Expression of Age-Related Resistance in Pepper Plants Infected with *Phytophthora capsici*

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ABSTRACT

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Eight pepper cultivars with different degrees of resistance to *Phytophthora capsici* were evaluated on the basis of a disease severity rating for age-related resistance under controlled environmental conditions. With increasing age of pepper plants, all cultivars tested became gradually resistant to Phytophthora blight. High inoculum concentrations of *P. capsici* resulted in symptoms on some resistant cultivars. The incubation periods of *P. capsici* in resistant pepper cultivars and old plants were longer than those in susceptible cultivars and young plants, respectively. The soil-drench method of inoculation was more reliable than the stem-inoculation method for evaluation of age-related resistance. Appropriate screening techniques for evaluation of age-related resistance are discussed.

Foliar and stem blight of pepper (*Capsicum annuum* L.), caused by *Phytophthora capsici* Leonian, is one of the most economically destructive soilborne diseases in many pepper-growing areas (1,6,10). In Korea, this disease occurs in all organs of the pepper plant (i.e., root, stem, leaf, and fruit), especially in fields excessively wet during prolonged periods of rainy weather from June to August.

Because the Phytophthora disease is not readily controlled by soil or foliar fungicides, effective control in the field can be achieved by growing resistant cultivars or by rotating crops as often as needed to reduce the disease pressure. Resistance in pepper to *P. capsici* was reported in 1960 by Kimble and Grogan (5). This resistance has been governed by two distinct dominant genes acting independently without additive effects. However, prolonged incubation periods or very high concentrations of inoculum of *P. capsici* can occasionally overcome resistance in pepper, resulting in symptoms on resistant plants (1). Inoculum concentrations and incubation periods also appear to greatly affect the expression of resistance in pepper plants (1).

Considerable research has been done to identify a stable and durable type of resistance to *P. capsici* for use in breeding programs (1,5,7,9). Age-related resistance, which is distinctly expressed as pepper plants mature, may be very effective in reducing damage from this disease. Seedling pepper plants at one- through eight-leaf stages are usually susceptible to *P. capsici*, regardless of host genotype.

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In the present study, symptom development on pepper plants was examined to select cultivars with age-related resistance. Resistance was evaluated by comparing susceptible and resistant cultivars inoculated by various techniques.

MATERIALS AND METHODS

Pepper cultivars used for evaluation of age-related resistance to foliar and stem blight were Kumkangkimjang, Kingkun, Damoakun, Taeyangkun, Hanbyul, Hongsanho, Jinpum, and Champion. Ten seeds of all cultivars were sown at 20-day intervals in a plastic pot (5 × 15 × 10 cm) containing steam-sterilized loam soil, sand, and peat (3:5:2, v/v). Seedlings (28-day-old) were transplanted into 10-cm-diameter plastic pots (one plant per pot) containing the above soil mix. Fertilizer was applied at the rate of 0.27-0.27-0.13 g of actual N-P-K per pot at 3-wk intervals after planting. Pepper plants were grown on a greenhouse bench at 25 ± 5 C.

An isolate of *P. capsici* obtained from the Department of Plant Pathology, Institute of Agricultural Sciences, Suweon, Korea, was grown on oatmeal agar in petri plates at 25 ± 1 C for 7 days and incubated under fluorescent light at 28 ± 1 C for 48 hr to induce sporulation. Mycelia and sporangia were harvested with sterilized water. Zoospore release was induced by chilling harvested sporangia at 4 C for 40–60 min. Zoospore discharge occurred within 30–60 min after the sporangial suspension was returned to room temperature. The suspension was decanted through two layers of cheesecloth, and zoospores were counted with a hemacytometer. Sterile tap water was used to adjust suspensions to the required concentrations.

Cultivars were screened for resistance by either soil-drench, stem-wound, or foliar-spray inoculation. Plants at the two-, four-, six-, and eight-leaf stages and the first-branched flowering stage were inoculated by drenching the soil with zoospore suspensions. Twenty milliliters of a suspension of motile zoospores (10^4/ml) were poured uniformly over the surface of the soil in each pot. Immediately after soil-drenching, pots were placed in a large plastic tray filled with tap water and stored for 2 wk in the greenhouse. 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 = stem lesions extending to cotyledons, defoliated first and second leaves, damping-off occurring in seedling plants, or 30–50% of entire plant diseased; 3 = stem lesions extending to second leaves, yellowing or defoliation of some upper leaves, brownish lesions developing to the petioles in seedling plants, or 50–70% of entire plant diseased; 4 = long, brownish lesions on stems extending up to at least 10 cm from the soil, all leaves except the uppermost leaf defoliated, seedling tissues collapsing and shoots wilted, or 70–90% of entire plant diseased; 5 = plant dead.

In the wound-inoculation procedure, plant stems were wounded by making a 1-cm longitudinal slit in the stem 2 cm from the soil surface. A small quantity of sterile cotton soaked for 30 min in a zoospore suspension (10^4/ml) was placed on the wounded slits in stems. Inoculation sites were then covered with plastic tape to maintain moist conditions favorable for penetration of the fungus into the stem tissue. Disease severity was rated based on a 0–5 scale, where 0 = no visible symptoms; 1 = brownish lesion at the inoculation point; 2 = stem lesion extending 1–3 cm from inoculation point to cotyledons; 3 = stem lesion progression up to half of the plant height; 4 = stem lesion progressing toward the shoot apex; 5 = plant dead.

In the procedure for inoculation by foliar spray, various concentrations of zoospores were sprayed to runoff on the upper leaves of pepper plants of various maturity levels. Inoculated plants were placed in a moist chamber at 95% relative humidity and 28 ± 1 C for 24 hr in the dark. They were then transferred to a growth chamber at 80 ± 10% relative humidity and 28 ± 2 C with 10,000 lx

illumination for 16 hr a day. Disease severity was rated every day after inoculation on the basis of a 0–5 scale, where 0 = no visible symptoms; 1 = small, circular, or irregular spots on upper leaves; 2 = leaf-enlarged symptoms with brownish lesions beginning to appear on stems and less than 25% of the plant wilted; 3 = leaves defoliated with lesions on leaves covering half of a leaf and 25–50% of the plant wilted moderately; 4 = leaves defoliated or dried, with rapidly expanding stem lesions and 50–70% of the plant wilted severely; 5 = plant dead.

Hanbyul (susceptible at all plant growth stages) and Kingkun (susceptible at an early growth stage but resistant later) were reevaluated more precisely for age-related responses to *P. capsici* by using the soil-drench, stem-wounding, and foliar-spray inoculation methods. To determine the inoculum density required to cause foliar blight, Kingkun pepper plants were inoculated with the various zoospore concentrations at the six-leaf and first-branched flowering stages.

Disease severity ratings were used to calculate areas under disease progress curves (AUDPC), according to the formula described by Shaner and Finney (8): AUDPC = $\Sigma_{i=1}^{n} (X_{i+1} + X_i)(t_{i+1} + t_i)/2$, where X_i = disease severity index at the ith observation, t_i = time (days) at the ith observation, and n = total number of observations. Data were analyzed statistically, and means were compared by Duncan's multiple range test ($P = 0.05$).

All data are the means of three replicates of 10 plants inoculated at the various ages. All experiments were performed twice, and each experiment had three replicates. Because the two experiments showed similar results, the results of only one experiment are reported.

Table 1. Areas under disease progress curves (AUDPC)[x] on eight pepper cultivars inoculated at different growth stages with *Phytophthora capsici* by either soil-drenching or stem-wounding[y]

	AUDPC				
	Soil-drench			Stem-inoculation	
Cultivar	2-Leaf	6-Leaf	First-branched	6-Leaf	First-branched
Jinpum	100.0 a[z]	5.1 a	0.0 a	85.0 a	55.0 a
Kingkun	108.5 bc	13.3 b	3.2 a	89.5 b	54.3 a
Champion	106.4 b	26.5 c	23.7 b	92.5 c	59.3 ab
Kumkangkimjang	115.1 d	81.8 d	47.4 c	96.5 d	86.8 c
Damoakun	108.7 bc	90.5 e	53.8 d	98.3 e	68.7 b
Taeyangkun	109.6 bc	83.3 d	69.8 d	99.0 e	92.8 c
Hongsanho	110.8 c	91.4 e	72.5 e	98.9 e	88.3 c
Hanbyul	107.7 bc	96.5 f	83.0 f	99.5 e	89.3 c

[x]AUDPCs were calculated using a disease severity rating based on a 0–5 scale, as described in Materials and Methods.
[y]Pepper plants at two-leaf, six-leaf, and first-branched flowering stages were simultaneously inoculated with a zoospore suspension (10^4/ml) by using soil-drench and stem-wound techniques.
[z]Numbers within a column followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

Susceptible plants had blighted foliage, leaf defoliation, crown rot, rapidly growing stem lesions, or damping-off in juvenile plants. Many plants classed as resistant were symptomless or had foliage blight crown rot and patches of superficial brownish-to-purple speckling that developed slowly on the stems. At the two-leaf stage and with the soil-drench technique, large differences in disease severity were not observed among the eight cultivars tested, although most of the cultivars were susceptible (Table 1). In general, all cultivars became resistant as plants grew older. Based on AUDPC calculations of disease severity ratings at the six-leaf and first-branched flowering stages of plants, ranking (in order from greatest to least) was as follows: Hanbyul, Hongsanho, Taeyangkun, Damoakun, Kumkangkimjang, Champion, Kingkun, Jinpum. The cultivars Champion, Kingkun, and Jinpum were exceptionally more resistant as the plants matured. Therefore, these cultivars were considered resistant to Phytophthora stem blight at the later growth stages, especially after the first-branched flowering stage.

Disease severity on Hanbyul and Kingkun differed at the first-branched flowering stage. Kingkun had become highly resistant at this stage. Symptom development of Phytophthora blight on

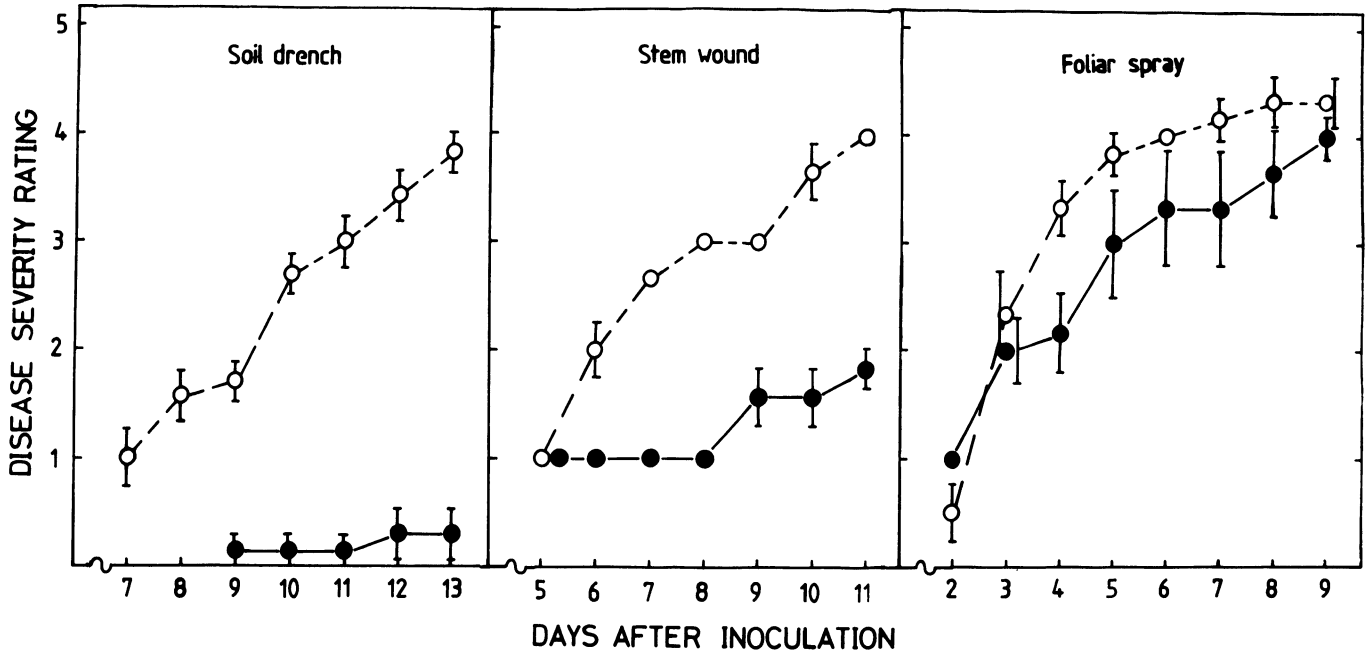

Fig. 1. Disease severity curves for two pepper cultivars, Hanbyul (susceptible O—O) and Kingkun (resistant ●—●), inoculated with 10^4 zoospores per milliliter of *Phytophthora capsici* by using the soil-drench, stem-wound, and foliar-spray inoculation methods at the first-branched flowering stage. Disease severity rating based on a 0–5 scale, as described in Materials and Methods. Each value represents a mean ± 1 standard deviation of three replicates of 10 plants.

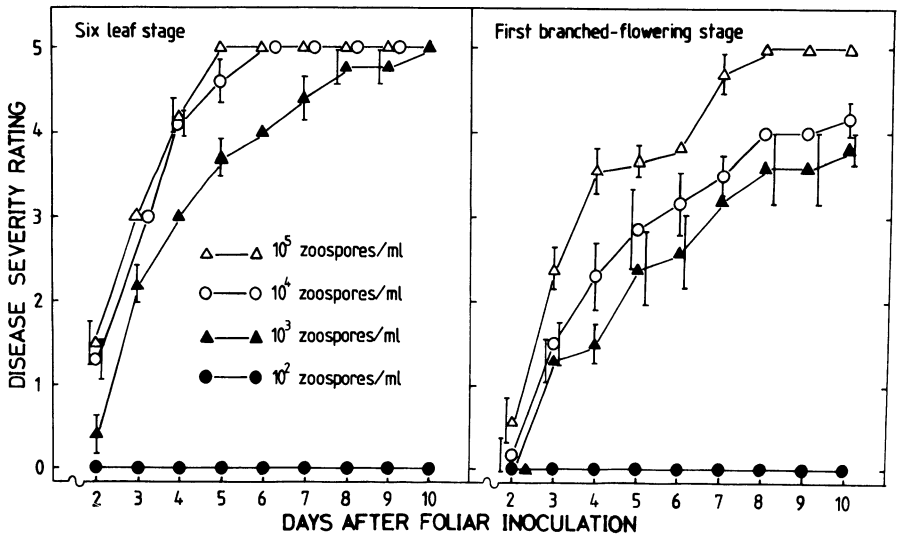

Fig. 2. Disease severity curves of age-related resistant cultivar Kingkun inoculated with 10-fold serial dilutions from 10^2 to 10^5 zoospores per milliliter of *Phytophthora capsici* by foliar spraying. Disease severity rating based on a 0–5 scale, as described in Materials and Methods. Each value represents a mean ± 1 standard deviation of three replicates of 10 plants.

the two pepper cultivars inoculated by stem-wounding was similar to that on plants inoculated by soil-drenching (Fig. 1). With inoculation by foliar spray, slight differences were observed in disease severity between the two cultivars at the first-branched flowering stage of plants. However, at the four- to eight-leaf stages with all three inoculation techniques, there were no differences between the two cultivars in levels of resistance to Phytophthora blight.

With the lowest inoculum level used in foliar sprays (10^2 zoospores per milliliter), symptoms were not induced on the leaves of Kingkun pepper plants at the six-leaf and first-branched flowering stages (Fig. 2). At 10^4–10^5 zoospores per milliliter, all the juvenile plants at the six-leaf stage were killed (severity rating of 5) within 5–10 days after inoculation. At the first-branched flowering stage, resistance to *P. capsici* became more evident. Disease development was at intermediate levels and was progressively more severe with increasing concentrations of zoospores.

DISCUSSION

Screening pepper cultivars for age-related resistance to *P. capsici* could be done effectively under greenhouse conditions provided that either roots or stems of plants were exposed to zoospores by the soil-drench or stem-wound inoculation methods, and that the plants were at the first-branched flowering stage. All cultivars were susceptible to the disease at the two-leaf stage and became increasingly resistant as plants grew older, when expression of age-related responses became apparent. The differences between the susceptible and resistant responses to *P. capsici* were more quantitative rather than qualitative, because some of the symptoms developed more slowly on resistant plants.

Expression of resistance in pepper plants to *P. capsici* was affected by the inoculation concentrations and incubation periods. High inoculum concentrations of *P. capsici* resulted in symptoms on some resistant cultivars, as previously observed (1), but the incubation periods of *P. capsici* in resistant pepper cultivars and older plants were longer than those in susceptible cultivars and young plants. Smith et al (9) also found that resistance of pepper to crown end root rot could be overcome by prolonged exposure to the fungus.

Evaluation of pepper cultivars for resistance by using the stem-wound inoculation methods produced results similar to the soil-drenching technique. For evaluating age-related resistance specifically, however, the soil-drench method was more reliable than the stem-inoculation method. It should be noted that age-related resistance was not observed on the leaves of peppers because there were no large differences in disease severity between the younger and older leaves when the inoculation was by foliar spray. These data suggest that foliar inoculation may be inappropriate for precise screening of pepper cultivars for resistance to *P. capsici*, and also that the same genetic factors that govern susceptibility or age-related resistance in root or stem may not be operable in leaves, as demonstrated on soybeans by Keen and Horsch (4). By contrast, Barksdale et al (1) reported recently that screening of pepper lines for resistance to foliar blight by using the foliar inoculation method gave similar results to planting in infested soil in the greenhouse or field, and that the foliar inoculation method was simpler to manage.

Kingkun, classed as age-related resistant, was as susceptible as Hanbyul at the two- to three-leaf stages. Kingkun was symptomless and highly resistant at the first-branched flowering stage when inoculated by soil-drench and stem-wound methods. These results would imply that age-related resistance in pepper plants may be a generalized result of physiological changes in tissues of root and stem during aging, which are primarily dependent on the genotype of the plant (2,3). Further physiological and morphological studies will be necessary to elucidate causes of age-related resistance of pepper plants to Phytophthora blight. It also is suggested that an appropriate concentration of inoculum, a precise inoculation method, and the use of test pepper plants at the proper growth stage may be very important for evaluation of resistance to *P. capsici* under controlled environmental conditions.

ACKNOWLEDGMENT

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