used to identify physiological specialization of the pathogens were based on Cohen *et al*., 2003. The differential hosts (Table 1) were grown under greenhouse conditions. Plants were grown in 9 cm^2 plastic pots using sterilized planting materials Krassmann KTS2 (Germany) composed of peat moss and vermiculite. Plants were watered twice daily and fertilized as needed. Plants were placed in greenhouse conditions until they had two cotyledons that could be used for pathogenicity tests with *P. cubensis* isolates.

Pseudoperonospora cubensis isolates

Seventeen *P. cubensis* isolates were collected from cucumber cultivated in commercial production areas of northern Thailand (Chiangrai, Chiangmai, Lampun, Lampang and Tak provinces) from 2005 through 2008. These isolates were tested at the Lampang Agricultural Research and Training Center, Rajamangala University of Technology, Lanna from July 2008-Febuary 2009. The isolates were maintained in 20% glycerol in a freezer at -20°C.

Pathogenicity testing

Pathogenicity tests were conducted with the plant species listed in Table 1. Tests were conducted with 12 plants per cultivar per assay and repeated two times. The plants were inoculated with a suspension of *P. cubensis* (10^4 sporangia ml^{-1}) at the two cotyledon stage. The adaxial and abaxial surfaces of the cotyledons were sprayed with the inoculum suspensions until incipient run-off. Inoculated plants were placed in the dark at 20°C and high humidity for 21 hours. Subsequently, they were transferred to the greenhouse under ambient conditions of 24-28°C and 80-90% relative humidity. After 7 days, the disease reactions on the host plants were recorded. Sporulation was rated qualitatively as compatible or incompatible. A set of the host plants without fungal inoculation were used as a control.

Table 1. The differential hosts used for pathogenicity tests with *Pseudoperonospora cubensis* isolates.

No.	Scientific name	Cultivar/ accession number	Country of origin
1	*Cucumis sativus*	C1, Malai 759, Toto, Rantong	Thailand
2	*Cucumis melo* var.*reticulatus*	Singto, Chiatai	Thailand
3	*Cucumis melo* var.*conomon*	Kamini, PI532830(SHIDAO XIAN)*	Thailand, China (Shaanxi)
4	*Cucumis melo* var.*acidulus*	PI200819 *	Myanmar (Mandaley)
5	*Citrullus lanatus*	Black melon	Thailand
6	*Cucurbita maxima*	Singto	Thailand
7	*Lagenaria vulgaris*	Advance	Thailand
8	*Luffa acutangula*	Chiatai	Thailand
9	*Benincasa hispida*	Chiatai	Thailand
10	*Momordica charantia*	Chiatai	Thailand
11	*Trichosanthes sp.*	Advance	Thailand

Note * The seeds were obtained from National Plant Germplasm System, GRIN USDA. (http://www.ars-grin.gov/npgs/orders.html)

Results and Discussion

The downy mildew reactions of the host species with seventeen *P. cubensis* isolates from the northern region of Thailand were observed (Table 2). These isolates were classified into four pathotypes (Thomas *et al.*, 1987 and Cohen *et al.*, 2003) as follows:
Pathotype 1: Downy mildew symptoms were identified and their reactions were highly compatible with *Cucumis sativus* and *Cucumis melo* var. *reticulatus* in 1 isolate, N-Lpu 3/1.
Pathotype 2: Downy mildew symptoms were identified and their reactions exhibited highly compatible reaction with *Cucumis sativus*, *Cucumis melo* var. *reticulatus* and *Cucumis melo* var. *conomon* in 3 isolates, N-Cr 3/1, N-Cm1/2 and N-Lpa6/1.

Pathotype 3: Downy mildew symptoms were identified and their reactions were highly compatible with *Cucumis sativus*, *Cucumis melo* var. *reticulatus*, *Cucumis melo* var. *conomon* and *Cucumis melo* var. *acidulus* in 11 isolates, N-Cm1/1, N-Cm1/10, N-Cm3/1, N-Cm6/1, N-Lpu3/2, N-Lpu6/1, N-Lpa1/6, N-Lpa 2/1, N-Lpa2/3, N-Lpa 4/1 and N-Tak6/1.

Pathotype 4: Downy mildew symptoms were identified and their reactions were highly compatible with *Cucumis sativus*, *Cucumis melo* var. *reticulatus*, *Cucumis melo* var. *conomon*, *Cucumis melo* var. *acidulus* and *Citrullus lanatus* in 2 isolates, N- Lpa 2/4 and N- Lpa 2/5 (Figure 1).

The results of our study showed that Pathotype 3 was present in 64.7 percent of all tested isolates. Pathotypes 1, 2, and 4 were present in 17.6, 11.8 and 5.9 percent, of all isolates, respectively. Pathotype 3 was diverse [found or "detected on samples from four out of the five provinces"??] in four provinces (except Chiangrai). Pathogenicity testing of each isolate collected from the northern regions showed that they could be grouped according to their physiological specialization. Furthermore, all isolates were similar in morphology when compared with the Palti and Korea isolates (data not shown). This evidence suggests that host specificity of *P. cubensis* isolates could be better used to identify downy mildew rather than by its morphological character. Therefore, host specificity of *P. cubensis* isolates requires further study to facilitate and streamline the identification of isolates.

Table 2. The pathotypes of *Pseudoperonospora cubensis* isolates collected from the northern region of Thailand.

Pathotype	Number of Pathotypes						% of all isolates
	Chiangrai	Chiangmai	Lumpun	Lumpang	Tak	Total	
1	0	0	1	0	0	1	5.88
2	1	1	0	1	0	3	17.64
3	0	4	2	4	1	11	64.70
4	0	0	0	2	0	2	11.76
Total	**1**	**5**	**3**	**7**	**1**	**17**	**100**

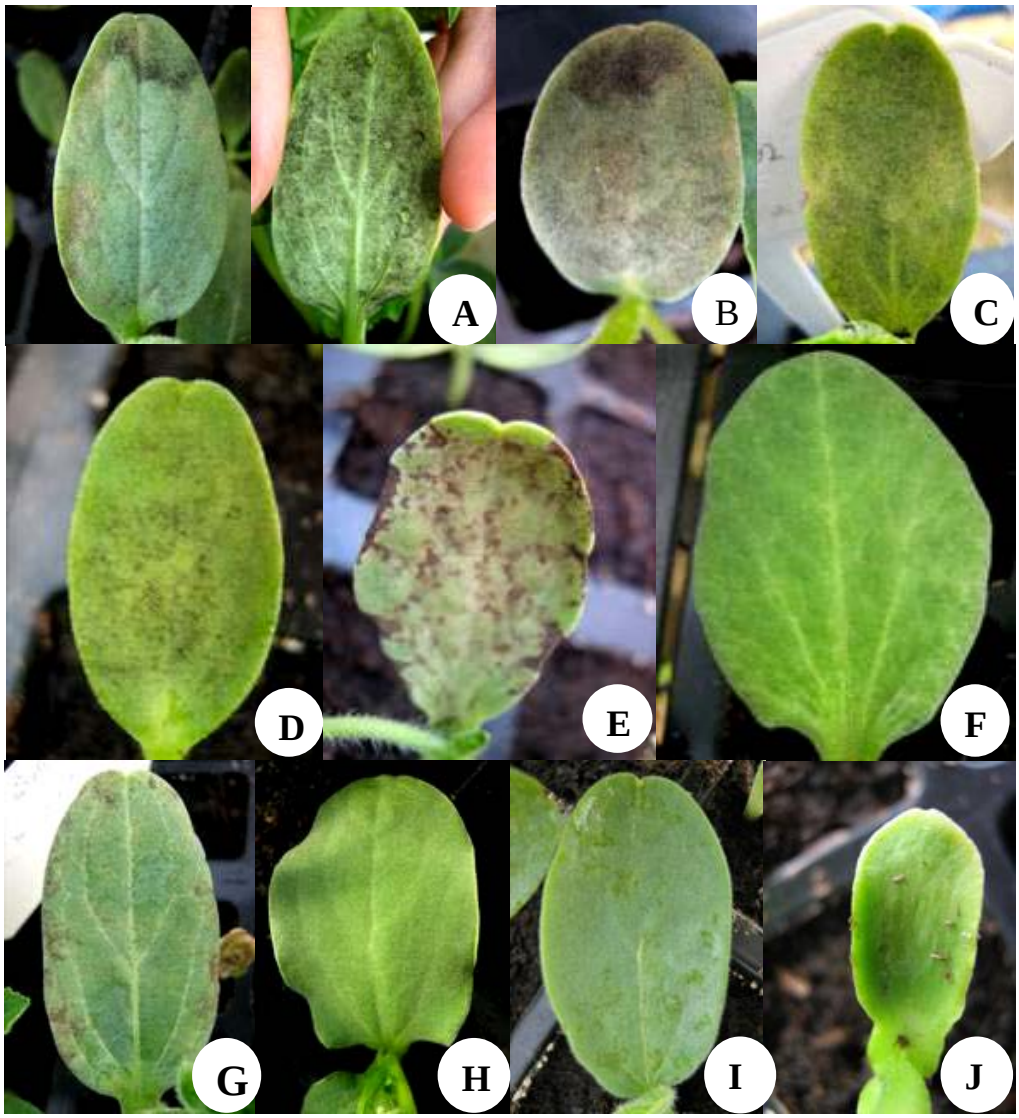

Figure 1. The disease reaction of *Pseudoperonospora cubensis* N-Lpa2/5 isolate on the cotyledons of A, *Cucumis sativus*; B, *Cucumis melo* var. *reticulatus*; C, *Cucumis melo* var. *conomon*; D, *Cucumis melo* var. *acidulous*; E, *Citrullus lanatus*; F, *Cucurbita maxima*; G, *Lagenaria vulgaris*; H, *Luffa acutangula*; I, *Benincasa hispida*; and J , *Momordica charantia* under greenhouse conditions at 7 days, post inoculation (dpi).

Conclusion

Seventeen *P. cubensis* isolates collected during 2005-2008 from cucumber cultivated in northern Thailand were evaluated for their host specificity on differential hosts, after artificial inoculation under greenhouse conditions and then assessed for symptoms seven days after inoculation. It was found that pathotypes 1, 2, 3 and 4 had 1, 3, 11 and 2 isolates of cucumber downy mildew, respectively. The four pathotypes of *P. cubensis* present in the target region deserve further study to identify strategies for durable resistance and management.

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