Virulence and Relative Density of Three Root Rotting Organisms; *Pythium ultimum, Pythium echinocarpum* and *Rhizoctonia solani* in Alpine Soils in Chinese Cabbage

KIM, CHOONG-HOE AND WON-DAE CHO

김충회 • 조원대 : 고냉지 배추 재배지역 토양에서 배추에 뿌리 썩음을 일으키는 *Pythium ultimum, Pythium echinocarpum, Rhizoctonia solani*균의 병원력 및 토양내 상대밀도

Korean J. Plant Prot. 25(3) : 183-189(1986)

ABSTRACT Fifty three diseased root samples of Chinese cabbage and 22 soil samples were taken from six alpine areas in Kangwon province to examine the organisms causing root rot of Chinese cabbage. Among thirteen microorganisms detected from diseased roots samples, *Pythium ultimum, Pythium echinocarpum* and *Rhizoctonia solani* were detected frequently and were pathogenic to Chinese cabbage. Five to 100% of young Chinese cabbage plants grown in the sample soils taken from alpine cabbage fields were infected either alone or together with *P. ultimum, P. echinocarpum* and *R. solani*, depending upon the orgin of sample soils. When relative density of the three organsims in the sample soils was estimated based on the infection frequency on the plants grown in those sample soils, *P. ultimum* was prevalent only in Chahang-i-li area. *P. echinocarpum* was prevalent in Yongsan-il-li, Hoengwe-li, and Munmaek areas. *R. solani* was solely found in Maebongsan area where both *P. ultimum* and *P. echinocarpum* were not detected. In the three areas where both *P. ultimum* and *P. echinocarpum* were present, density of *P. echinocarpum* was generally greater than that of *P. ultimum.*

Seedlings of thirty major Chinese cabbage varieties were inoculated independently with the three organisms in the greenhouse. Six varieties were resistant to *P. ultimum.* Three and two varieties were moderately resistant to *P. ultimum* and *P. echinocarpum*, respectively. All varieties were highly susceptible to *R. solani.*

INTRODUCTION

Recently, Chinese cabbage has been cultivated at large scale in alpine area around Daekwan-lyong, Pyongchang-gun, Samchuck-gun, and Taebaek-si in Kangwon province, to supply Chinese cabbages to suburban areas during summer periods from June to August. In these areas, Chinese cabbage is often being cultivated continuously for several years in the same fields because of limitation of cultivation acreage. This continuous cropping system often resulted in severe outbreak of disease.

Disease problems occurred in alpine area in Jeonbuk province were reported previously.[2] In that study, Fusaium damping off and a root rot presummed to be caused by *Aphanomyces* sp. were listed as the diseases associated with roots of Chinese cabbage in the alpine area. Root diseases in Chinese cabbage caused by *Rhizoctonia solani, Pythium ultimum* and *Aphanomyces raphani* have been reported in Japan(see reference 2).

During the disease survey conducted in Pyongchang-gun, Samchuck-gun and Taebaek-si in Kangwon province in 1981 and 1982, a root disease was found to be prevalant in Chinese cabbage fields. Diseased root was rotten in dry form and usually did not have root hairs. In cross section, inner part of diseased root was partially discolored. In the early stage of growth, infected plants became wilted and rate of growth of those plants de-

Department of Plant Pathology, Agricultural Sciences Institute, Suwon 170 Korea (농업기술연구소 병리과)

creased gradually. In the severe cases, young plants often died soon. In the later growth stages, heads of infected cabbage plants were subjected to be separated easily from roots by outer physical forces such as wind or rainfall, due to insufficient root development to support above ground parts of Chinese cabbage.

This study was conducted to examine the organisms accociated with this disease.

MATERIALS AND METHODS

Sample collection. Twenty two soil samples and 53 diseased roots were taken from 22 Chinese cabbage growing fields in six alpine areas in Pyongchang-gun and Tabaek-si in Kangwon province during July in 1982. Approximately 20~30kg of soil was taken from soil surface in each field and put into plastic bags. Sample soils were preserved in the laboratory until they were used in the greenhouse.

Detection, isolation, and identification of microorganisms. Diseased root samples were thoroughly washed under a running tab water and cut into small pieces using razor blades. The pieces were surface-sterilized in chlorox 1% solution for 3 min and put into petri dishes containing 1% water agar. Some pieces of diseased samples were put into test tubes each containing 10 ml of sterilized distilled water with one or two boiled corn grains to detect water molds. Five day after incubation at 28°C, microorganisms growing from the diseased root pieces or corn grains were indentified under the microscope or were isolated into corn meal agar (corn meal 20 g, agar 10 g, water 1 *l*) by transferring hyphal tip from the fungal colonies.

Species of fusaria were identified based on the classification system developed by Snyder and Hansen.[3] *Pythium* species were identified following the key of Waterhouse.[4] *Rhizoctonia solani* was identified using the method of Parmeter.[1]

Pathogenecity test. The Chinese cabbage variety 'Samjin' was planted one in each in clay pots(10 cm diam. 12 cm high) containing sterilized vermiculite. The plants were raised in the greenhouse at 19~35°C until inoculation.

Each of thirteen fungal isolates was grown in 9 cm diam. petri dishes containing corn meal agar. The culture petri dishes were incubated at 24°C for 26 days. Conidial suspension of fusaria, *Gloeosporium* sp., *Monilia* sp., *Alternaria alternata* and *Mucor* sp. was made by brushing the colony surface of the culture with small volume of sterile water. Mycelial suspension was made with *R. solani*, both species of *Pythium*, *Saprolenia* sp. and an unidentifed fungus by homogenizing the colony surface removed from the culture at the rate of 250 ml of sterile water per pertri dish culture. Concentration of conidial suspension was adjusted 30~50 conidia in the 100x microscopic field. Inoculation was performed at the 2~3 leaf stage by drenching the inoculum around the plant at the rate of 2 ml per plant. After inoculation, the plants were kept in a plastic covered chamber to maintain humid condition. Temperature inside the chamber ranged from 22 to 34°C during the experiment. Nine days after inoculation, number of infected plants was examined in each fungus.

Pot test. The Chinese cabbage variety 'Samjin' was used in the study. Seeds were planted in plastic square boxes (40x50x10cm) containing sterilized soils (sand: peat: clay=2 : 1 : 2) in the greenhouse. At the 3~4 main leaf stage the plants were transplanted, three plants per each, into clay pots (18 cm diam., 12 cm high) containing the soil taken from alpine areas. A total of 20 pots was made with one soil sample, hence 60 plants were grown with

one soil sample. After transplanting, plants were kepts in the plastic-covered chamber in the greenhose. A weaven rice straw carpet was placed on the top of the chamber to shade sunlight in the day time. A humidifier was placed inside the chamber to maintain humid condition. Temperature varied from 19 to 35°C inside the chamber during the test period. In the day time of clear day, one side of plastic chamber was openned to prevent temperature rise. Three weeks after transplanting, number of infected plants was recorded.

Roots of infected plants were collected from the pots. Causal organisms were determined in the same way described in the previous section. Frequency of infection by each pathogen was examined with each soil sample.

Varietal resistance. To prepare inocula, *Pythium ultimum*, *P. echinocarpum* and *R. solani* were grown on potato sucrose agar (potato 250 g, sucrose 3 g, agar 10 g, water 1 l) in 9 cm diam. petri dishes at 28°C for 7 days. Colony surface was then removed from each culture using a long razor blade and was homogenized after adding 250 ml of sterile water per petri dish culture to make mycelial suspension. The suspension was kept at 4°C until inoculation.

Thirty Chinese cabbage varieties were obtained from the Horticultural Experiment Station in Suwon. One hundred seeds of each variety were planted in a plastic square box (40x50x10 cm) containing steamed soil (sand: peat: clay=2 : 1 : 2). There were 3 replications (=boxes) for each of three pathogens. The plastic boxes were placed on the greenhouse bench until inoculation. Fourteen days after planting, plants were inoculated with each of three pathogens by drenching the inoculum suspension around the plants at the rate of 1 ml per plant. After inoculation, plants were placed in the plastic chamber described in the previous section. Number of diseased plants in each variety was counted 10 days after inoculation.

RESULTS

Microorganisms associated with diseased roots. Thirteen different species of fungi including one unidentified species and several bacteria and nematodes were detected from diseased root samples (Table 1). Among the fungi detected, fusaria were most frequent species found with diseased root samples. *Pythium ultimum* and *P. echinocarpum* were also frequently detected from the root samples. *Rhizoctonia solani* was found on the five root samples. *Saprolegnia* species was detected from sterilized corn grains that were used as baits for water molds. Other fungi such as *Gloeosporium* sp., *Monilia* sp., *Mucor* sp. and *Alternaria* sp. were found with less than five

Table 1. Frequency of detection of microorganisms from 53 diseased root samples of Chinese cabbage taken from six alpine areas in Kangwon province, and pathogenecity of the microorganisms on seedlings of Chinese cabbage

Microorganisms	Frequency of detection	Pathogenecity (No. of infected plants/total no. of plants)[a]
Pythium ultimum	11	6/6
P. echinocarpum	12	6/6
Fusarium oxysporum	19	0/6
F. solani	7	0/6
F. equisetti	1	0/6
Fusarium sp.	10	0/6
Rhizoctonia solani	5	6/6
Gloeosporium sp.	1	0/6
Monilia sp.	1	0/6
Alternaria alternata	4	0/6
Mucor sp.	2	0/6
Saprolegnia sp.	3	0/6
Unknown fungus	1	0/6
Bacteria	4	-[b]
Nematode	4	-

[a] The variety, Samjin was planted in the pots (10 cm diam. 12 cm high) and was inoculated with microorganism suspension at the 2~3 main leaf stage.

[b] The organism was not tested.

root samples. Bacteria and nematode were detected usually along with the fungi described above. Most of bacterial colonization was very limited in the periphery of the specimen.

Among the thirteen fungi tested, *P. ultimum*, *P. echinocarpum* and *R. solani* were pathogenic to Chinese cabbage plants. The roots of infected plants were completely rotten by these pathogens. In particular, *R. solani* affected not only roots but also leaf and stems. Bacteria and nematodes were not included in the pathogenecity test.

Table 2. Degree of infection of Chinese cabbage plants grown in the soil samples taken from Chinese cabbage fields in alpine area in Kangwon province in 1982

Area sampled[a]	No. of years of continued cabbage cultivation[b]	% infection[c]
Pyongchang-gun		
Do-am, Suha-li Ⅰ	—	8
Do-am, Suha-li Ⅱ	—	33
Do-am, Suha-li Ⅲ	—	66
Do-am, Suha-li Ⅳ	—	92
Yongsan-il-li	—	68
Chahang-i-li	—	85
Hoengwe-li Ⅰ	10	66
Hoengwe-li Ⅱ	1	30
Hoengwe-li Ⅲ	5	80
Hoengwe-li Ⅳ	3	88
Hoengwe-li Ⅴ	3	96
Hoengwe-li Ⅵ	3	100
Hoengwe-li Ⅶ	1	30
Hoengwe-li Ⅷ	3	84
Hoengwe-li Ⅸ	5~6	80
Hoengwe-li Ⅹ	1	78
Hoengwe-li Ⅺ	10	40
Hoengwe-li XII	2	20
Hoengwe-li XIII	1	92
Taebaek-si		
Maebong-san Ⅰ	—	33
Maebong-san Ⅱ	—	42
Munmaek	—	33
Check Ⅰ	—	0
Ⅱ	—	0
Ⅲ	—	0

[a] About 20~30kg of soil samples was taken from the soil surface. In the check treatment, the same amount of sterilized soils was used.
[b] Values were obtained from interviews with farmers who own that cabbage field.
[c] Values are the percentage of infected plants from a total of 60 plants examined. The Chinese cabbage plant, Samjin was transplanted into the soil samples at the 3~4 leaf stage in the greenhouse. Number of infected plants was examined 3 wks later.

Infectivity of plant in soil samples. Origin of soil samples and severity of infection on those soil samples are shown in Table 2. Percentage of infection of Chinese cabbage plants grown in the sample soils varied from 5 to 100%, depending upon the origin of soil. There was no obvious difference in percentage of infection between soils from the six alpine areas. In general, plants grown in the soil samples from Taebaek-si area were less diseased compared to those from other area. Severity of infection also varied greatly between soil samples within one area.

Correlation between infection severity and number of years of continuous cropping was low ($r=0.29$) and was not statistically significant.

Infections of Chinese cabbage plants in the soil samples were all caused by *P. ultimum* *P. echinocarpum* and *R. solani*. Frequency of infection of these three pathogens varied with origin of soil sample (Table 3). No infections occurred by *R. solani* with soil samples from Pyongchang-gun and Munmaek in Taebaek-si. Infections of plants in the sample soil from other than Maebong-san area were caused either alone or together by *P. ultimum* and *P. echinocarpum*. In the sample soils where both species of *Pythium* were present, infection frequency of *P. echinocarpum* was generally greater than that of *P. ultimum*.

Varietal resistance. There were significant differences in level of infections among 30 varieties to *P. ultimum*, *P. echinocarpum* and *R. solani*. All thirty varieties were highly susceptible to *R. solani* with 87 to 100% infection severity (Table 4). Only two varie-

Table 3. Frequency of isolation of *Pythium ultimum*, *P. echinocarpum* and *Rhizoctonia solani* from diseased plants of Chinese cabbage grown in the soil sampled from Chinese cabbage fields in the alpine area in Kangwon province in 1982

Origin of soil sample	Total no. of diseased plants examined[a]	Frequency of infections		
		P. ultimum	*P. echinocarpum*	*R. solani*
Pyongchang-gun				
Do-am, Suha-li	50	24	26	0
Yongsan-il-li	23	4	19	0
Chahang-i-li	27	27	0	0
Hoengwe-li	31	6	25	0
Taebaek-si				
Maebong-san	28	0	0	28
Munmaek	28	0	28	0

[a] Diseased roots were examined directly under the microscope, or examined after 2 days incubation on corn meal agar at 26°C.

Table 4. Virulence of *Pythium ultimum*, *P. echinocarpum* and *Rhizoctonia solani* on seedlings of 30 Chinese cabbage varieties grown in the greenhouse

Variety	% infected plants by		
	P. ultimum	*P. echinocarpum*	*R. solani*
Sambok	84.6	88.5	100
Wonkyo 204	29.2	89.4	100
Wonkyo 205	6.5	51.2	95.1
Wonkyo 206	23.3	57.7	98.5
Miwonyolm	75.3	63.2	92.2
Kosanjiyolm	64.7	62.7	96.1
Konaengjiyolm	92.5	93.2	97.3
Yolmdaehyungkalak	78.7	93.6	95.7
Naebyunghawang	59.1	83.7	100
Kumkangyolm	71.4	96.1	100
Naebyung-60-il	71.9	87.7	96.5
Kowon	56.4	73.1	100
Samikalak	68.4	100	100
Naebyungwolha	39.3	88	98
Naeseosamkye	73.5	97.1	100
Konangjichosengyolm	2.6	49.4	86.6
Naebyungyolm	88.4	93.7	94.7
Danobom	38.3	98.3	96.7
Manchun	36.9	50.8	96.1
Ipchunkalak	87.7	91.8	100
Naeseobaeklo	97.6	96.4	98.8
Samjin	66.7	96.7	100
Kangliokdaehyungkalak	78.2	77.0	100
Kalaksinilho	77.3	82.7	98.7
Konong-3-ho	78.3	86.4	100
Miho-70-il	86.1	88.6	100
Miho-1-ho	80.3	93.4	100
Kangliok-60-il	75.0	85.5	100
Changwonbaechu	19.7	95.0	100
Wolbokyolm	21.2	45.2	100

[a] Seeds were obtained from the Horticultural Experiment Station.

[b] Number of inoculated seedlings varied from 26 to 100 depending on the germination ability of the varieties and averaged 72 throughout the experiment.

ties; Konaengichosengyolm and wolbokyolm were moderately resistant to *P. echinocarpum*. Remaining twenty eight varieties were highly susceptible to this pathogen. Six out of 30 varieties were resistant to *P. ultimum* with 2 to 29% infection severity. Three varieties were moderately resistant and twenty one varieties were highly susceptible to this pathogen.

DISCUSSION

Among the three fungus isolates pathogenic to Chinese cabbage plants, *P. ultimum* and *R. solani* have been reported previously in Chinese cabbage (see reference 2). These two pathogens were reported to be a problem in alpine areas particularly when cabbage cultivation continued for several years in the same fields. In the present study, typical symptoms observed in cabbage fields were not reproduced with young cabbage plants in the greenhouse. Cabbage plants at the 3~4 main leaf stage might not be aged enough to see the typical symptom under the condition of heavy inoculum level. Nevertheless, there are several indications suggesting the possibility of association of *P. ultimum*, *P. echinocarpum* and *R. solani* with the root disease observed in alpine area. *P. ultimum*, *P. echinocarpum* and *R. solani* were frequently isolated from the diseased root samples taken from the alpine fields, and were highly pathogenic to young cabbage plants. Further, plants grown in the sample soil taken from the fields in alpine area were overwelmly infected solely with these pathogens. This results suggest that *P. ultimum*, *P. echinocarpum* and *R. solani* might involve the root rotting phenomenon. These pathogens may cause the disease either alone or collectively.

Aphanomyces raphani was also reported as a cause of root rot in Chinese cabbage.[5] In our study, this fungus was not detected either in the diseased root samples or in the soil samples taken from the alpine cabbage fields. Instead, a similar water mold *Saprolegnia* sp. was found from diseased root samples but not pathogenic to Chinese cabbage plant. This indicates little possibility of association of *A. raphani* with the root rot we observed in the alpine area.

When the relative density of populations of *P. ultimum*, *P. echinocarpum* and *R. solani* in the soil sampled from alpine areas was estimated based on infection frequency of these three pathogens on Chinese cabbage plants grown in those soil samples, *P. ultimum* appeared to be prevalent in Chahang-i-li area, whereas *P. echinocarpum* appeared to be prevalent in Yongsan-il-li, Hoengwe-li and Munmaek in Taebaek-si area. Density of *R. solani* appeared high only in Maebong-san area. In the areas where both *P. ultimum* and *P. echinocarpum* were present, density of *P. echinocarpum* appeared greater than that of *P. ultimum*.

In the varietal resistance study, most or all the varieties cultivated currently in alpine areas were susceptible to either one of the three pathogens. This might contribute to severe incidence of this disease in the alpine area. In the present study, young plants were used for inoculation in the greenhouse. Level of resistance of mature plants, however, may be different from those of young plants. In this aspect, screening of resistance with mature plants conducting in the alpine area may be most desirable.

적 요

강원도 고냉지 배추재배 지역에서 53개의 이병 뿌리와 22개 토양 표본을 채취하여 배추 생육 초기에 뿌리 썩음을 일으키는 병원균을 조사하였다.

이병된 뿌리에서 검출된 13개 곰팡이 중에서 *Pythium ultimum*, *Pythium echinocarpum*,

*Rhizoctonia solani*의 3종이 자주 검출 되었으며 배추에 병원성이 있었다. 채취한 토양에 본엽 3~4엽기의 배추를 이식하여 발병 유무를 온실에서 조사 하였을 때 토양 표본에 따라 5내지 100%의 식물이 이들 균에 의하여 감염 되었다. 토양내 이들 3종의 균의 밀도를 배추 식물의 감염 빈도에 의하여 조사 하였을 때 차항 2리 지역의 토양에서는 *P. ultimum*이 우점종 이었고 *P. echinocarpum*은 용산 2리, 횡계리, 문백 지역에서 주로 밀도가 높았다. 매봉산 지역의 토양에서는 *R. solani* 균만이 단지 분포하고 있었으며 *P. ultimum*과 *P. echinocarpum*이 동시 분포하는 지역에서는 대체로 *P. echinocarpum*균의 밀도가 *P. ultimum*에 비하여 더 높았다.

주요 30품종의 유묘를 가지고 온실에서 품종 저항성을 조사한 결과 6개 품종이 *P. ultimum*에 대하여 저항성이었고 3개 품종이 중도 저항성 이었다. *P. echinocarpum*에 대해서는 저항성 품종이 없고 2개 품종이 중도 저항성 이었으며 *R. solani*균에 대해서는 모두가 이병성 이었다.

LITERATURES CITED

1. Parmeter, J.R. Jr. 1970. *Rhizoctonia solani*, Biology and Pathology. University of California Press, Berkeley. 255pp.
2. So, I.Y., S.H. Lee, H.M. Kim, and W.H. Lee. 1981. Disease incidences in radish and chinese cabbage. I. Major diseases in radish and chinese cabbage grown in alpine areas in Jeonbuk Province. (in Korean) Korean J. Pl. Prot. 20 : 135~145.
3. Tousson, T.A., and P.E. Nelson. 1976. A Pictorial Guide to the Indentification of Fusarium Species According to the Taxonomic System of Snyder and Hansen. 2nd edition. University Park, Pennsylvania State University press. 43 pp.
4. Waterhouse, G.M. 1967. Key to Pythium Pringsheim. CMI Mycological Papers No. 109. 25 pp.
5. Yokosawa, R., A. Ogoshi and R. Sakai. 1972. On the mechanisms of zoospore invasion of *Aphanomyces raphani* Kendrik to the host plant. (in Japanese). Ann. Phytopath. Soc. Japan 38 : 284~288.