The Host Range of Downy Mildew, *Peronospora parasitica*, from *Brassica oleracea*, Cabbage and Broccoli Crops

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Abstract

We collected three cabbage plants infected with *Peronospora parasitica* in Kagawa, two broccoli plants in Mie and one broccoli plant in Tottori. Five single spore isolates from each sample were prepared, and the host range of thirty single spore isolates in total was examined. Test plants used for the host range tests were *Brassica oleracea* (eighteen cultivars of cauliflower, cabbage and broccoli), *B. campestris* (eight cultivars of Pak-choi, mizuna, rape, Chinese cabbage and turnip), *B. juncea* (one cultivar of leaf mustard), *B. napus* (one cultivar of rutabaga) and *Raphanus sativus* (two cultivars of Japanese radish). In all the test plants, cotyledons with hypocotyl were used for the inoculation tests. All the *B. oleracea* plants except two cultivars of cabbage were highly susceptible to all the tested isolates, and it is considered that this is a host species of the fungus. *B. napus* was moderately susceptible, and suggested to be a possible host species. *B. campestris*, *B. juncea* and *R. sativus* were resistant, and considered to be non-host species. Therefore, all the isolates were considered to belong to the same strain of the fungus and the strain to be specific to *B. oleracea* and possibly to *B. napus*. Among *B. oleracea* plants, two cultivars of cabbage cv. Golden Best and cv. YR-Sawamidori were resistant. Difference in pathogenicity was not found among the tested single spore isolates.

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Key words : *Peronospora parasitica*, downy mildew, *Brassica oleracea*, parasitism.

INTRODUCTION

Downy mildew fungus (*Peronospora parasitica*) of Cruciferae plants is well-known to comprise many strains with different host species specificities^{1-7,11,12}. Although the strains are principally species specific, they often show various degrees of pathogenicity to plants of other species, and the host ranges of the strains often overlaps each other. Therefore, to investigate certain problems of the disease, such as the epidemiology of the fungus on some crops and their cultivar resistance, first of all, it is necessary to know the host range of the tested isolates.

Only few information is available of the strains of the fungus distributing in Japan. Long before in 1934, Hiura and Kanegae⁵⁾ reported a particular investigation, in which they used downy mildew derived from Japanese radish (*Raphanus sativus*) and some *Brassica* plants, and the isolates were not pathogenic to cabbage (*B. oleracea*). In Japan, few report was found on host range of downy mildew on *B. oleracea* plants. Recently, we have observed the occurrence of downy mildew of broccoli and reported the host range of the fungus¹¹). In this report, we showed the identity of the host range of the downy mildew fungus of cabbage and broccoli, and

found resistant cultivars among *B. oleracea* plants using single spore isolates of the fungus.

MATERIALS AND METHODS

Fungal isolates. Three cabbage plants in Kagawa, two broccoli plants in Mie and one broccoli plant in Tottori which were infected with *Peronospora parasitica* were collected for the inoculation tests (Table 1). The downy mildew samples were brought to the laboratory in a cool box. One lesion was cut out and shaken in distilled water to prepare a conidial suspension. The suspension was spread on a plate of 1.5% water agar and one agar piece with a single conidium was cut off under a light microscope. The agar piece was transplanted onto the undersurface of a broccoli cotyledon (cv. Banrei) prepared on moist filter papers in petri dishes and incubated for seven days at 20°C in a growth cabinet (5000 lux, 12 hr light/12 hr dark). Thirty agar pieces per one downy mildew sample were transplanted. The proportions of successful inoculation varied depending on the samples and ranged from 16.7% (5/30) to 60% (18/30). Five single spore isolates per each diseased sample were arbitrarily selected from the diseased cotyledons (Table 1). The isolates were maintained by periodical inoculation on broccoli cotyledons or preserved at -80°C

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Table 1. Single spore isolates of *Peronospora parasitica* used for the inoculation tests and their origin

Single spore isolates	Downy mildew samples for single spore isolation			
	Crop	Sample No.	Location	Date of collection
CPP5a,b,c,d,e	Cabbage	CPP5	Kotonami, Kagawa	Nov. 1992
CPP6a,b,c,d,e	Cabbage	CPP6	Kotonami, Kagawa	Nov. 1992
CPP7a,b,c,d,e	Cabbage	CPP7	Kotonami, Kagawa	Nov. 1992
BPP1a,b,c,d,e	Broccoli	BPP1	Ano, Mie	Jan. 1990
BPP2a,b,c,d,e	Broccoli	BPP2	Daiei, Tottori	Nov. 1992
BPP3a,b,c,d,e	Broccoli	BPP3	Ureshino, Mie	Nov. 1992

in a deep freezer¹⁰⁾ until use for the inoculation tests to avoid contamination with other downy mildew fungus.

Test plants. The Cruciferae plants used for the host range tests were *Brassica oleracea* (eighteen cultivars of cauliflower, cabbage and broccoli), *B. napus* (one cultivar of rutabaga), *B. campestris* (eight cultivars of Pak-choi, mizuna, rape, Chinese cabbage and turnip), *B. juncea* (one cultivar of leaf mustard) and *Raphanus sativus* (two cultivars of Japanese radish) as shown in Table 2 and 3. These test plants were sown in vermiculite in 7.5 cm plastic pots and grown for seven days in the growth cabinet to prepare seedlings at the stage of cotyledons for the inoculation tests under the condition described above.

Inoculation conditions. The seedlings of the test plants were cut at the hypocotyl, and the cotyledons, still remaining of hypocotyl, were placed in plastic cases (usually ten seedlings per each). The hypocotyls were winded with a piece of cotton wetted with distilled water or modified Knop's solution⁹⁾ to keep the freshness

of the cotyledons. In some tests, to simplify the managements, cotyledons were transplanted in 1.5% water agar settled in plastic cases. Inoculum was prepared from the broccoli cotyledons with sporulation. Conidia were washed into distilled water and the concentration was adjusted to $1-5 \times 10^4$ conidia/ml with a Thoma hemacytometer. Five μ l of the conidial suspension was placed on each cotyledon. Inoculated cotyledons were incubated for seven days under the conditions described above in a growth cabinet to develop disease lesions. Inoculation tests were replicated two to three times.

Disease assessment. The disease lesions of the inoculated cotyledons were observed macroscopically, and the disease indexes was calculated as the average of the percentage of diseased cotyledons as follows; 4: sporulation on more than 50% of inoculated cotyledons, 3: 20% to 49%, 2: less than 19%, 1: sparse sporulation or large necrosis, 0: no sporulation or limited necrosis.

Table 2. Host reactions to single spore isolates of *Peronospora parasitica* on cotyledons in a differential host range set containing the four major cultivated species of *Brassica* and the synthetic genus *Raphanus*

Plants inoculated			Fungal isolates								
			Cabbage				Broccoli				
			CPP5		CPP6		CPP7		BPP1		
Scientific name	Crop	Cultivar	a	bcde	abcde	abcde	abcde	abcde	abcde	abcde	abcde
<i>B. campestris</i> (chinensis group)	Pak-choi	Yukishiro	0 ^{a)}	0000	00000	00000	00010	01000	00000		
<i>B. campestris</i> (japonica group)	mizuna	Shirokukisenkin	0	0020	00000	00000	00000	00000	00000	00000	
<i>B. campestris</i> (narinosa group)	rape	Shinbansei	0	0000	00000	00000	00000	02000	00000	00000	
<i>B. campestris</i> (pekinensis group)	Chinese cabbage	Kashin	0	0000	00000	00000	00000	00000	00000	02200	
<i>B. campestris</i> (pekinensis group)	Chinese cabbage	Nozaki-No. 2	0	0000	00000	00000	00000	00000	00000	00000	
<i>B. campestris</i> (rapifera group)	turnip	Hinona	0	0000	00000	00000	00000	00000	00000	00000	
<i>B. campestris</i> (rapifera group)	turnip	Komatsuna	0	0000	00000	00000	00000	00000	00000	00200	
<i>B. campestris</i> (rapifera group)	turnip	Wasekanamachi	0	0101	22001	20000	13000	01100	00200		
<i>B. juncea</i> (cernua group)	leaf mustard	Ha-karashina	0	0000	00202	00000	00000	10000	00000		
<i>B. oleracea</i> (botrytis group)	cauliflower	Nozakiwase	4	4444	44433	42442	33220	44343	43433		
<i>B. oleracea</i> (botrytis group)	cauliflower	Snowball	4	4444	44444	44434	44344	44444	44444		
<i>B. oleracea</i> (botrytis group)	cauliflower	Snow Crown	4	4444	33323	40343	20323	23332	34433		
<i>B. oleracea</i> (capitata group)	cabbage	Shikidori	4	4444	44444	44444	44442	44444	44444		
<i>B. oleracea</i> (italica group)	broccoli	Banrei	4	4444	44444	44334	34343	44444	44444		
<i>B. napus</i> (napobrassica group)	rutabaga	Green Top	3	0003	33123	32222	20322	33433	22312		
<i>R. sativus</i> (daikon group)	Japanese radish	Miyashige	0	0000	00000	00000	00000	00000	00000		
<i>R. sativus</i> (daikon group)	Japanese radish	Nerima	0	0000	00000	00000	00000	00000	00000		

a) Disease index: 0, no sporulation or limited necrosis on inoculated cotyledons; 1, sparse sporulation or large necrosis on inoculated cotyledons; 2, sporulation on less than 19% of inoculated cotyledons; 3, sporulation on 20% to 49% of inoculated cotyledons; 4, sporulation on more than 50% of inoculated cotyledons.

Table 3. Host reactions to single spore isolates of *Peronospora parasitica* on differential cultivars of *Brassica oleracea*

<i>B. oleracea</i> plants inoculated		Fungal isolates					
		Cabbage		Broccoli			
		CPP5		CPP6	BPP1	BPP2	BPP3
Crop	Cultivar	d	e	ab	ad	ac	bd
Cauliflower	Akizuki	4 ^{a)}	4	44	44	44	44
Cauliflower	Bridal	4	4	44	44	44	44
Cauliflower	Early Snowball A	3	3	44	34	44	33
Cauliflower	Nozakiwase	4	4	44	33	43	33
Cauliflower	Snowball	4	4	44	44	44	44
Cauliflower	Snow Crown	4	4	33	23	33	43
Cabbage	Delight Ball	4	4	44	44	44	44
Cabbage	Golden Best	0	0	00	00	00	01
Cabbage	Kuroba-Succession	4	4	44	44	44	44
Cabbage	Savoy King	4	4	44	33	44	44
Cabbage	Shikidori	4	4	44	44	44	44
Cabbage	YR-Sawamidori	0	0	00	00	00	10
Broccoli	Banrei	4	4	44	34	44	44
Broccoli	De Cico	4	4	44	33	44	44
Broccoli	Ryokuyo	4	4	44	33	34	44
Broccoli	Shinkai	4	4	44	44	44	44
Broccoli	Tenrai	4	4	44	44	44	44
Broccoli	Three Seven	4	4	44	44	44	44

a) Disease index: 0, no sporulation or limited necrosis on inoculated cotyledons; 1, sparse sporulation or large necrosis on inoculated cotyledons; 2, sporulation on less than 19% of inoculated cotyledons; 3, sporulation on 20% to 49% of inoculated cotyledons; 4, sporulation on more than 50% of inoculated cotyledons.

RESULTS

Host range

The results of the host range tests, using the thirty single spore isolates of the downy mildew fungus and the cotyledons of five species of Cruciferae plants, are compiled in Table 2. *Brassica oleracea* was susceptible. The major disease indexes of cv. Banrei of broccoli, cv. Shikidori of cabbage and cv. Snowball of cauliflower were 4, indicating that these cultivars are highly susceptible. These results clearly shows that *B. oleracea* is a host species of the fungus. *B. napus* (cv. Green Top of rutabaga) was moderately susceptible. The disease indexes were mostly 2 and 3. This result suggests that *B. napus* is a possible host species of the fungus. *B. campestris* was resistant. Among the eight cultivars of five crops, the highest disease indexes was 2 in a few cases, most of the disease indexes were 0. These results indicate that *B. campestris* is a non-host species. *B. juncea* (cv. Ha-karashina of leaf mustard, most disease indexes, 0) and *Raphanus sativus* (cv. Miyashige and cv. Nerima of Japanese radish, all disease indexes, 0) were also resistant, and considered to be non-host species. As a whole, it was considered that all the test isolates belong to one strain which is host-specific to *B. oleracea* and possibly to *B. napus*.

Cultivar resistance

Further inoculation tests were carried out to find resistant cultivars using ten isolates of the downy mildew fungus and each six cultivar of cauliflower,

cabbage and broccoli. The results are compiled in Table 3. Two cultivars of cabbage, cv. Golden Best and cv. YR-Sawamidori, were resistant (most of disease indexes, 0). All the other cultivars were susceptible (most of disease indexes, 4). Variation of pathogenicity was not found among the single spore isolates.

DISCUSSION

To test the pathogenicity of some downy mildew fungus of Cruciferae plants, it is common to use the method of spraying inoculation to seedlings. In a preliminary inoculation tests of downy mildew fungus, we obtained the results (data not shown) that the disease reaction between cotyledons and leaves corresponded each other very well, suggesting that cotyledons are also a useful material for the host range tests. Therefore, as some researchers used cotyledons for inoculation of downy mildew fungus^{2,6,7,12)}, too, we used cotyledons in the present experiments. Moreover, we also found that conidial suspensions with more than 10^4 conidia/ml were sufficient for the sprouting of the downy mildew fungus on most of the broccoli cotyledons (data not shown). Host range of downy mildew of *Brassica oleracea* was examined based on these informations.

Evidence based on host range studies on downy mildew^{1-7,11,12)} and other biotrophic fungi indicates that host specificity occurs at different levels. Many experiments have been carried out at the genus, species and cultivar levels in cruciferous plants. In Japan, physiological specialization of the downy mildew fungus was

observed only in 1934⁵⁾. Recently, we have examined the pathogenicity of downy mildew of *B. oleracea* to some Cruciferae crops¹¹⁾. The present results shows that the downy mildew fungus of broccoli and cabbage distributing in Japan belongs to one strain and host-species is specific to *B. oleracea*. *B. napus* may be a host species, but it is necessary to investigate particularly to confirm the possibility. These results were in agreement with previous reports indicating that downy mildew derived from *B. oleracea* generally displays a high host-species specificity^{1,3,11,13)}. Hiura and Kanegae⁵⁾ reported formerly that the downy mildew isolates derived from Japanese radish (*Raphanus sativus*) and some *Brassica* plants were not pathogenic to cabbage (*B. oleracea*). Therefore, some strains of downy mildew fungus of Cruciferae plants are considered to be distributing in Japan, and it is a future problem to draw full figures on the strains.

In the inoculation tests of different cultivars of *B. oleracea*, two cultivars of cv. Golden Best and cv. YR-Sawamidori, were found to be resistant, while other cultivars found to be a high to moderate susceptibility. It was suggested that these two cultivars may be useful materials for breeding resistant varieties of *B. oleracea* crops. Although the tested isolates were not infective to the cultivars, physiological races which is infective to the resistant cultivars may occur. In the United States of America, downy mildew fungus pathogenic to resistant cultivars have been detected and races have been differentiated^{8,13)}. The survey of such races is another future problem.

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Literature cited

1. Chang, I.H., Shih, N.L. and Chiu, W.F. (1964). A preliminary study on the physiological differentiation of the downy mildews (*Peronospora parasitica* (Pers.) Fr.) of Chinese cabbage and other cruciferous vegetables in the vicinity of Peking and Tientsin. *Acta Phytopathol. Sin.* 7: 33-44.
2. Dickinson, C.H. and Greenhalgh, J.R. (1977). Host range and taxonomy of *Peronospora* on crucifers. *Trans. Br. Mycol. Soc.* 69(1): 111-116.
3. Dzhanuzakov, A. (1962). Specialization and variability of certain *Peronospora* fungi. *Bot. Zh. USSR* 47: 862-867.
4. Gäumann, E. (1926). Über die Spezialisierung des fals-

chen Mehltaus (*Peronospora brassicae* Gm.) auf dem Kohl und seinen Verwandten. *Landw. Jbr. der Schweiz.* 40: 463-468.

5. Hiura, M. and Kanegae, H. (1934). Studies on the downy mildews of cruciferous vegetables in Japan 1. *Trans. Sapporo Nat. Hist. Soc.* 13: 125-133.
6. Kluczewski, S.M. and Lucas, J.A. (1983). Host infection and oospore formation by *Peronospora parasitica* in agricultural and horticultural *Brassica* species. *Trans. Br. Mycol. Soc.* 81(3): 591-596.
7. McMeekin, D. (1969). Other hosts for *Peronospora parasitica* from cabbage and radish. *Phytopathology* 59: 693-696.
8. Natti, J.J., Dickson, M.H. and Atkin, J.D. (1967). Resistance of *Brassica oleracea* varieties to downy mildew. *Phytopathology* 57: 144-147.
9. Ohguchi, T., Yoshida, K., Ismail, M.Y. and Asada, Y. (1989). A laboratory method for propagation of downy mildew fungus (*Peronospora parasitica*) using the cotyledon or true leaf of Japanese radish seedlings. *Ann. Phytopathol. Soc. Jpn.* 55: 561-566.
10. Satou, M. and Fukumoto, F. (1993). Preservation of conidia of broccoli downy mildew fungus with cryogenic protectants by freezing at -80°C . *Ann. Phytopathol. Soc. Jpn.* 59: 492-499.
11. Satou, M., Hagiwara, Y., Ishii, M. and Fukumoto, F. (1991). Downy mildew of broccoli caused by *Peronospora brassicae*. *Proc. Kansai Pl. Prot. Soc.* 33: 67-68 (in Japanese).
12. Sherriff, C. and Lucas, J.A. (1990). The host range of isolates of downy mildew, *Peronospora parasitica*, from *Brassica* crop species. *Plant Pathol.* 39: 77-91.
13. Thomas, C.E. and Jourdain, E.L. (1990). Evaluation of broccoli and cauliflower germplasm for resistance to race 2 of *Peronospora parasitica*. *Hortscience* 25(11): 1429-1431.

和 文 摘 要

佐藤 衛・福本文良：キャベツおよびブロッコリー(*Brassica oleracea*)から分離したべと病菌(*Peronospora parasitica*)の宿主範囲

香川県の3点のキャベツ、三重県の2点および鳥取県の1点のブロッコリーから *Peronospora parasitica* のサンプルを集め、各サンプルから5菌株、合計30の単胞子分離株を調製し、これらの宿主範囲を調査した。供試植物として、*Brassica oleracea* (カリフラワー、キャベツおよびブロッコリー18品種)の他、*B. campestris* (タイサイ、ミズナ、アブラナ、ハクサイおよびカブ8品種)、*B. juncea* (カラシナ1品種)、*B. napus* (ルタバガ1品種)および *Raphanus sativus* (ダイコン2品種)を用いた。接種試験の結果、分離源と同種の植物である *B. oleracea* の3作物の16品種は高い感受性を示し、本種は宿主植物と考えられた。また、*B. napus* は中程度の感受性を示したことから宿主となる可能性が示唆されたが、*B. campestris*、*B. juncea*、*R. sativus* は抵抗性を示したことから非宿主と考えられた。供試したべと病菌はすべて同じ系統に属し、*B. oleracea* (*B. napus* も含む可能性がある)を宿主とする系統と考えられた。*B. oleracea* の中でキャベツの2品種、ゴールデンベストおよびYR-さわかみどりは抵抗性を示した。供試した単胞子分離菌株で病原性に違いは見られなかった。