Resistance to Gray Leaf Spot in *Capsicum* Peppers

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Abstract. Of 467 accessions of *Capsicum* pepper (*Capsicum annuum* L.) tested for resistance to gray leaf spot, KC321, KC220, KC208, KC47 (PI244670), KC43 (PI244670), KC380, and KC319 were highly resistant to both *Stemphylium solani* and *S. lycopersici*, the causal agents of gray leaf spot.

Gray leaf spot, caused by *Stemphylium solani* Weber, was first reported on tomatoes by Weber (1930) in Florida. Hannon and Weber (1955) found that another closely related species of *Stemphylium* also caused gray leaf spot on tomatoes (*Lycopersicon esculentum* Mill.), symptoms of which were indistinguishable from those caused by *S. solani*. They named this new species *S. floridanum*; its current name is *Stemphylium lycopersici* (Enjoji) Yamamoto (Ellis and Gibson, 1975). Both *S. solani* and *S. lycopersici* are also pathogenic on pepper (Hannon and Weber, 1955; Weber, 1930).

Gray leaf spot on pepper caused by *S. solani* was first observed in a natural field condition in Louisiana by Sinclair et al. (1958) and later in Florida by Blazquez (1969), but its occurrence on pepper caused by *S. lycopersici* has not been reported previously.

We have observed severe leaf spot and defoliation on pepper plants caused by gray leaf spot in Korea since 1994. The disease was more prevalent on plants growing in relatively cool mountainous terrain, such as Subi-Myun, Youngyang in Kyungpook province. Two different species of *Stemphylium* were isolated from the diseased leaves, depending on the specimen collected. The causal fungi were identified as *Stemphylium solani* and *S. lycopersici*, respectively (Cho, 1997).

Studies on resistance to the disease have been conducted since 1996 in a wide range of genetic resources. Good levels of resistance have been observed and the results are reported here.

Materials and Methods

Expt. 1. Preliminary mass screening. Preliminary evaluation for resistance to *Stemphylium lycopersici* was conducted with three sets of plantings and inoculations. Included were 463 accessions of *C. annuum* L., two of *C. pubescens* Luiz & Pavon and one each of *C. baccatum* L. and *C. frutescens* L. from the B.S.K.'s collections from all over the world, including 93 U.S. Plant Introduction lines and 107 local Korean cultivars. Seeds were sown in Barokur[®] mix (Seoul Agricultural Materials Co., Seoul, Korea) in 128-cell trays on a hot bed within a greenhouse. About 1 month after seeding, seedlings were transplanted to 32-cell trays filled with Barokur[®] mix in the greenhouse (first set), to an outdoor nursery (second set), or to 72-cell trays in the greenhouse (third set), where they were maintained until completion of the test. Of each accession, 8–12 plants were used in each test. A commercial hybrid 'Kumtap' was included as a susceptible control in every set.

An isolate of *S. lycopersici* collected from Ipseok, Andong, Kyungpook province was used in the preliminary screening. *Stemphylium lycopersici* was initially cultured on plates of agar containing V8[®] juice (Campbell Soup Co., Camden, N.J.). Mycelial blocks ≈ 5 × 5 mm were cut from the fresh marginal area of the initial culture on the agar plates, plated on agar containing calcium carbonate (Sahin and Shepard, 1979), and incubated at 15 °C in the dark. *Stemphylium lycopersici* sporulated profusely on the surface of the mycelial blocks within 72 h, whereas *S. solani* sporulated only sparsely. Therefore, *S. lycopersici* was used first in the preliminary tests for resistance. Spores of *S. lycopersici* were collected by gently rubbing the surface of the agar blocks with a hair brush after flooding in sterile distilled water, and the suspension was filtered through four layers of gauze. Spore concentrations were measured with a hemocytometer, and adjusted to ≈ 10⁴ spores per mL.

Pepper seedlings were inoculated 50–60 d after seeding by spraying the spore suspension on the foliage using a compressed air sprayer until both sides of the leaves were wet. The inoculated plants were incubated for 2 nights

in humid conditions generated by wetting the bottom carpet or soil of the nursery bed with water and covering it with a plastic film.

Disease was scored 5 to 7 d after inoculation using an arbitrary scale based on the number of spots and discoloration on the most diseased leaves on a plant: 1 = no spots found; 2 = one to three spots; 3 = four to six spots; 4 = 7 or more spots but no discoloration; and 5 = 7 or more spots with discoloration or defoliation.

*Expt. 2. Confirmation evaluation for resistance to both *S. solani* and *S. lycopersici*.* A total of 23 accessions, consisting of 20 lines selected as potentially resistant in preliminary mass screening and three susceptible checks (Kumtap, Subi and Chilsung), were sown in Barokur[®] mix in 128-cell trays in the greenhouse. The germinated seedlings were transferred to 32-cell trays filled with Barokur[®] mix 1 month after seeding. Two sets of a maximum of 10 plants for each accession were grown, one set for inoculation with *S. solani* and the other for inoculation with *S. lycopersici*.

Plants of each set of accessions were inoculated with spore suspensions of isolates of either *S. solani* or *S. lycopersici* collected from Ipseok, Andong, Kyungpook province. For sporulation, both species of *Stemphylium* were seeded on V8[®] juice agar plates with three to four mycelial pieces in each plate and cultured at 20 to 25 °C and a 12-h photoperiod provided by fluorescent lights. Plastic petri dishes were used instead of the glass ones made in Korea. Both *S. solani* and *S. lycopersici* sporulated abundantly. Spores were collected from 3-d-old cultures on the plates and spore suspensions were prepared as in Expt. 1.

Pepper seedlings were inoculated and incubated as in Expt. 1. Disease was scored 16 d after inoculation based on two scales, disease index as in the preliminary mass screening, and the average number of spots formed on the three most diseased leaves of each plant, counted with a mechanical counter. The experiment was conducted on seedlings grown in trays and 6 to 10 plants were tested per accession. Therefore, a completely randomized design was used, considering the individual plants in each line as replications. The SAS GLM and MEANS procedure was used for statistical analysis.

Results and Discussion

Expt. 1. Preliminary mass screening. A total of 101 accessions were tested in the first set, of which eight showed mean disease indices <2.0 (Table 1). No spots were found on KC47 'PI244670', KC174 'Early Jalapeno', or KC380 'Habukung'. Accessions showing resistance in the first set were evaluated in an outdoor nursery in the second set. However, bacterial spot and Phytophthora blight interfered with disease readings and reduced the number of usable plants. The six least-diseased accessions of the 206 tested are listed in Table 1. Two of the best three accessions were among those selected in the first set. In the third set, 13 accessions of the 171 tested showed mean disease indices <2.0, with KC380, KC47

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and KC319, which were included as resistant controls, ranking highest among resistant lines (Table 1).

Expt. 2. Confirmation evaluation for resistance to both *S. solani* and *S. lycopersici*. Twenty accessions selected in preliminary evaluations and three susceptible controls were tested again for resistance to both *S. solani* and *S. lycopersici*. KC320, KC220, KC208, KC47 (PI244670), KC43 (PI241670), KC380, and KC319 were highly resistant to both *S. solani* and *S. lycopersici* (Table 2) as expected, based on results of the preliminary tests. Numerous spots developed on the susceptible controls such as 'Subi', 'Chilsung' and 'Kumtap', causing discoloration and defoliation. Some other accessions were also less affected than the susceptible controls. The resistant accessions, which were initially selected only for resistance to *S. lycopersici*, showed resistance to both *S. solani* and *S. lycopersici*. The phenotypic correlations between both the mean disease indices and the mean numbers of spots formed on the three most diseased leaves for the two *Stemphylium* species (0.92 and 0.87, respectively) were highly significant. The coefficients of variation for disease index and mean number of spots on a leaf were 21% and 52%, respectively, for inoculation with *S. solani* and 29% and 70%, respectively, for inoculation with *S. lycopersici*. Thus, disease index was the better indicator of resistance. Counting the number of spots on the leaves was time-consuming and tedious.

PI244670 and PI241670 were resistant to *Stemphylium* and have been reported to possess quantitative resistance to bacterial spot (Cook and Stall, 1963; Kim, 1988; Sowell, 1960). KC14 (PI201234) has resistance to Phytophthora blight (Kim, 1986, 1988; Kimble and Grogan, 1960) and was also moderately resistant to gray leaf spot. All of the resistant lines and the other genotypes used in this study are maintained by the second author and available to interested parties.

Although Weber (1930) reported that *S. solani* sporulated well on potato dextrose agar, we had difficulty in inducing sporulation. However, *S. lycopersici* sporulated abundantly on mycelial blocks when incubated on water agar with CaCO₃ under cool, dark conditions as reported for *Alternaria* species (Sahin and Shepard, 1979). This was the reason why we used *S. lycopersici* in the preliminary evaluations, although *S. solani* is the dominant species in Korea (Cho, 1997). Later, we found that the glass lids of domestically produced petri dishes inhibited sporulation, possibly by blocking transmission of ultraviolet light. Abundant sporulation was obtained when plastic petri dishes were used. We also found that both species were sporulating well on V8® juice agar in Pyrex® glass petri dishes.

Gray leaf spot is an important disease in pepper fields located in mountainous terrain in Korea, and the resistant lines selected will be used in breeding for resistance.

Table 1. Resistance to *Stemphylium lycopersici* in the top class accessions and in the susceptible control 'Kumtap' in the three sets of plantings.

KC no.	Cultivar	First planting		Second planting		Third planting	
		No. plants tested	Mean disease index ^z	No. plants tested	Mean disease index ^z	No. plants tested	Mean disease index ^z
47	PI244670*	10	1.0 a ^y	---	---	12	1.1 a
174	Early Jalapeno	10	1.0 a	3	1.7 a	---	---
380	Habuksung*	10	1.0 a	---	---	12	1.1 a
319	Unknown*	10	1.2 a	8	1.1 a	12	1.2 ab
43	PI241670	---	---	8	1.3 a	---	---
158	Red chili	---	---	---	---	12	1.3 a-c
320	Unknown	---	---	---	---	12	1.4 a-d
318	Albaregia	---	---	---	---	12	1.5 a-d
157	Papri Sweet	---	---	---	---	6	1.5 a-d
14	PI201234	11	1.6 b	---	---	---	---
220	Beopjeon	11	1.6 b	---	---	---	---
40	PI241641	---	---	---	---	5	1.6 a-d
314	Hungary	11	1.7 b	---	---	---	---
208	Jinan	---	---	8	1.7 a	---	---
304	Tam Mild Jalapeno	---	---	---	---	12	1.7 a-d
312	Kecskeszarv	11	1.8 b	---	---	---	---
52	PI257047	---	---	7	1.8 a	---	---
6	PI164677	---	---	6	1.8 a	---	---
390	Dochigi-Sandaka	---	---	---	---	12	1.8 b-d
195	Seodong	---	---	---	---	12	1.8 b-d
322	Navator F ₁	---	---	---	---	11	1.8 cd
305	Tam Mild Chile	---	---	---	---	8	2.0 d
F ₁ hybrid	Kumtap	11	4.9 c	4	4.0 b	12	4.7 e

^z1 = no spots; 2 = 1–3 spots on a leaf; 3 = 4–6 spots; 4 = 7 or more spots developed on a leaf but no yellowing; 5 = 7 or more spots per leaf with yellowing.

^yMean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

*Resistant controls.

Table 2. Disease ratings for selected lines of pepper after inoculation with *Stemphylium solani* and *S. lycopersici*

KC no.	Cultivar	No. plants tested	<i>S. solani</i>		<i>S. lycopersici</i>	
			No. spots per leaf ^z	Mean disease index ^y	No. spots per leaf ^z	Mean disease index ^y
320	Unknown	10	0.2 a*	1.4 a	0.0 a	1.0 a
220	Beopjeon	10	0.4 a	1.4 a	0.0 a	1.0 a
208	Jinancho	8	1.4 a	1.4 a	1.0 a	0.0 a
47	PI244670*	10	1.9 a	2.5 b	1.2 a	0.3 a
43	PI241670	7	2.4 a	2.1 ab	1.6 ab	0.7 a
380	Habuksung*	10	2.4 a	1.6 a	1.1 a	0.0 a
319	Unknown*	10	7.4 ab	2.0 ab	2.2 bc	3.0 ab
390	Dochigi-Sandaka	10	15.8 a-c	3.9 de	3.3 d-f	20.3 cd
174	Early Jalapeno	10	16.8 a-c	3.6 cd	2.5 cd	2.7 ab
195	Seodong	10	22.7 bc	3.2 c	2.6 cd	16.5 b-d
158	Red chili	10	24.3 b-d	4.1 de	3.3 d-f	8.1 a-c
326	Kecskeszarv	10	30.0 c-e	4.1 de	2.9 e-g	18.5 cd
305	Tam Mild chile	6	31.8 c-e	4.0 de	3.6 e-g	27.3 d
14	PI201234	10	31.9 c-e	4.0 de	3.9 fg	21.2 cd
304	Tam Mild Jalapeno	9	41.0 d-f	4.0 de	3.3 d-f	7.1 a-c
40	PI241641	10	41.4 d-f	4.0 de	3.9 fg	27.9 d
6	PI164677	10	43.4 ef	4.0 de	3.3 ab	2.8 c-e
322	Navator F ₁	8	44.7 ef	4.3 de	55.7 e	4.3 g
312	Kecskeszarv	10	54.8 fg	4.2 de	44.7 e	4.1 fg
157	Papri Sweet	10	62.6 g	4.1 de	28.4 d	4.0 fg
202	Subi	10	86.1 h	4.6 e	77.6 f	4.5 g
F ₁ hybrid	Kumtap	10	96.1 hi	4.3 de	56.8 e	4.5 g
201	Chilsung	10	107.3 i	4.6 e	48.4 e	4.3 g

^zMean number of spots formed on the three most diseased leaves.

^yDisease index of the most diseased leaf on a plant: 1 = no spots; 2 = 1–3 spots; 3 = 4–6 spots; 4 = 7 or more spots formed but no yellowing; 5 = 7 or more spots on a leaf with discoloration and defoliation.

*Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

*Resistant controls.

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