Quantitative Evaluation of Resistance of Korean Tomato Cultivars to Isolates of *Phytophthora capsici* from Different Geographic Areas

JAE SOON HWANG, Graduate Student, and BYUNG KOOK HWANG, Professor, Department of Agricultural Biology, Korea University, Seoul 136-701

ABSTRACT

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The resistance of 10 Korean tomato cultivars to seven isolates of *Phytophthora capsici* was evaluated by using stem wounding and foliar spraying methods under controlled environmental conditions. The response of tomato cultivars tested to Phytophthora blight was quantitative rather than qualitative. There were highly significant differences among the isolates in virulence to the Korean tomato cultivars, indicating some pathogenic variation in *P. capsici*. Significant differences were also observed among cultivar × isolate interactions in the analysis of variance. When the inoculation was by stem wounding, no symptoms developed in the stems of mature tomato plants. Expression of the age-related resistance to Phytophthora blight was more apparent in tomato stems than in tomato leaves. The foliar spraying inoculation method was effective in screening tomato cultivars for resistance to *P. capsici*, whereas the soil drenching and stem wounding inoculation techniques were less reliable for evaluating cultivar resistance on old tomato plants.

Phytophthora capsici Leonian is one of the most destructive soilborne pathogens and has a wide host range. The fungus is pathogenic on some species of Cucurbitaceae, Leguminosae, and Solanaceae, including tomato (*Lycopersicon esculentum* Mill.) (14). In tomato, the pathogen not only infects roots and fruits but also causes crown rot disease (9,14). Recently, Hartman and Huang (4) reported that some isolates of *P. capsici* caused foliar blight on foliar-inoculated tomato plants but did not cause basal stem blight on soil-inoculated ones. This causal agent has been responsible for major losses of tomato production (14), but control strategies have been limited because of difficulties in effectively controlling the soilborne disease. Chemical applications, good water management, and crop rotation have been considered effective control measures (6,13). The use of resistant cultivars is also a strategy for controlling diseases caused by *Phytophthora* spp. (16). Resistance has been identified in only some tomato genotypes in the *P. parasitica*- and *P. capsici*-tomato systems (1,3). Age-related resistance, which is distinctly expressed as plants mature, may be useful in controlling Phytophthora blight in tomato fields. The resistance level of mature tomato plants in the field to *P. parasitica* could be predicted by screening the seedling resistance (2). Although tomato plants at the seedling stage were used for resistance screening in most of the studies, little is known about the expression of plant resistance at different growth stages.

In the present study, we selected Korean tomato cultivars resistant to *P. capsici* by using various inoculation methods at different growth stages of the plants. The pathogenic variations of *P. capsici* isolates from different geographic areas on Korean tomato cultivars were also examined to determine the existence of differential interactions between *P. capsici* isolates and tomato cultivars.

MATERIALS AND METHODS

Fungal isolates and inoculum. Five Korean isolates of *P. capsici*—87E1, 87L1, 87L19, 88J1, and 88Su1—were obtained from the Department of Plant Pathology of the Agricultural Sciences Institute at Suweon, Korea; the Italian isolate CBS 178.26 was obtained from the Centraal Bureau voor Schimmelcultures at Baarn, Netherlands; and the French isolate S197 was supplied by E. Pochard of the Plant Pathology Station, Institut National de la Recherche Agronomique at Montfavet, France. All isolates were grown on oatmeal agar at 28 C for 7–10 days and then incubated under fluorescent light at 28 C for 2 days to obtain sporangia. Sterilized tap water was poured into the plates to harvest sporangia. The sporangial suspension was chilled at 4 C for 30–60 min to release zoospores and then decanted through four layers of cheesecloth. Zoospore suspension was adjusted to the required concentration with sterilized tap water.

Tomato plants. Seeds of tomato cultivars Alchan, Ilkwang, Kangyuk, Kwangmyung, Kwangsu, Kwangyang, Suhkwang, Sunmyung, Wolkwang, and Youngsu, which are intensively cultivated in Korea, were sown in plastic trays (55 × 35 × 15 cm) containing steam-sterilized loam soil, sand, and peat (4:4:2, v/v). Seedlings at the two-leaf stage were transplanted to plastic pots (5 × 15 × 10 cm) containing the same soil mix. Fertilizer (N-P-K, 0.27, 0.27, 0.13 g per pot) was applied at 4-wk intervals after planting. Tomato plants were grown in a growth room under 16-hr day illumination at 25 ± 2 C.

Inoculation procedures. Tomato plants at the four- and eight-leaf stages were inoculated by the foliar-spray and stem-wound methods. In the foliar-spray inoculation procedure, a zoospore suspension (1×10^4/ml) was sprayed over both sides of leaves. Inoculated plants were placed in a moist chamber at 28 C for 24 hr and then returned to the growth room for disease evaluation. In the stem-wound inoculation procedure, 1-cm longitudinal slits were made 1 cm from the soil surface in the stems of tomato plants at the four- and eight-leaf stages. Sterile cotton was dipped in a zoospore suspension (1×10^5/ml) and then placed on the stem slits. Inoculation sites were covered with plastic tape to maintain a moist condition.

Disease evaluation and data analysis. Disease development of 10 tomato cultivars was recorded for 14 successive days after inoculation. To evaluate infection of foliar-spray inoculated plants, disease severity was rated on a 0–5 scale, where 0 = no visible symptoms, 1 = small irregular spots on young leaves and appearance of stem lesion, 2 = leaf lesion enlarged and <25% of the plant wilted, 3 = leaves beginning to be blighted and about 50% of the plant wilted, 4 = leaves blighted and >50% of the plant wilted with rapidly growing stem lesion, and 5 = plant dead. Disease severity of stem-wound inoculated plants was also rated on a 0–5 scale, where 0 = no visible symptoms, 1 = appearance of lesion at inoculation point, 2 = stem lesion extending 1–5 cm from the inoculation point and <25% of the plant wilted, 3 = stem lesion progressed up to half of the plant and about 50% of the plant wilted, 4 = stem lesion extended continuously and >50% of the plant wilted, and 5 = plant dead. Disease severity ratings were used to calculate areas under disease progress curves (AUDPCs) according to the formula previously described by Shaner and Finney (15): $\Sigma_{i=1}^{n} (X_{i+1} + X_i)(t_{i+1} - t_i)/2$, where X_i = disease severity

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Table 1. Areas under disease progress curves (AUDPCs)[a] on 10 tomato cultivars foliar-spray inoculated at the four-leaf stage with seven *Phytophthora capsici* isolates[b]

Isolate	AUDPCs on different cultivars										
	Youngsu	Sunmyung	Kwangmyung	Kangyuk	Alchan	Kwangsu	Kwangyang	Wolkwang	Ilkwang	Suhkwang	Av.
S197	19.1	40.6	38.5	35.0	37.1	43.2	37.6	53.5	53.4	52.4	41.0
88Sul	23.5	28.0	40.1	32.6	46.4	42.6	49.1	41.0	45.0	44.5	39.3
87E1	39.0	32.3	32.6	40.2	32.6	36.8	34.3	31.5	46.9	38.5	36.5
87L19	15.8	4.8	11.0	15.6	27.5	23.7	24.4	36.2	36.8	27.8	22.4
CBS 178.26	6.6	5.9	14.1	8.8	12.9	15.2	31.1	22.7	30.0	30.8	17.8
87L1	6.6	5.4	2.7	12.4	1.1	0.0	13.4	13.6	2.7	19.6	7.8
88J1	1.6	0.0	0.0	9.7	2.0	1.8	0.9	0.0	0.0	4.2	2.0
Av.	16.0	16.7	19.9	22.0	22.8	23.3	27.3	28.4	30.7	31.1	

[a] AUDPCs were calculated using a disease severity rating based on a 0–5 scale, where 0 = no visible symptoms and 5 = plant dead.
[b] Plants were inoculated with a zoospore suspension (10^4/ml).

Table 2. Areas under disease progress curves (AUDPCs)[a] on 10 tomato cultivars stem-wound inoculated at the four-leaf stage with seven *Phytophthora capsici* isolates[b]

Isolate	AUDPCs on different cultivars										
	Youngsu	Kwangmyung	Kangyuk	Ilkwang	Suhkwang	Sunmyung	Kwangyang	Kwangsu	Wolkwang	Alchan	Av.
S197	23.3	38.5	34.1	43.2	41.3	42.3	36.0	42.9	36.7	41.7	38.0
87E1	24.3	16.7	21.7	34.8	36.8	42.8	38.8	38.8	25.5	43.8	32.4
87L1	3.3	27.2	16.6	15.5	34.1	42.7	19.3	22.0	36.5	38.0	25.5
88Sul	6.0	9.7	35.8	2.9	4.3	0.0	21.3	33.0	21.5	39.3	17.4
87L19	11.8	6.0	2.1	9.3	10.9	8.2	25.8	7.7	4.5	3.2	9.0
CBS 178.26	2.0	6.8	6.1	12.2	5.0	4.0	7.0	2.3	24.8	4.5	7.5
88J1	0.0	0.0	0.0	3.3	0.0	0.0	0.0	1.6	0.0	6.5	1.1
Av.	10.1	15.0	16.6	17.3	18.9	20.0	21.2	21.2	21.4	25.3	

[a] AUDPCs were calculated using a disease severity rating based on a 0–5 scale, where 0 = no visible symptoms and 5 = plant dead.
[b] Plants were inoculated with a zoospore suspension (10^5/ml).

index at the *i*th observation, t_i = time (days) at the *i*th observation, and n = total number of observations. All data are the means of six plants inoculated at different growth stages. All experiments were performed twice, with similar results. AUDPCs from one experiment were analyzed statistically by the analysis of variance procedures.

RESULTS

In tomato plants inoculated by foliar spray with a zoospore suspension of *P. capsici*, brownish, irregularly shaped, water-soaked lesions were produced on the leaves. Infected leaves were curled and wilted as disease became severe, but defoliation did not occur. No hypersensitive symptoms were observed in the leaves. In stem-wound inoculated plants, the pathogen invaded the vascular system through the wounds (*data not presented*) and caused rapidly expanding stem lesions. The stem lesions were initially dark brown and moist, then later became dry. No symptoms were induced in the stems of tomato plants inoculated at the eight-leaf stage.

AUDPCs were used to evaluate the virulence of seven isolates of *P. capsici* on 10 Korean tomato cultivars. When the foliar-spray inoculation method was used, the mean AUDPCs based on disease severity ranked Suhkwang > Ilkwang > Wolkwang > Kwangyang > Kwangsu > Alchan > Kangyuk > Kwangmyung > Sunmyung > Youngsu for the cultivars and S197 > 88Sul > 87E1 > 87L19 > CBS 178.26 > 87L1 > 88J1 for the *P. capsici* isolates (Table 1). When the stem-wound inoculation technique was used, the mean AUDPCs (which differed slightly from those with the foliar-spray inoculation method) ranked Alchan > Wolkwang > Kwangsu > Kwangyang > Sunmyung > Suhkwang > Ilkwang > Kangyuk > Kwangmyung > Youngsu for the cultivars and S197 > 87E1 > 87L1 > 88Sul > 87L19 > CBS 178.26 > 88J1 for the *P. capsici* isolates (Table 2). The cultivar Youngsu was consistently highly resistant to all the isolates tested, and isolate S197 was generally highly virulent to all the cultivars tested, whereas isolate 88J1 was less virulent or avirulent.

The analysis of variance of the AUDPCs obtained by foliar-spray and stem-wound inoculation methods showed highly significant differences among cultivars, isolates, and cultivar × isolate interactions (Tables 3 and 4). The tomato cultivars and the *P. capsici* isolates interacted differently, and the inoculation methods elicited individual expressions of disease.

The cultivars Kwangyang (susceptible to Phytophthora blight) and Youngsu (resistant to Phytophthora blight) were reevaluated by foliar-spray and stem-wound inoculation at different leaf stages with three isolates that differed in virulence. In general, disease severities on the two cultivars were much less in plants inoculated at the eight-leaf stage than in those inoculated at the four-leaf stage (Fig. 1). The age-related resistance expressed at mature plant stages was more pronounced in Youngsu than in Kwangyang. The development of Phytophthora blight on the two tomato cultivars inoculated at the four-leaf stage by stem wounding (Fig. 2) was similar to that on the plants inoculated by foliar spraying (Fig. 1). With inoculation by the stem-wound technique at the eight-leaf stage, however, no symptoms were observed on any part of tomato plants of either cultivar (*data not presented*). The two cultivars showed no difference in level of resistance to *P. capsici* when inoculated by either method at the four-leaf stage

Table 3. Analysis of variance for areas under disease progress curves on 10 tomato cultivars foliar-spray inoculated with seven isolates of *Phytophthora capsici*

Source of variation	df	Mean square	*F* value	*P*
Cultivar	9	853.1	12.4	<0.01
Isolate	6	9,850.8	143.1	<0.01
Cultivar × isolate	54	168.5	2.5	<0.01

Table 4. Analysis of variance for areas under disease progress curves on 10 tomato cultivars stem-wound inoculated with seven isolates of *Phytophthora capsici*

Source of variation	df	Mean square	*F* value	*P*
Cultivar	9	473.3	8.6	<0.01
Isolate	6	6,276.5	113.6	<0.01
Cultivar × isolate	54	235.1	4.3	<0.01

with the highly virulent isolate S197 or the weakly virulent isolate CBS 178.26. All the juvenile plants of the two tomato cultivars were dead at 10 days after inoculation with isolate S197, but disease developed slowly after inoculation with isolate CBS 178.26.

DISCUSSION

The resistance of 10 Korean tomato cultivars to seven isolates of *P. capsici* was evaluated by stem-wound and foliar-spray inoculations done under controlled environmental conditions. The response of tomato cultivars to Phytophthora blight was quantitative rather than qualitative, as observed in interactions of pepper and *P. capsici* (7). For instance, Phytophthora blight developed more slowly on the resistant cultivar Youngsu than on the other cultivars tested. In particular, no hypersensitive reactions were observed in any of the Korean tomato cultivars inoculated with *P. capsici* isolates of different virulences. There were highly significant differences among the isolates in virulence to the Korean tomato cultivars (Tables 3 and 4), indicating that some pathogenic variation has occurred naturally in *P. capsici*, as previously demonstrated in the interactions of pepper and *P. capsici* (5,7). The French isolate S197, which showed a high level of virulence on the French and Korean pepper cultivars (7,11,12), was also most virulent to all the Korean tomato cultivars tested (Tables 1 and 2). However, the Italian isolate CBS 178.26, with the weakest virulence to the Korean pepper cultivars (7), was moderately virulent to the Korean tomato cultivars. The Korean isolate 88J1 could not attack tomato cultivars, although it showed a considerable level of virulence on Korean pepper cultivars (7). This finding suggests that some isolates of *P. capsici* may infect both pepper and tomato and also that other isolates may attack pepper but not tomato. Although significant differences were also observed among cultivar × isolate interactions in the analysis of variance of this study, we could not define these interactions as differential or race-specific, because a few samples of fungal isolates and tomato cultivars were insufficient for such a decision. We also were not sure whether the inoculation methods and scales for disease assessment used in this experiment were appropriate to differentiate tomato and *P. capsici* interactions.

All tomato cultivars became increasingly resistant as plants aged. Expression of the age-related resistance to Phytophthora blight was more apparent in tomato stems than in tomato leaves. Stem symptoms did not develop in tomato plants inoculated by stem wounding at the eight-leaf stage, suggesting a possible gene expression for age-related resistance only at the mature plant stage. Recently, Kim et al (8) reported the expression of age-related resistance in pepper plants infected with *P. capsici*.

The foliar-spray inoculation method could well screen tomato cultivars for resistance to *P. capsici*, especially at the late growth stage of plants. However, soil-drench and stem-wound inoculations may not be appropriate for evaluating cultivar resistance on old tomato plants, because no symptoms were induced on stems or roots of plants more than 4 wk old. In our study, soil drench with zoospore suspensions of *P. capsici* did not cause any symptoms on stems and roots of tomato plants, regardless

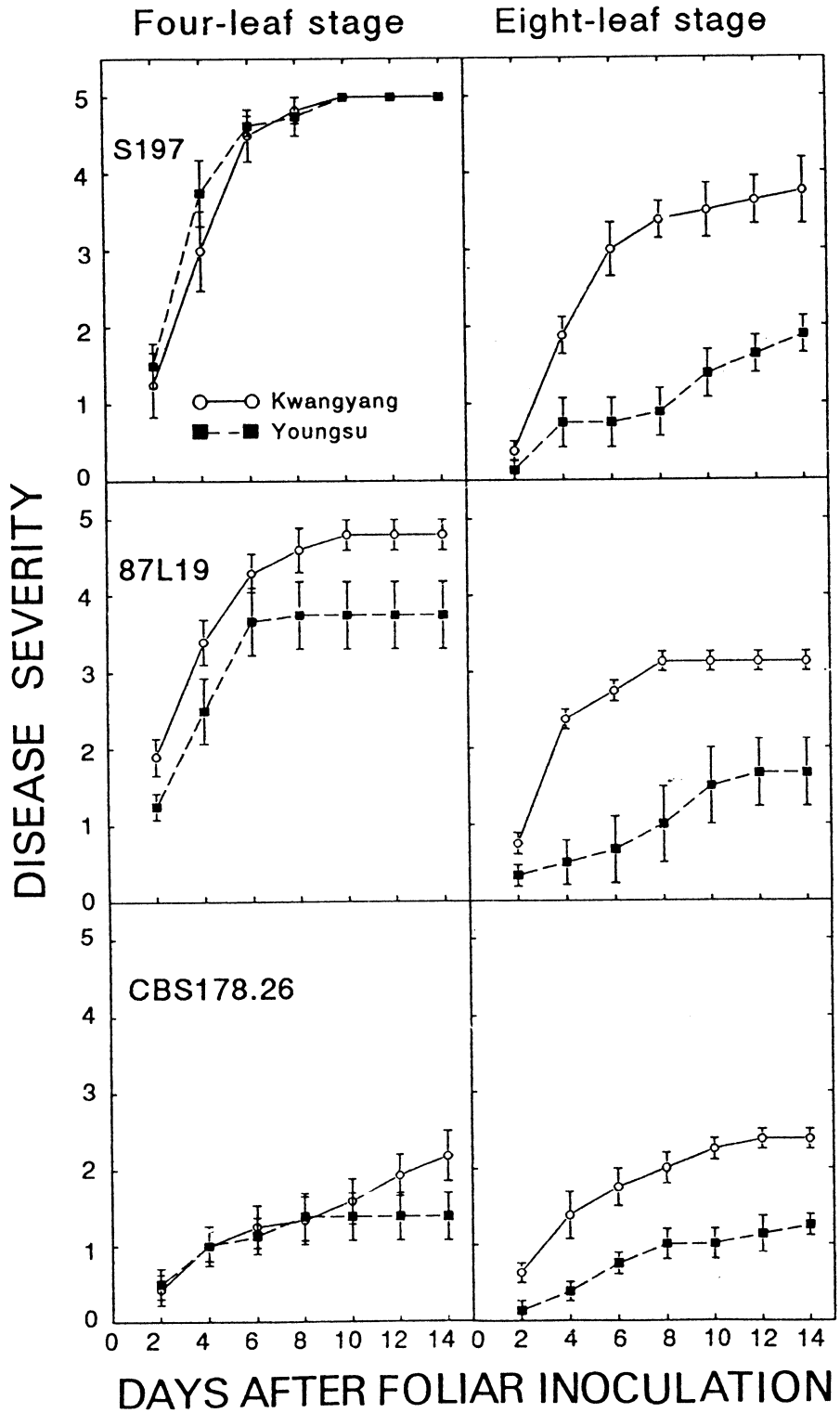

Fig. 1. Disease severity curves for two tomato cultivars, Kwangyang (susceptible) and Youngsu (resistant), inoculated by foliar spraying at the four- and eight-leaf stages with three *Phytophthora capsici* isolates of different virulence—S197, 87L19, and CBS 178.26. Disease severity is based on a 0–5 scale, where 0 = no visible symptoms and 5 = plant dead. Each value represents a mean ± 1 standard deviation of six plants.

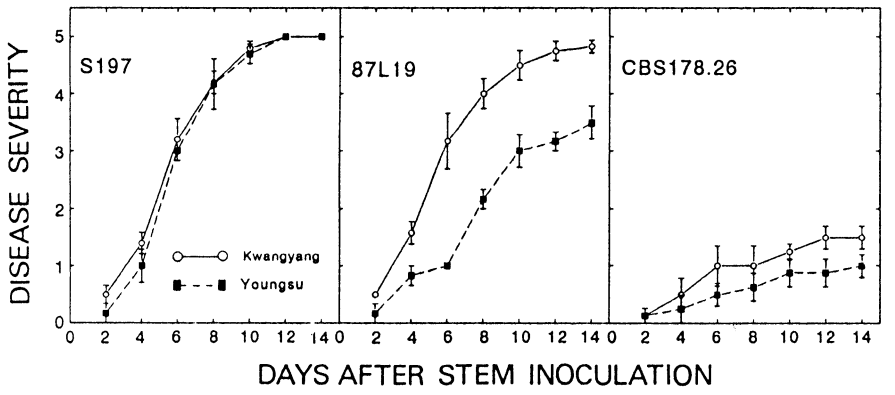

Fig. 2. Disease severity curves for two tomato cultivars, Kwangyang (susceptible) and Youngsu (resistant), inoculated by stem wounding at the four-leaf stage with three *Phytophthora capsici* isolates of different virulence—S197, 87L19, and CBS 178.26. Disease severity is based on a 0–5 scale, where 0 = no visible symptoms and 5 = plant dead. Each value represents a mean ± 1 standard deviation of six plants.

of isolate and plant growth stage (*data not presented*). Our findings agree well with the recent observations of Hartman and Huang (4), in which Taiwan isolates of *P. capsici* did not produce stem blight on soil-drenched tomato plants. The levels of virulence of *P. capsici* isolates used may also affect the precise screening of tomato cultivars for resistance to Phytophthora blight. Inoculation of juvenile plants with the highly virulent isolate S197 did not distinguish between resistant and susceptible cultivars, whereas the moderately virulent isolate 87L19 precisely differentiated these cultivars tested (Figs. 1 and 2). These data suggest that use of the foliar-spray inoculation method and a moderately virulent *P. capsici* isolate may be recommended for screening of tomato cultivars for resistance to *P. capsici* in breeding programs. Inoculum level and environmental conditions should also be appropriate for the precise evaluation of tomato cultivars (7,10,13).

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