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Phytophthora Root Rot of Chinese Cabbage and Spinach Caused by *P. drechsleri* in Korea

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***Phytophthora* root rot of Chinese cabbage and spinach is reported for the first time in Korea. The diseases ocurred at Yangju, Seosan and Yeocheon in Korea from 1995 through 1998, mainly in lowland and submerged areas. Symptoms consisted of stunt, yellows, wilt and eventual death due to root rot. Fourteen isolates collected from naturally infected plants were all identified as *P. drechsleri* based on mycological characteristics. PCR-RFLP analysis of rDNA of the isolates confirmed the above result, since the restriction band patterns of the small subunit and internal transcribed spacers were identical to *P. drechsleri* and *P. cryptogea*, but distinct from closely related species of *P. erythroseptica, P. cambivora, P. sojae* and *P. megasperma.* The pathogen showed strong pathogenicity to Chinese cabbage, moderate to spinach, radish, cabbage and tomato, and weak or none to brown mustard, kale, chicory and pepper in pathogenicity tests.**

Keywords : Chinese cabbage, spinach, PCR-RFLP, *Phytophthora drechsleri*, r-DNA.

Chinese cabbage and spinach are major green vegetables in Korea based on cultivation acreage, consumption amount and cash value for farmers. Especially, Chinese cabbage is extensively used for preparation of a favorite Korean dish, Kimchi. The production area of Chinese cabbage covers ca. 50,000 ha and consumption per capita is estimated ca. 60 kg annually (Anonymous, 1998).

In recent years, club root caused by *Plasmodiophora brassicae* emerged as a greatest threat to safe production of the plant in Korea. During a survey on the soil-borne disease from 1995 through 1998, Chinese cabbage and spinach showing wilt with no distinct club roots were observed in several cultivation areas. Unexpectedly, *Phytophthora* sp. was consistently isolated from the discolored root tissues.

According to Jee (1998), 15 species of *Phytophthora* affect numerous important crops in Korea. Vegetables belonging to Solanaceae and Cucurbitaceae are vulnerable to the pathogen, but no *Phytophthora* diseases on other vegetables in Cruciferae, Liliaceae, Compositae and Umbelliferae have been reported (Korean Society of Plant Pathology, 1998), although a few *Phytophthora* diseases are listed in '*Compendium of Vegetable Diseases with Color Plates*' published by the authors (Cho et al., 1997).

In general, *Phytophthora* diseases are considered less significant on green vegetables than other soil-borne diseases, however, *Phytophthora* root rot on cabbage, spinach, lettuce, kale, tyfon, cauliflower and broccoli caused by *P. megasperma, P. drechsleri,* or *P. cryptogea* have been reported in several countries (Downes and Loughnane, 1969; Karakaya et al. 1995; Sherf and Macnab, 1968; Thompson and Phillips, 1988). In this study, the causal pathogens of Chinese cabbage and spinach root rot were identified and Koch's postulates were confirmed to report *Phytophthora* root rot on the green vegetables for the first time in Korea.

Materials and Methods

Isolation and identification. Chinese cabbage and spinach showing wilt due to root rot were collected from 1995 to 1998 at Yangju, Seosan and Yeochon, Korea. Roots were washed under running tap water, cut into small pieces and disinfected with 0.1% NaClO for ca. 10 sec., placed on water agar and incubated at 25°C for 2 days in the dark. Mycelial tips growing out from the tissues were transferred to 10% V8 juice agar for further study.

To investigate morphological characteristics of the fungus, isolates were cultured on 10% V8 agar for 3~4 days and sporulated as described by Jee et al. (1997, 1998). Effect of temperature on mycelial growth was examined on corn meal agar (CMA) in the dark at 2~5°C intervals from 5 to 38°C. Mating types and sexual reproduction structures of the isolates were examined using Ko's (1978) polycarbonate membrane method (PC MB 90 mm, 0.2 μm, Nucleopore Co., USA) as described by Jee et al. (1997). Nine agar disks were made from 5-day-old cultures of a testee isolate growing on 20% V8 agar by a cork borer (9 mm in dia.) and distributed evenly in a new petri plate. A sterilized PC membrane

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was placed on the top of the disks, and three disks of A1 and A2 mating types of *P. parasitica* (=*P. nicotinae*) for each were laid upside down on the top. Three disks of the testee isolate remained without mating to examine self fertilization. The mating type standards of *P. parasitica* were supplied by Dr. W. H. Ko, Dept. of Plant Pathology, Univ. of Hawaii at Manoa, USA. After incubation at 20°C for 14 days in the dark, the PC membrane was removed along with the disks on the top, and oospores formed on the bottom agar disks were examined.

DNA isolation. *Phytophthora* isolates from Chinese cabbage and spinach and closely related six species of *Phytophthora* were used. All isolates were obtained from the culture collections at

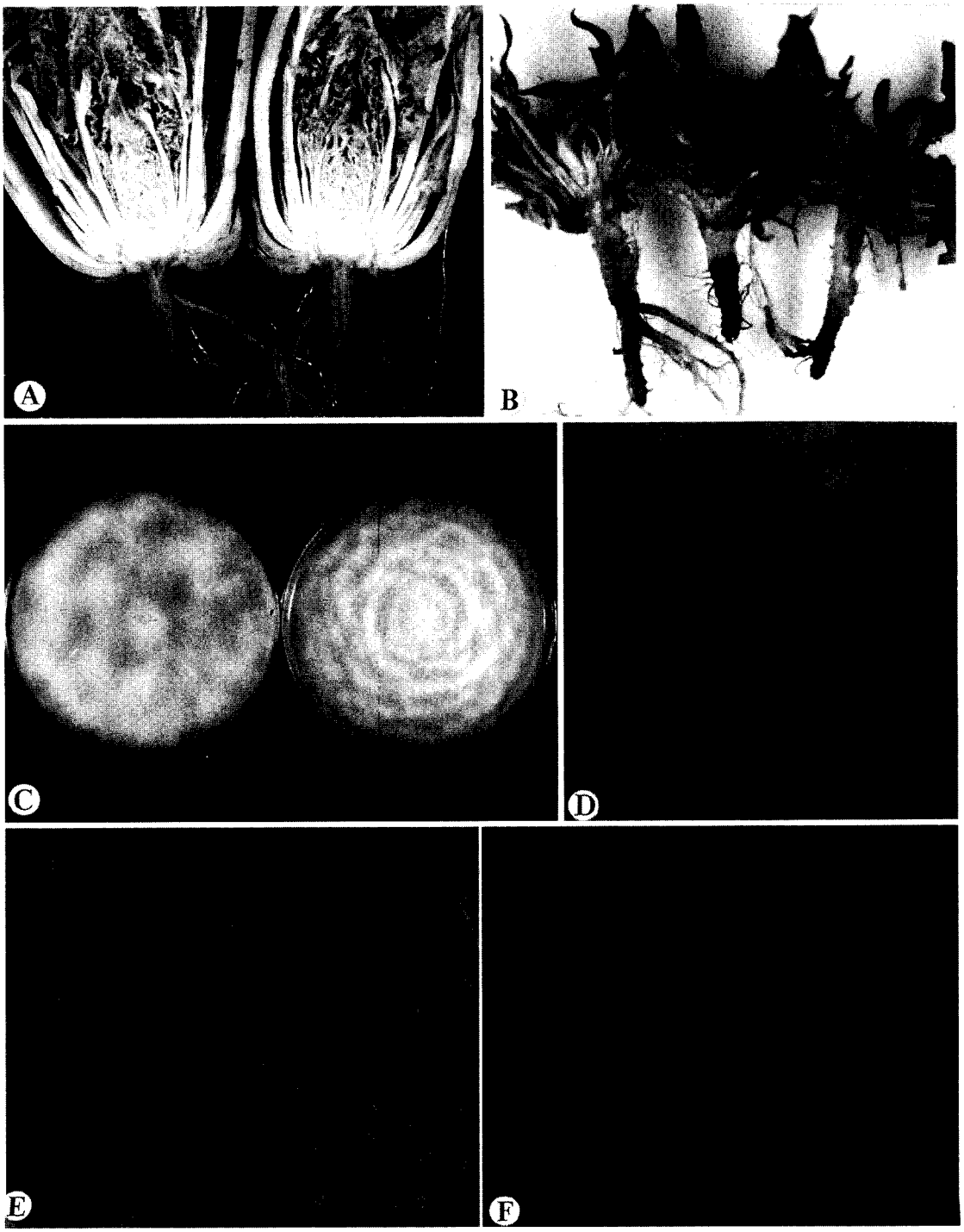

Fig. 1. Chinese cabbage and spinach infected by *Phytophthora drechsleri* and features of the fungus. A; Infected Chinese cabbage showing discolored inner root tissues, B; Infected spinach showing severe rot on root, C; Colony patterns on 10% V8A (left) and PDA (right), D; Hyphal swellings, E; Sporangia, F; Oospores of the pathogen. Scale bar=20 μm.

Plant Pathology Div., National Institute of Agricultural Science and Technology, RDA, Korea (Jee, 1998). *P. drechsleri* (P-9615), *P. cryptogea* (P-9533), *P. erythroseptica* (P-96117), *P. cambivora* (Pb-06), *P. sojae* (P-9662), and *P. megasperma* (P-9608) were originated from tomato, gerbera, arrowroot, apple, soybean and tomato, respectively.

Isolation procedure of total DNA basically followed the method of Lee and Taylor (1990). Young mycelia grown in clarified 10% V8 juice broth for 2 days were rinsed twice with distilled water prior to isolation of DNA as follows: 1) Mycelia in 400 μl of DNA extraction solution (3% SDS, 50 mM EDTA, 50 mM Tris-HCl, pH 7.2, and 1% 2-mercaptoethanol) were macerated with a glass rod and incubated at 65°C for 1 hr. 2) Four-hundred μl of phenol:chloroform (v/v, 1:1) was added to the tube, vortexed and centrifuged at 13,000×g for 10 min 3) After collecting supernatant (aqueous phase) to a new 1.5 ml-tube, 20 μl of 3 M sodium acetate and 0.54 volume (220 μl) of isopropanol were added and centrifuged at 12,000×g for 5 min to precipitate DNA. 4) The pellet was rinsed twice with 70% ethanol, vacuum-dried for 5 min and dissolved in 50 μl of TE buffer (10 mM Tris-Cl, pH 8.0, and 1 mM EDTA). 5) RNase (1.0 μl) was added to the total DNA extract and reacted for 30 min at 37°C.

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Primers used in this study were designed by White et al. (1990) for the amplification of internal transcribed spacers (ITS) including 5.8S rDNA in the nuclear DNA repetitive unit. The primers consisted of ITS1: 5'-TCCG-TAGGTGAACCTGCGG-3', ITS4: 5'-TCCTCCGCTTATTGA-TATGC-3', NS1: 5'-GTAGTCATATGCTTGTCTC-3', and NS8: 5'-TCCGCAGGTTCACCTACGGA-3'. Each template DNA (100 ng) prepared as above was added in the reaction mixture [1 ×buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% triton X-100), each dNTP (0.1 mM), each primer [1 pM), $MgCl_2$ (1.5 mM), *Taq* DNA polymerase (2 units) (Promega)]. The thermal cycles were performed 35 times with profile of 95°C, 58°C and 72°C for 1 min, 1 min and 2 min, respectively. The first denaturation and the last extension time were extended to 4 min and 8 min, respectively. The success of amplification was monitored by analyzing 5 μl of the reactant on 1% agarose gel electrophoresis.

PCR-amplified rDNA regions of each isolate were digested separately with two restriction enzymes, *Hae*III and *Hha*I, according to the manufacturer's instructions (Takara, Japan). The digested fragments were separated by 3% MetaPhor agarose (FMC Bio-products) with TAE buffer (40 mM Tris-acetate, pH 8.0, 1 mM EDTA).

Pathogenicity test. Four cultivars of Chinese cabbage, spinach, radish, cabbage, kale, chicory, brown mustard, tomato and pepper were used in the pathogenicity test. About 50-day-old plants grown in pots (18×12 cm) in a greenhouse were inoculated with zoospore suspension of the Chinese cabbage isolate (P-9509) and the spinach isolate (P-9818). Fifty ml of the zoospore suspension (10^4-10^5/ml) prepared as described by Jee et al. (1997, 1996) was soil-drenched into pots and disease severity was recorded 14 days after inoculation. At least three plants were tested for each treatment and the experiment was repeated twice.

Results

Isolation and identification. *Phytophthora* root rot of Chinese cabbage and spinach occurred at Yangju, Seosan and Yeochen, Korea from 1995 through 1998, especially in lowland and submerged areas. Affected plants showed retarded growth and leaf yellows followed by wilt and eventual death. Tap and lateral roots had brown rot with discoloration of inner vascular tissues (Fig. 1). From the infected plants, five isolates of *Phytophthora* sp. were collected from Chinese cabbage and nine from spinach. The disease incidence ranged from 5% in the field Chinese cabbage to 64% on the plant cultivated in the greenhouse (Table 1).

All isolates grew well on common media such as 10% V8A, potato dextrose agar (PDA), CMA and oat meal agar. The fungus produced fluffy aerial mycelia and slightly rosaceous colony pattern on PDA (Fig. 1). Sporangia formed only in water were non-papillate, often internally or externally proliferated, elongated ovoid to obpyriform,

Table 1. Survey on *Phytophthora* root rot of Chinese cabbage and spinach, and collection of isolates

Host	Field location	Disease rate[a] (%)	No. of isolates collected	Collection date
Chinese cabbage	Yangju	64	2	Sep. 1995
(*Brassica campestris*)	Seosan	5	3	Jun. 1997
Spinach	Yeocheon	30	9	Mar. 1998
(*Spinacia oleraceae*)				

[a]Plants showing wilt caused by root rot were considered as infected. Thirty plants in each of randomly selected 3 plots were counted in a field.

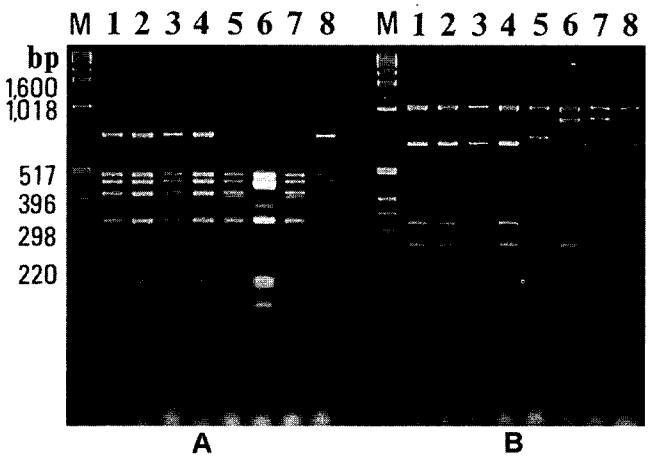

Fig. 2. Restriction patterns of the small subunit and ITS regions of *Phytophthora* spp. digested with *Hae* III (A) and *Hha* I (B). Lane 1; Chinese cabbage isolate P-9509, 2; Spinach isolate P-9818, 3; *P. drechsleri* P-9615, 4; *P. cryptogea* P-9533, 5; *P. erythroseptica* P-96117, 6; *P. cambivora* Pb-06, 7; *P. sojae* P-9662, 8; *P. megasperma* P-9608.

Table 2. Characteristics of the *Phytophthora* causing root rot of Chinese cabbage and spinach in comparison with *P. drechsleri*

Investigated characteristics		Features of representative isolates originated from		*P. drechsleri* [a]
		Chinese cabbage (P-9509)	Spinach (P-9819)	
Sporangium	Formation	Only in water Inter or external Single or sympodium	Only in water Inter or external Mostly single	Only in water Inter or external Single, lax sympodium
	Papillium	None	None	None
	Shape	Obpyriform, ovoid	Elongated ovoid	Broadly obpyriform, ovoid, ellipsoidal
	Base	Round or tapered	Tapered	Tapered
	Caducity	None	None	None
	Size (μm)	36-60×28-38 (av. 47.8×30.2)	34-60×20-32 (av. 48.5×25.7)	40-71×22-34 (av. 52.0×28.0)
	L/B ratio	1.58	1.89	1.9 (1.4-2.5)
Chlamydospore		None	None	None
Hyphal swelling		Common	Rare	Sometimes
Cultural pattern	10% V8	Fluffy, aerial	Fluffy, aerial	No distinct, fluffy
	PDA	Slightly rosaceous	Floral	Slightly floral
Sexuality		Heterothallic, A1	Heterothallic, A1	Heterothallic, some self fertile
Oogonium	Shape	Smooth, spherical	Smooth, spherical	Spherical
	Size (μm)	26-40 (av. 33.4)	30-40 (av. 34.8)	28-38 (av. 33)
Oospore	Filling	Plerotic	Plerotic	Plerotic or aplerotic
	Size (μm)	24-36 (av. 28.5)	21-38 (av. 30.4)	16-37 (av. 28.0)
Antheridium	Type	All amphigynous	All amphigynous	Amphigynous
Growth (°C)	Minimum	7	7	5-10
	Optimum	28	28	25-30
	Maximum	35	35	35

[a] Ho and Jong (1986).

rounded or slighted tapered base, mostly single and ranged 34-60×20-38 μm (av. 47.8-48.5×25.7-30.2 μm). The fungus grew between 7 and 35°C and maximally at 28°C. The fungus was heterothallic since oospores were formed only when mated with either A1 or A2 mating type standard. While all spinach and two Chinese cabbage isolates were A1 type, three Chinese cabbage isolates originated from Seosan were A2 type. Antheridia were all amphigynous. Sizes of oogonia and oospores were measured as 26-40 μm (av. 33.4-34.8 μm) and 21-38 μm (av. 28.5-30.4 μm), respectively (Table 2).

PCR-RFLP of ribosomal DNA. The primers, ITS1 and ITS4, successfully amplified the small subunit and ITS regions of rDNA of all the isolates used in this study. The amplified small subunit and ITS regions of the *Phytophthora* species were about 2,600 bp (data not shown). RFLP analysis of the amplified fragments when digested with *Hae*III or *Hha*I showed that the Chinese cabbage and spinach isolates (P-9509 and P-9819) were identical to those of *P. dreshcelri* (P-9615) and *P. cryptogea* (P-9533), but distinct from other species such as *P. erythroseptica* (P-96117), *P. cambivora* (Pb-06) and *P. sojae* (P-9662). However, the band patterns of *P. megasperma* (P-9608) were distinguished from those of the two present isolates, *P. dreshcelri* (P-9615) and *P. cryptogea* (P-9533) only when digested with *Hha*I but not with *Hae* III.

Table 3. Pathogenicity of the *Phytophthora* isolates of Chinese cabbage and spinach to vegetables

Tested plants	Cultivar	Degree of root rot by representative isolates[a]		Estimated phenotype[b]
		Chinese cabbage (P-9509)	Spinach (P-9819)	
Chinese cabbage	Gorangji	3.7	2.7	S
	Mi-in	4.0	3.7	S
	Yeorumsingwan	3.3	3.7	S
	Banjeom	3.7	3.0	S
Radish	Sinjinju	2.0	2.0	M
	Yeorumyeolmu	1.3	1.3	M
Spinach		2.0	1.3	M
Tomato		1.5	1.0	M
Cabbage		1.0	1.3	M
Kale		0.3	0.7	R
Chicory		0.5	0.5	R
Brown mustard		0.5	0	R
Pepper		0.5	0	R

[a] Degree of root rot: 0; healthy, 1; weak, 2; moderate, 3; severe, 4; death.
[b] S; susceptible, M; moderately resistant, R; resistant.

Pathogenicity. Chinese cabbage was the most susceptible among tested plants and the susceptibility was not different significantly among cultivars tested. Spinach, radish and

tomato were also infected by the fungus moderately. However, cabbage and kale were hardly infected, and chicory, brown mustard and pepper were not affected by the fungus (Table 3).

Discussion

Phytophthora diseases on green vegetables have not been reported in Korea previously, although the diseases on cabbage, spinach, cauliflower, turnip, rutabaga, kale and Brussels sprouts have been reported in several other countries (Downes and Loughnane, 1969; Karakaya et al. 1995; Sherf and Macnab, 1968; Thompson and Phillips, 1988). The young Chinese cabbage grown in the greenhouse at Yangju and spinach in the field at Yeochon were rather heavily affected by the pathogen. However, it may be concluded from our survey that the disease occurrence was confined to flooded or poorly drained soil, but not commonly encountered during the cultivation.

In the genus of *Phytophthora, P. cryptogea, P. drechsleri* and *P. megasperma* were reported as one of casual pathogens of spinach and crucifers (Downes and Loughnane, 1969; Karakaya et al. 1995; Sherf and Macnab, 1968; Thompson and Phillips, 1988). All of the mycological characters of the present isolates examined were in accordance with *P. drechsleri* described by different authors (Erwin and Ribeiro, 1996; Ho and Jong, 1986; Ho et al. 1995; Stamps et al. 1990). Morphological characteristics of the present isolates, especially the Chinese cabbage isolates, also resembled *P. cryptogea* (Erwin and Ribeiro, 1996; Ho and Jong, 1986; Jee et al., 1996; Stamps et al. 1990). Distinctive criteria between the two species have been ambiguous for a long time. Ho & Jong (1986) insisted the two species should be combined because of their indistinguishable morphology. However, Mills et al. (1991) reported that at least seven distinct genetic entities represented within the *P. cryptogea-P. drechsleri* complex group. Since variations between some groups were as great as those among other valid species in the genus, they alleged that the two species should not be merged into one.

The genetic diversity of Korean isolates of *P. drechsleri* and *P. cryptogea* was investigated by Hong et al. (1998) based on PCR-RFLP of rDNA. Twenty-one isolates of *P. drechsleri* originated from 15 different host plants were divided into three distinct groups, designated as *Pd*G1, *Pd*G2 and *Pd*G3. Four isolates of *P. cryptogea* previously identified by Jee (1998) and Jee et al. (1996) came under *Pd*G1 and *Pd*G2, and the two groups presented over 95% homology. PCR-RFLP analysis of rDNA conducted in our study indicated that band patterns of the present isolates were identical to *P. drechsleri* and *P. cryptogea*, but clearly differed from closely related species of *P. erythroseptica, P. cambivora P. sojae* and *P. megasperma*. Based on the study and literature review (Erwin and Ribeiro, 1996; Ho and Jong, 1986; Hong et al. 1998; Jee et al. 1996; Mills et al. 1991), we are judging that *P. drechsleri* and *P. cryptogea* are not clearly separable morphologically and genetically, but several groups exist in the complex. Therefore, we identify the causal pathogen of Chinese cabbage and spinach root rot as *P. drechsleri,* which has a wide host range and more commonly found pathogen on the vegetables. Since the pathogen distributed worldwide causing mainly root rots of hundreds of important crops (Erwin and Ribeiro, 1996; Ho et al. 1995; Jee, 1998) and Chinese cabbage was susceptible to the pathogen, the *Phytophthora* root rot on the plants could be a potential hazard if environmental conditions are favorable for the disease development.

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