Virulence to Korean Pepper Cultivars of Isolates of *Phytophthora capsici* from Different Geographic Areas

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ABSTRACT

Kim, E. S., and Hwang, B. K. 1992. Virulence to Korean pepper cultivars of isolates of *Phytophthora capsici* from different geographic areas. Plant Dis. 76:486-489.

The relative virulence of 14 isolates of *Phytophthora capsici* from diverse geographic origins, including France, the Netherlands, Bulgaria, Italy, New Mexico, and Korea, was evaluated on 12 Korean pepper cultivars under controlled environmental conditions. No hypersensitive symptoms were observed in any of the cultivars inoculated with any of the 14 isolates. Highly significant cultivar, isolate, and cultivar × isolate effects were observed, indicating a differential host-pathogen interaction. Significant differences in virulence among the isolates tested were found but were not dependent on the country of origin. Isolate CBS 178.26, which has been maintained since 1926, was the most weakly virulent. To evaluate resistance, the soil-drench and stem-wound methods were more reliable than the foliar-inoculation method when highly virulent isolates were used. The stem-wound and foliar-spray methods were more appropriate than the soil-drench method when weakly virulent isolates were used.

Foliar and stem blight of pepper (*Capsicum annuum* L.), caused by *Phytophthora capsici* Leonian, is one of the most widespread and destructive soilborne diseases, occurring in many pepper-growing areas all over the world (2,8,19). In Korea, this disease, which occurs in all organs of pepper plants (i.e., root, stem, leaf, and fruit), causes severe damage in pepper production, especially during prolonged periods of rainy weather from June to August. Because of the difficulties in controlling Phytophthora blight with soil or foliar fungicides, growing resistant pepper cultivars and rotating crops are recommended to farmers. The pathogen also infects eggplant, cucumber, honeydew melon, pumpkin, tomato, watermelon, and cocoa (5,7,13,16,17,20).

Resistance of pepper to *P. capsici* was reported to be governed by two distinct dominant genes that act independently without additive effects (15) or by a single dominant gene with modifiers (2). Polach and Webster (11) demonstrated that the ability of *P. capsici* to overcome resistance of pepper plants is controlled by at least two genes. The pathogenic variation of *P. capsici* isolates in Korea was shown by Yang et al (21). This variability in pathogenicity may occur by nuclear exchange through sexual recombination based on the two compatibility types, designated A1 and A2 (18). Recently, Bowers and Mitchell (3) demonstrated that oospore progenies from pairings of pathogenic isolates of *P. capsici* differed in their ability to cause disease in pepper plants. Similarly, in the *P. infestans*-potato system, Romero and Erwin (12) crossed an A1 compatibility type (race 1, 2, 3, and 4) with an A2 race 0 and obtained some single-oospore isolates that differed in race from either parent. In addition to sexual recombination, long-term maintenance of cultures of *Phytophthora* spp. in vitro may be another cause of pathogenic variation. Apple (1) suggested that *Phytophthora parasitica* var. *nicotianae* loses its virulence during culturing.

In this study, we examined the pathogenic variation on Korean pepper cultivars of *P. capsici* isolates from diverse geographic origins to determine whether or not there is a differential interaction between *P. capsici* isolates and pepper cultivars. The isolates and inoculation methods most appropriate for resistance-screening of pepper genotypes were also determined.

Accepted for publication 18 September 1991 (submitted for electronic processing).

MATERIALS AND METHODS

Plant, fungal isolate, and inoculum. Twelve pepper cultivars were used for evaluating virulence of 14 isolates of *P. capsici*: Kingkun, Mankang, Jinsol, Hongilpum, Dabogkun, Taeyangkun, Saerona, Churaehong, Hongsanho, Ilwolkun, Juctoma, and Hanbyul. Seeds of each cultivar were sown in plastic trays (55 × 35 × 15 cm) containing a steam-sterilized soil mix of a commercial compost soil (peat moss, perlite, and vermiculite, 5:3:2, v/v/v), sand, and loam soil (1:1:1, v/v/v). Six seedlings at the six-leaf stage were transplanted to plastic pots (5 × 15 × 10 cm) containing the above described soil mix. Fertilizer (N-P-K, 0.27, 0.27, 0.13 g per pot) was applied at 3-wk intervals after planting. Pepper plants were raised in a growth chamber at 25±2 C with 5,000 lx illumination for 16 hr/day.

The geographic origin, sources, and characteristics of the 14 isolates of *P. capsici* used in this study are described in Table 1. The eight isolates from Korea were isolated from pepper-growing areas in 1987 and 1988. They were identified as *P. capsici* and confirmed to be pathogenic on pepper plants by the Department of Plant Pathology of the Agricultural Sciences Institute at Suweon, Korea. The four CBS isolates originated from Italy, Bulgaria, the Netherlands, and New Mexico, and were obtained from the Centraalbureau voor Schimmelcultures at Baarn, the Netherlands. The two isolates from France were supplied by E. Pochard (Plant Pathology Station, Institut National de la Recherche

Table 1. Origins and characteristics of *Phytophthora capsici* isolates used in this study

Isolate	Origin	Source[a]	Compatibility type
87E1	Korea	ASI	A1
88E10	Korea	ASI	A1
87JU5	Korea	ASI	A1
87K1	Korea	ASI	A1
87KS1	Korea	ASI	A1
87L19	Korea	ASI	A2
88J1	Korea	ASI	A2
PC-Nong	Korea	ASI	A1
CBS178.26	Italy	CBS	A1
CBS370.72	New Mexico, USA	CBS	A2
CBS422.77	Bulgaria	CBS	A1
CBS521.77	Netherlands	CBS	A1
S101	France	INRA	A1
S197	France	INRA	A1

[a]ASI = Department of Plant Pathology, Agricultural Science Institute at Suweon, Korea. CBS = Centraalbureau voor Schimmecultures at Baarn, the Netherlands. INRA = Supplied by E. Pochard, Plant Pathology Station, Institut National de la Recherche Agronomique at Montfavet, France.

Agronomique at Montfavet, France), who had used them in studies of resistance of pepper plants to *P. capsici* (9,10). All isolates were grown on V8 juice agar plates at 25±1C, checked for their pathogenicity on pepper plants, and evaluated for compatibility type.

The isolates were grown on oatmeal agar at 25±1 C for 7 days and then moved under fluorescent light at 28±1 C for 48 hr. Mycelia and sporangia were gently brushed into sterile water with an artist's paintbrush, and zoospore release was induced by chilling the suspension at 4 C for 30–60 min. Zoospore discharge occurred within 30–60 min after the suspension was returned to room temperature. The suspension was decanted through four layers of cheesecloth to remove mycelial fragments. Zoospore concentration was determined with a hemacytometer, and inoculum densities were adjusted with sterile tap water to give suspensions containing 1×10^5 zoospores per milliliter.

Inoculation procedures and disease evaluation. In the wound-inoculation procedures, the stems of pepper plants at the second-branch stage were wounded by cutting a 1-cm longitudinal slit 1 cm from the soil surface. A small quantity of sterile cotton dipped in 1×10^5 zoospores per milliliter for 30 min was placed on the wounded slits of stems, which were then covered with plastic tape. To evaluate virulence of 14 isolates of *P. capsici* to 12 pepper cultivars, lesion development on the infected stems of pepper plants was recorded for 13 successive days after inoculation. Lesion development was expressed as: percentage of lesion length = lesion length (cm) divided by plant height (cm) times 100. Percentages of lesion length were also used to calculate areas under disease progress curves (AUDPCs) according to the formula described by Shaner and Finney (14): $\text{AUDPC} = \Sigma_{i=1}^{n} (Xi_{+1} + Xi)(ti_{+1} - ti) / 2$, where Xi = percent lesion length at the ith observation, ti = time (days) at the ith observation, and n = total number of observations. Data were analyzed statistically by Duncan's multiple range test ($P = 0.05$). All data are the means of six plants inoculated with each of 14 isolates. All experiments were performed twice with similar results; data are presented from one experiment only.

For precise comparison of the highly virulent isolate S 197 and the less virulent isolate CBS 178.26, the pepper cultivars Hanbyul (highly susceptible) and Kingkun (highly resistant) were inoculated with a zoospore suspension (1×10^5/ml) at the first-branch stage by using the soil-drench, stem-wound, and foliar-spray inoculation methods previously developed by Kim et al (6). Disease severity was rated daily after inoculation based on a 0–5 scale, where 0 = no visible disease symptoms, 1 = leaves slightly wilted with brownish lesions beginning to appear on stems, 2 = 30–50% of entire plant diseased, 3 = 50–70% of entire plant diseased, 4 = 70–90% of entire plant diseased, 5 = plant dead.

RESULTS

The relative virulence of the isolates of *P. capsici* on the 12 Korean pepper cultivars was evaluated by using AUDPCs (Table 2). Susceptible plants had blighted foliage, leaf defoliation, crown rot, and expanded stem lesions. Some plants within cultivars were symptomless or had foliage blight, crown rot, and patches of a superficial, brownish purple speckling that developed slowly on the stems. No hypersensitive symptoms were observed in any of the cultivars. The ranks of mean AUDPCs based on the percentage of lesion length were consistently in the order Hanbyul > Juctoma > Ilwolkun > Hongsanho > Churaehong > Saerona > Taeyangkun > Dabogkun > Hongilpum > Jinsol > Mankang > Kingkun for the pepper cultivars tested and S197 > CBS 521.77 > 87Ju5 > CBS 422.77 > 87E1 > PC-Nong > 88E10 > 87L19 > S101 > CBS 370.72 > 87KS1 > 88J1 > 87K1 > CBS178.26 for the isolates of *P. capsici*. Significant differences among the isolates tested were found in virulence, regardless of country origin. For example, the French isolate S197 was highly virulent to all the cultivars tested, whereas the French isolate S101 was moderately virulent. The eight Korean isolates also exibited different levels of virulence to the pepper cultivars. The analyses of variance of the AUDPC in this experiment (Table 3) showed highly significant effects of cultivar, isolate, and cultivar × isolate on disease expression. There was in general a differential interaction between the pepper cultivars and *P. capsici* isolates when the different cultivars were inoculated with each of the different isolates.

The disease progress curves for the two selected cultivars Hanbyul (highly susceptible) and Kingkun (highly resistant) after inoculation with 14 isolates of *P. capsici* at the second-branch stage were plotted (Fig. 1). Stem lesions of the cultivar Hanbyul were more rapidly enlarged than those of the cultivar Kingkun. The inoculated plants of the cultivar Hanbyul died at 13 days after inoculation with the highly virulent isolate S197, but in the cultivar Kingkun the lesions reached about 46% of the plant height.

The cultivars Hanbyul and Kingkun, which were the most susceptible and the most resistant, respectively, among the cultivars tested, were reevaluated by inoculation of the virulent isolate S197 and the avirulent isolate CBS178.26 using the soil-drench, stem-wound, and foliar-spray inoculation methods (Fig. 2). Disease severities on Hanbyul and Kingkun differed at the first-branch stage. When inoculated with each of the two isolates, Kingkun was more resistant at this stage than Hanbyul. With all three

Table 2. Areas under disease progress curves (AUDPC)[a] on 12 pepper cultivars in an evaluation of virulence of 14 *Phytophthora capsici* isolates from various geographic origins by using the stem-wound inoculation method[b]

	AUDPC on different cultivars											
Isolate	**Kingkun**	**Mankang**	**Jinsol**	**Hongilpum**	**Dabogkun**	**Taeyangkun**	**Saerona**	**Churaehong**	**Hongsanho**	**Ilwolkun**	**Juctoma**	**Hanbyul**	**Average**
S197	235	337	357	430	386	434	319	451	383	388	454	623	400
CBS 521.77	220	199	243	376	361	441	378	450	481	512	561	452	389
87Ju5	170	291	379	256	276	364	476	348	323	424	365	451	344
CBS 422.77	161	217	345	367	350	323	415	276	462	382	344	474	343
87E1	142	103	162	347	204	342	293	412	435	442	522	490	325
PC-Nong	134	258	260	282	310	266	417	340	381	422	393	398	322
88E10	144	188	190	270	245	299	330	292	362	334	357	523	295
87L19	176	80	50	256	316	355	319	374	344	339	383	364	280
S101	143	162	264	186	305	187	214	262	247	252	333	313	239
CBS 370.72	125	133	174	296	285	228	299	225	152	197	214	423	229
87KS1	114	108	49	153	193	229	163	239	188	256	419	436	212
88J1	44	56	39	136	116	281	196	241	368	279	294	273	194
87K1	13	23	103	148	130	99	93	165	170	401	359	216	160
CBS 178.26	17	115	23	5	219	24	46	31	135	321	100	147	99
Average	131	162	188	251	264	277	283	293	317	354	364	399	

[a] AUDPCs were calculated using the percentage of stem lesion length rated every day after inoculation. Percent lesion length = lesion length (cm)/plant height (cm) × 100.

[b] Pepper plants at second-branch stage were inoculated with a zoospore suspension (10^5/ml) on wounded slits 1 cm above the soil surface by using the stem-wound technique.

inoculation techniques, significant differences were found between the two isolates in level of virulence to the two pepper cultivars. However, the soil-drench inoculation with the avirulent isolate CBS178.26 and the foliar inoculation with the virulent isolate S197 could not distinguish between the two cultivars in the level of resistance to Phytophthora blight.

Table 3. Analysis of variance for the areas under disease progress curves (AUDPC) on 12 pepper cultivars inoculated with 14 isolates of *Phytophthora capsici*

Source of variation	df	Mean square[a]
Cultivar	11	361,454.9
Isolate	13	345,216.2
Cultivar × isolate	143	16,781.6

[a] F value highly significant ($P < 0.01$).

DISCUSSION

The virulence of 14 isolates of *P. capsici* from diverse geographic origins, including France, the Netherlands, Bulgaria, Italy, New Mexico, the United States, and Korea, was evaluated on the 12 Korean pepper cultivars under controlled environmental conditions. The differences between the susceptible and resistant responses to Phytophthora blight were more quantitative than qualitative, as previously observed (5), because *P. capsici* caused symptoms in all the cultivars tested. The symptoms caused by various isolates developed more slowly on the resistant cultivars. No hypersensitive symptoms were observed in any of the Korean pepper cultivars inoculated with different isolates of *P. capsici*. Highly significant cultivar, isolate, and cultivar × isolate effects were found, indicating a differential host-pathogen interaction.

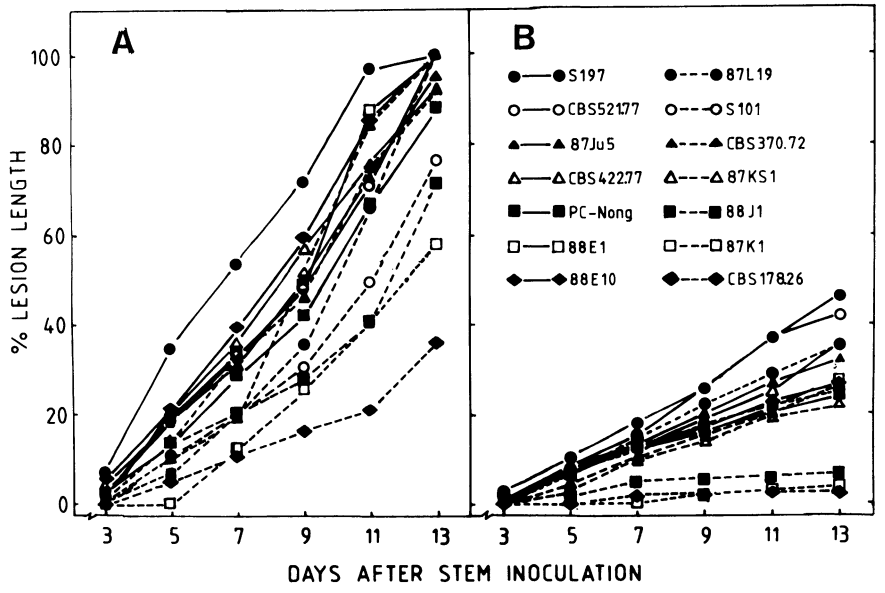

Fig. 1. Disease progress curves on the pepper cultivars **(A)** Hanbyul (susceptible) and **(B)** Kingkun (resistant) in an evaluation of 14 *Phytophthora capsici* isolates from various geographic origins by using the stem-wound inoculation method.

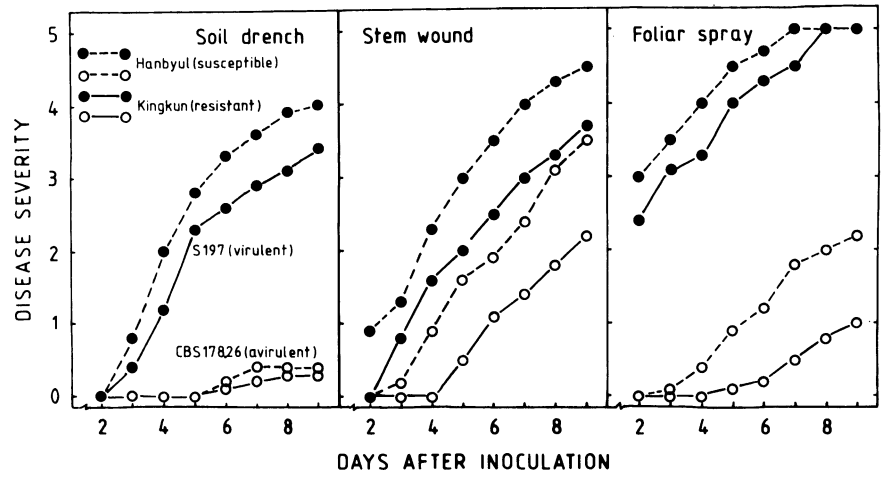

Fig. 2. Disease severity curves for the pepper cultivars Hanbyul (susceptible) and Kingkun (resistant) inoculated with a virulent (S197) and an avirulent (CBS178.26) isolate of *Phytophthora capsici* by different methods.

Significant differences between the isolates tested were found in virulence to the Korean pepper cultivars, regardless of country of origin. The French isolate S197, which showed a high level of virulence on the French pepper cultivars (9,10), was also most virulent to all the Korean cultivars tested, as compared to the other isolates of diverse geographic origins. The isolate CBS178.26, which has been maintained in CBS since isolation in 1926 from pepper plants in Italy, was the most weakly virulent to the pepper cultivars. This suggests that in vitro, long-term maintenance of *P. capsici* isolates may cause the loss or decrease of virulence on pepper plants. The fact that there was a great variation in virulence among the Korean isolates would reflect the possible occurrence of pathogenic specialization of *P. capsici* on the various pepper genotypes grown in Korea for a long period. Recently, Yang et al (21) reported the pathogenic variation of *P. capsici* on pepper plants in Korea, indicating that the pathogenic specificity of the fungus may operate horizontally rather than vertically because of the lack of differential interaction between cultivars and isolates. Similarly, considerable variation in virulence was observed in the European isolates.

Our findings of a great genetic variation in these same isolates of *P. capsici* from Korea, Europe, and New Mexico by examining restriction fragment length polymorphisms of mitochondrial DNA (4) supports the existence of variation in virulence of naturally occurring *P. capsici* isolates. *P. capsici* isolates differed in pathogenicity to Korean pepper cultivars quantitatively rather than qualitatively, indicating only a quantitative degree of physiologic specialization in *P. capsici* isolates under study. Highly significant isolate × cultivar interactions in the analysis of variance of our experiment may occur because of the lack of a proper scale for measuring disease severity depending on environment and plant age. To ascertain whether or not pathogenic races really exist in the pepper-*P. capsici* system in nature, however, further extensive studies should be carried out using various genotypes of pepper plants and *P. capsici* originating from diverse genetic sources of the world.

The soil-drench, stem-wound, and foliar-spray inoculation methods greatly affected the precise evaluation of pepper cultivars for resistance using different isolates of *P. capsici*. We suggest that the soil-drench and stem-wound methods may be more reliable than the foliar-spray inoculation method for evaluating cultivar resistance using highly virulent isolates, but, for the inoculation of weakly virulent isolates, the stem-wound and foliar-spray methods may be more appropriate than the soil-drench method.

Therefore, inoculation methods should be chosen for screening of pepper cultivars for resistance to *P. capsici* that are primarily dependent on the levels of virulence of the isolates used in breeding programs. Our earlier studies also suggested that an appropriate concentration of inoculum and the use of a test pepper plant at the proper growth stage may be very important for evaluation of cultivar resistance to Phytophthora blight (6).

ACKNOWLEDGMENT

We thank G. L. Hartman of the Asian Vegetable Research and Development Center, Taiwan, for his critical review of this manuscript.

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