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Occurrence of Clubroot in Cruciferous Vegetable Crops and Races of the Pathogen in Korea

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Cruciferous vegetable crops grown in several locations in Korea were surveyed from 1996 to 2000. Clubroot severely occurred up to a maximum of 100% in Chinese cabbage fields in 15 out of 42 locations, and in cabbage fields in 5 out of 13 locations surveyed. The disease also severely occurred up to a maximum of 40% in radish fields in 6 out of 35 locations, and up to a maximum of 40% and 100% in turnip and brown mustard fields in one each out of the few locations surveyed, respectively. The disease occurred less than 1% in one kale field in one out of two locations surveyed. A total of 268 isolates of *Plasmodiophora brassicae* was obtained from six cruciferous vegetable crops. The isolates were classified into 13 races based on their pathogenicity to the differential varieties of cabbage and rutabaga. There were 13 races found in isolates from Chinese cabbage, while 6 races each were found in isolates from cabbage and radish. There were five and three races found in turnip and brown mustard isolates, respectively. One isolate from kale was identified as race 8. Race 8 was the most frequently isolated from five cruciferous vegetable crops, except brown mustard. Races 3 and 14 were isolated only from Chinese cabbage.

Keywords **:** clubroot, cruciferous vegetable crop, *Plasmodiophora brassicae*, race.

Clubroot caused by *Plasmodiophora brassicae* Woronin occurs in crucifers worldwide. The disease is characterized by hypertrophy and stunt of the infected plants resulting in severe yield loss in the field. In Korea, clubroot has occurred in cruciferous crops for more than 80 years now since the disease was first found on Chinese cabbage in 1920 by Nakata and Takimoto (1928). Recently, the disease became a major problem because of the wide distribution and difficulty of control in major cultivating areas of Chinese cabbage and some other cruciferous crops in the country (Cho et al., 1997; Kim et al., 1999; Kim and Oh, 1997). Kim and Oh (1997) also reported that Korean hybrids of Chinese cabbage are highly susceptible to the disease.

Diverse physiologic races have been reported in the pathogen based on the pathogenicity of the isolates to cruciferous species and varieties (Ayers, 1957; Buczacki et al., 1975; Johnston, 1968; Tanaka et al., 1998; Williams, 1966). Information on races of the pathogen attacking cruciferous crops and on the distribution of the races in the field is very important in breeding for resistant varieties and in establishing cropping systems for the control of the disease. There has been no report on race grouping of *P. brassicae* occurring in cruciferous crops in Korea.

This study was conducted to determine the occurrence and distribution of the disease in the country and to identify races of the pathogen attacking cruciferous vegetable crops.

Materials and Methods

Survey and collection of diseased roots. Cruciferous vegetable crops grown in several locations in Korea were surveyed from 1996 to 2000. Incidence of clubroot on the crops was investigated. Severity of the disease was rated in terms of percentage of infected plants among 100 plants observed with three to five replicates in each infected field. Root galls of the diseased plants were collected, washed in water, air-dried for 1 day, and then stored in polyethylene bags in a freezer at -20°C.

Preparation of inoculum. Suspension of resting spores was obtained from the collected root galls by a modified procedure of the Williams's method (1966). Ten to 30 g each of the root gall was crushed with the use of a mortar and pestle, and 50 ml of distilled water was added. The crushed gall suspension was squeezed through eight layers of cheesecloth. The resulting suspension was centrifuged at 2,500 rpm for 5 minutes. After the supernatant was discarded, the pellet containing resting spores was suspended and washed with 30 ml of distilled water three times by centrifugation. The final pellet was re-suspended in distilled water, and the suspension was adjusted to a concentration of $2\text{-}3\times10^7$ spores/ml using a haemocytometer. The spore suspension was stored at 4°C and used within 24 hours for the pathogenicity tests.

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Cultivation of differential varieties. Differential varieties, Jersey Queen and Badger Shipper of cabbage (*Brassica oleracea* L. var. *capitata* L.), and Laurentian and Wilhelmsburger of rutabaga (*Brassica napobrassica* Mill.), were used for the pathogenicity tests. Three seeds of each variety were sown in circular plastic pots (29 cm in height and 21 cm in diameter) containing sterile soil. All the pots were placed in a greenhouse at 18-28°C. One plant per pot was selected and cultivated in the greenhouse.

Pathogenicity test. Twenty-two-day-old plants of the crucifer varieties were used for the pathogenicity tests. For inoculation, the surface soil around the plant in the pot was dug at a depth of 1-2 cm, and 20 ml each of the spore suspension was poured around the plant. After the spore suspension was absorbed in the soil, exposed plant parts were covered with the original soil. Each test was performed with three replicates. The inoculated plants were watered regularly to keep constant soil moisture during cultivation in the greenhouse. Disease rating was made based on the formation of galls on the roots of the plants 55 days after inoculation. Race determination was made according to the race designation system of Williams (1966).

Results

Disease incidence. Clubroot was observed in Chinese cabbage [*Brassica campestris* L. ssp. *pekinensis* (Lour.) Olss.], cabbage, radish (*Raphanus sativus* L.), turnip (*Brassica rapa* L.), brown mustard [*Brassica juncea* (L.) Czern.], and kale (*Brassica oleracea* L. var. *acephala* DC.) in several locations in Korea during the disease survey from 1996 to 2000 (Table 1). The disease severely occurred up to a maximum of 100% in Chinese cabbage fields in 15 out of 42 locations, and in cabbage fields in 5 out of 13 locations surveyed. The disease also severely occurred up to a maximum of 40% in radish fields in 6 out of 35 locations, and up to a maximum of 40% and 100% in turnip and brown mustard fields in one each out of the few locations surveyed, respectively. The disease occurred less than 1% in one kale field in one out of two locations surveyed.

Identification and isolation frequency of races. A total of 268 isolates of *P. brassicae* was obtained from six cruciferous vegetable crops. The isolates were classified into 13 races based on their pathogenicity to the differential varieties of cabbage and rutabaga (Table 2). There were 13 races in the isolates from Chinese cabbage and 6 races each in the isolates from cabbage and radish. Meanwhile, there were five and three races in the isolates from turnip and brown mustard, respectively. One isolate from kale was identified as race 8. Race 8 was the most frequently isolated from the five cruciferous vegetable crops, except brown mustard. Races 3 and 14 were isolated only from Chinese cabbage.

Distribution of races. The races isolated from Chinese cabbage were the most widely distributed in the locations

Table 1. Occurrence of clubroot in fields of cruciferous vegetable crops in surveyed locations of Korea from 1996 to 2000

Host	Location	No. of fields investigated	No. of fields infected	% infected plants[a]
Chinese cabbage	Bongwha	21	2	2-5
	Cheongyang	15	1	30
	Chuncheon	19	0	0
	Damyang	15	1	20
	Dangjin	12	0	0
	Danyang	28	1	5
	Gangwha	18	0	0
	Geochang	12	9	2-100
	Gimcheon	12	0	0
	Goyang	11	0	0
	Haenam	30	12	10-100
	Hapcheon	22	0	0
	Hoengseong	78	0	0
	Hongcheon	18	0	0
	Iksan	15	0	0
	Jangsu	12	0	0
	Jeju	58	0	0
	Jeongju	40	0	0
	Jinan	22	0	0
	Muju	47	5	2-80
	Namwon	21	0	0
	Namyangju	15	0	0
	Nonsan	11	0	0
	Paju	60	11	20-100
	Pocheon	13	0	0
	Pyeongchang	82	30	2-100
	Pyeongtaek	40	18	10-80
	Samcheok	18	0	0
	Seosan	13	0	0
	Suncheon	14	0	0
	Suwon	12	3	5-60
	Taebaek	82	29	1-100
	Wanju	26	0	0
	Wonju	18	0	0
	Yangju	61	27	1-100
	Yangsan	14	1	less than 2
	Yangpyeong	18	0	0
	Yecheon	15	0	0
	Yeoju	11	0	0
	Yeoncheon	80	55	1-100
	Yeongcheon	11	0	0
	Yeongyang	13	0	0
Cabbage	Bongwha	12	3	2-50
	Hoengseong	6	0	0
	Hongcheon	14	0	0
	Jangsu	3	0	0

Table 1. Continued

Host	Location	No. of fields investigated	No. of fields infected	% infected plants[a]
Cabbage	Jeju	70	0	0
	Muju	5	0	0
	Namwon	2	0	0
	Paju	4	1	100
	Pyeongchang	61	22	2-100
	Samcheok	1	0	0
	Seosan	34	0	0
	Taebaek	10	4	1-90
	Yeoncheon	2	1	80
Radish	Bongwha	16	5	5-30
	Buyeo	66	0	0
	Cheolwon	15	11	1-40
	Cheongwon	37	0	0
	Daegu	20	0	0
	Danyang	17	0	0
	Gangwha	2	0	0
	Geumsan	10	0	0
	Hapcheon	8	0	0
	Hwacheon	2	0	0
	Hoengseong	9	0	0
	Hongcheon	39	1	2
	Iksan	11	0	0
	Jecheon	2	0	0
	Jeju	11	0	0
	Jeonju	33	0	0
	Jinan	3	0	0
	Muju	3	0	0
	Naju	12	0	0
	Namwon	10	8	1-30
	Nonsan	10	0	0
	Paju	27	0	0
	Pocheon	8	0	0
	Pohang	6	0	0
	Pyeongchang	23	1	40
	Seosan	2	0	0
	Suncheon	8	0	0
	Taebaek	4	0	0
	Wanju	10	0	0
	Yangsan	8	0	0
	Yecheon	11	0	0
	Yesan	22	0	0
	Yeoncheon	15	1	less than 2
	Yeongcheon	2	0	0
	Yeongyang	4	0	0
Turnip	Gangwha	23	0	0
	Yeoncheon	3	3	5-40
Brown mustard	Goyang	32	6	30-100
	Hongcheon	1	0	0
	Suncheon	1	0	0
	Yeocheon	17	0	0
Kale	Namyangju	22	0	0
	Yeoncheon	3	1	less than 1

[a] One hundred plants in each field were investigated with three to five replicates.

Table 2. Identification of *Plasmodiophora brassicae* races infecting cruciferous vegetable crops in Korea

Race[a]	No. of isolates identified as a designated race[b]						
	Chinese cabbage	Cabbage	Radish	Turnip	Brown mustard	Kale	Total
1	13	3	–	–	–	–	16
2	31	9	5	–	–	–	45
3	22	–	–	–	–	–	22
4	33	8	–	–	–	–	41
5	14	–	2	–	2	–	18
6	–	–	–	–	–	–	–
7	3	–	1	–	2	–	6
8	36	1	2	4	–	1	44
9	20	3	–	3	–	–	26
10	–	–	–	–	–	–	–
11	5	1	–	2	–	–	8
12	–	–	–	–	–	–	–
13	15	–	–	3	–	–	18
14	1	–	–	–	–	–	1
15	9	–	1	1	–	–	11
16	5	–	6	–	1	–	12
Total	207	25	17	13	5	1	268

[a] Designated by Williams (1966).
[b] – = not isolated.

investigated, and those isolated from the other cruciferous vegetable crops were rarely distributed (Table 3). Of the 13 races isolated from Chinese cabbage, race 8 was the most widely distributed in the locations investigated, followed by races 2 and 4. On the other hand, race 14 was the least distributed.

All the 13 races identified were distributed in Jeonnam province (Table 4). Twelve and 11 races were distributed in Gyeonggi and Chungnam provinces, respectively. Only two races were distributed in Chungbuk province. There was no isolate from Jeju province because clubroot did not occur in this province.

Discussion

Clubroot caused by *P. brassicae* commonly occurs in

Table 3. Distribution of *Plasmodiophora brassicae* races infecting cruciferous vegetable crops in Korea

Host	No. of locations examined	No. of locations with races[a]												
		1	2	3	4	5	7	8	9	11	13	14	15	16
Chinese cabbage	14	7	8	7	8	7	3	11	7	3	6	1	4	3
Cabbage	3	1	2	–	3	–	–	1	1	1	–	–	–	–
Radish	3	–	1	–	–	1	1	1	–	–	–	–	1	2
Turnip	1	–	–	–	–	–	–	1	1	1	1	-	1	-
Brown mustard	1	–	–	–	–	1	1	–	–	–	–	–	–	1
Kale	1	–	–	–	–	–	–	1	–	–	–	–	–	–

[a] – = no race identified.

Table 4. Occurrence of *Plasmodiophora brassicae* races in provinces in Korea

Province	No. of samples examined	Race of the isolates collected												
		1	2	3	4	5	7	8	9	11	13	14	15	16
Gyeonggi	69	+[a]	+	+	+	+	+	+	+	+	+	–	+	+
Gangwon	53	+	+	+	+	–	–	+	–	+	+	–	–	+
Chungbuk	3	–	–	–	–	+	–	+	–	–	–	–	–	–
Chungnam	49	–	+	+	+	+	+	+	+	+	+	–	+	+
Gyeongbuk	17	+	–	–	+	–	–	+	+	–	–	–	–	–
Gyeongnam	26	+	+	+	+	–	–	+	–	–	+	–	–	–
Jeonbuk	23	+	+	+	+	+	–	–	–	–	–	–	–	+
Jeonnam	28	+	+	+	+	+	+	+	+	+	+	+	+	+
Jeju	0	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] + = collected; – = not collected.

crucifers in temperate and northern regions of the world (Buczacki, 1979; Cubeta et al., 1998; Linnasalmi and Toiviainen, 1991). *P. brassicae* is soil-borne and can survive in soil for many years without host plants. The pathogen exists in soil as resting spores which result in the disease occurrence. Accordingly, effective detection methods of the pathogen from the soil have been studied (Faggian et al., 1999; Ito et al., 1999; Orihara and Yamamoto, 1998; Takahashi and Yamaguchi, 1987; Wakeham and White, 1996).

The disease severely occurred up to 100% in some cruciferous vegetable crops, and the pathogen was distributed in all provinces except in Jeju province, which is a big island and is one of the main areas producing cruciferous vegetable crops in Korea. The pathogen was not distributed in fields of cruciferous vegetable crops in that island. However, growers of cruciferous vegetable crops in the island should remain on the look out for infection of the pathogen in the field.

This study showed that there are 13 races of *P. brassicae* in the isolates from root galls of cruciferous vegetable crops in Korea. Williams (1966) designated races 1-16 of *P. brassicae* based on the reaction to infection of four crucifer varieties. He also examined 124 isolates of the pathogen from 16 countries and identified races 1-9. The 13 out of 16 races were identified in Korean isolates of *P. brassicae*, suggesting that diverse races of the pathogen are distributed in the fields of Korea. There were no races 6, 10, and 12 in the Korean isolates.

It has been reported that race 2 is the most commonly distributed in Finland (Linnasalmi and Toiviainen, 1991), and race 4 in Japan (Tanaka et al., 1998). Among the cruciferous vegetable crops, Chinese cabbage is the most important and the most widely cultivated in Korea. Thirteen races of *P. brassicae* exist in the isolates from Chinese cabbage. Especially, race 8 was the most widely distributed in the Chinese-cabbage-growing locations. Race 8 should be considered as a major group in breeding for resistance of the crop against the pathogen.

The race designation system of Williams (1966) has been commonly used worldwide. There is a possibility of mixed infection of some *P. brassicae* races in a root gall of the cruciferous vegetable crops as suggested by previous workers (Buczacki and Humphrey, 1973; Williams, 1966). The authors of this study were not sure whether there was a single infection of a race in the root gall. It is known that *P. brassicae* can not be cultured *in vitro*, although it is capable of at least a short-lived period of saprotrophic amoebal growth (Arnold et al., 1996). It is impossible to get single spore isolation from the root gall because of the uncultur-

able character of the pathogen. Narisawa et al. (1996) developed an efficient method of inoculation with single resting spores of the pathogen. If the method is applied to the single spore isolation, the race designation system of the pathogen would definitely be improved.

References

Arnold, D. L., Blakesley, D. and Clarkson, J. M. 1996. Evidence for the growth of *Plasmodiophora brassicae in vitro. Mycol. Res.* 100:535-540.

Ayers, G. W. 1957. Races of *Plasmodiophora brassicae. Can. J. Bot.* 35:923-932.

Buczacki, S. T. 1979. *Plasmodiophora brassicae.* CMI Descriptions of Pathogenic Fungi and Bacteria. No. 621.

Buczacki, S. T. and Humphrey, J. G. 1973. Problems associated with physiologic specialization in *Plasmodiophora brassicae,* with a description of two new races infecting *Brassica napus. Trans. Br. Mycol. Soc.* 60:588-590.

Buczacki, S. T., Toxopeus, H., Mattusch, P., Johnston, T. D., Dixon, G. R. and Hobolth, L. A. 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. *Trans. Br. Mycol. Soc.* 65:295-303.

Cho, W. D., Kim, W. G., Jee, H. J., Choi, H. S., Lee, S. D. and Choi, Y. C. 1997. Compendium of Vegetable Diseases with Color Plates. National Institute of Agricultural Science and Technology, Suwon, Korea. 447 p.

Cubeta, M. A., Cody, B. R. and Williams, P. H. 1998. First report of *Plasmodiophora brassicae* on cabbage in Eastern North Carolina. *Plant Dis.* 82:129.

Faggian, R., Bulman, S. R., Lawrie, A. C. and Porter, I. J. 1999. Specific polymerase chain reaction primers for the detection of *Plasmodiophora brassicae* in soil and water. *Phytopathology* 89:392-397.

Ito, S., Maehara, T., Maruno, E., Tanaka, S., Kameya, I. M. and Kishi, F. 1999. Development of a PCR-based assay for the detection of *Plasmodiophora brassicae* in soil. *J. Phytopathol.* 147:83-88.

Johnston, T. D. 1968. Clubroot in Brassicae: A standard inoculation technique and the specification of races. *Plant Pathol.* 17:184-187.

Kim, C. H., Cho, W. D. and Yang, J. M. 1999. Survey of field conditions of clubroot disease incidence of Chinese cabbage in major production areas and ecology of root gall development. *Plant Dis. Agric.* 5:77-83.

Kim, D. W. and Oh, J. H. 1997. Incidence, pathogenicity of clubroot fungus (*Plasmodiophora brassicae*) and varietal resistance in Chinese cabbage. *Korean J. Plant Pathol.* 13:95-99.

Linnasalmi, A. and Toiviainen, A. 1991. Occurrence of clubroot and *Plasmodiophora brassicae* Wor. races in Finland. *J. Agric. Sci. Finland* 63:415-434.

Nakata, K. and Takimoto, K. 1928. List of diseases of cultivated plants in Korea. *J. Agric. Exp. Stn., Govern.-Gen. Chosen* 15: 77-78 (In Japanese).

Narisawa, K., Kageyama, K. and Hashiba, T. 1996. Efficient root infection with single resting spores of *Plasmodiophora brassicae. Mycol. Res.* 100:855-858

Orihara, S. and Yamamoto, T. 1998. Detection of resting spores of *Plasmodiophora brassicae* from soil and plant tissues by enzyme immunoassay. *Ann. Phytopathol. Soc. Japan* 64:569-573.

Takahashi, K. and Yamaguchi, T. 1987. An improved method for estimating the number of resting spores of *Plasmodiophora brassicae* in soil. *Ann. Phytopath. Soc. Japan* 53:507-515.

Tanaka, S., Fujiyama, S., Shigemori, S., Nakayama, A., Ito, S. and Kameya, I. M. 1998. Pathogenesis of isolates of *Plasmodiophora brassicae* from Japan. (1) race and pathogenesis in clubroot resistant cultivars. *Proc. Assoc. Plant Protect. Kyushu* 44:15-19.

Wakeham, A. J. and White, J. G. 1996. Serological detection in soil of *Plasmodiophora brassicae* resting spores. *Physiol. Mol. Plant Pathol.* 48:289-303.

Williams, P. H. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. *Phytopathology* 56:624-626.