Inheritance of a Gene (Bs_3) Conferring Hypersensitive Resistance to *Xanthomonas campestris* pv. *vesicatoria* in Pepper (*Capsicum annuum*)

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ABSTRACT

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A gene is reported in PI 271322 that confers hypersensitivity to pepper strain race 1 of *Xanthomonas campestris* pv. *vesicatoria*. This gene is inherited as a single dominant gene and is named Bs_3.

Additional key words: bacterial spot

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is often a major problem in pepper (*Capsicum annuum* L.). Resistance to this disease has been reported in some cultivars as well as in lines introduced from other countries (9,13–16). Inheritance of this resistance has been reported to be dominant (1–3,9), recessive (7), or controlled by multiple factors (3,17). Hypersensitivity has also been reported (2,18) and has been introduced into a commercial cultivar (6).

Cook and Stall (4) in Florida have worked most extensively with this disease. They classified *X. campestris* pv. *vesicatoria* into three strains: a tomato strain to which all peppers are hypersensitively resistant, a pepper strain race 1 to which all peppers are susceptible, and a pepper strain race 2 to which a specific gene for hypersensitivity (found in PI 163192) confers resistance. They reported that pepper race 1 is distributed worldwide but pepper race 2 has been found only in Florida and Guadeloupe (5). Tests in Hawaii (10) have supported the conclusion that race 1, but not race 2, occurs in Hawaii. Hypersensitive resistance has also been found in PI 260435 (*C. chacoense*). This resistance was effective for both races 1 and 2 and controlled by a single dominant gene (2).

In this paper, we report the discovery of an additional gene in *C. annuum* that confers hypersensitivity, but this time, to pepper race 1.

MATERIALS AND METHODS

In a preliminary trial, seed of 166 plant introduction lines received from the Southern Regional Plant Introduction Station, Experiment, GA, were sprayed with a suspension of *X. campestris* pv. *vesicatoria* in the greenhouse and evaluated for resistance. The apparently resistant lines were transplanted along with susceptible check plants to a field with overhead irrigation to check further for possible disease development. PI 271322, a *C. annuum* line that originated in India, appeared resistant in both the greenhouse and field. One plant of this line was then transplanted to a bed for making crosses but died soon afterward, and the only progeny obtained from it came from pollen that had been used to pollinate flowers on the susceptible cultivar Keystone. This PI 271322 parent will be referred to as plant 1. Later, after the hypersensitive nature of the resistance in the progeny of plant 1 had been detected, more seeds of PI 271322 were grown and a second plant was used to make crosses with Keystone, backcrosses with both Keystone and PI 271322, and self-pollinations. This plant will be referred to as plant 2. Self- and cross-pollinations were done in the greenhouse or in an outside bed. Natural cross-pollination was prevented by wrapping the unopened buds with glassine bags.

Inoculum. One isolate of *X. campestris* pv. *vesicatoria* was used throughout this experiment. Six other isolates from Hawaii were tested by inoculating several lines and were found to act the same. All are presumed to be race 1 because they were able to infect plants with the PI 163192 gene that confers hypersensitivity to race 2 (4,10).

Inoculation. Bacterial cells for inoculations were obtained from a 48-hr-old culture of the bacterium on plates containing yeast extract, dextrose, calcium carbonate, and agar. A bacterial suspension of 10^8 cells per milliliter in distilled water was used for inoculation. One-month-old seedlings were inoculated by infiltrating the inoculum into the undersides of the two youngest fully expanded leaves with a DeVilbiss airbrush connected to a compressor set at 20 psi until an area at least 5 mm in diameter appeared water-soaked. Inoculated plants were left on the greenhouse bench without incubation. The infiltrated area of hypersensitive plants turned dark purple within 36–48 hr of inoculation and later became necrotic—clearly different from either the nonhypersensitive resistant or susceptible reactions (Fig. 1). The hypersensitive nature of this reaction

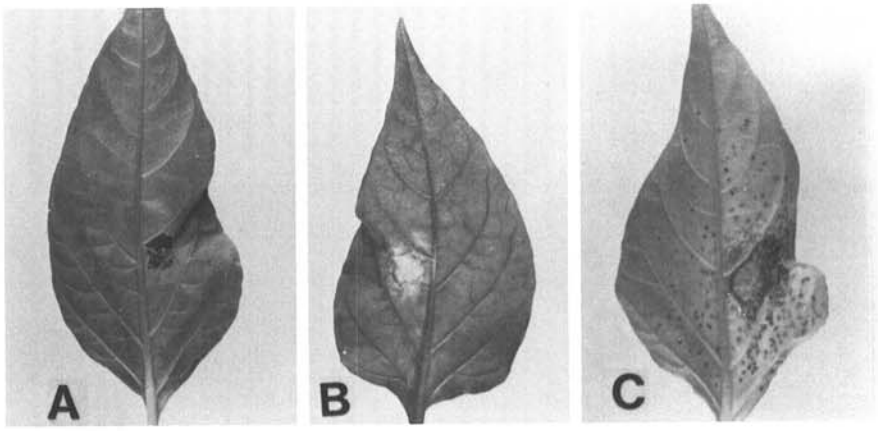

Fig. 1. Bacterial spot reactions on pepper leaves of 1-mo-old seedlings 7 days after inoculation by infiltration. **(A)** Hypersensitive reaction, **(B)** resistant nonhypersensitive reaction, and **(C)** susceptible nonhypersensitive reaction.

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was confirmed by an injection test (10). Within 24 hr of interveinal injection with a bacterial suspension of 10^8 cells per milliliter, tissue collapse characteristic of a hypersensitive reaction (11) appeared. The hypersensitive reaction in PI 271322 was confirmed by A. A. Cook in Florida (*personal communication*). Thus, each plant was then classified as hypersensitive or nonhypersensitive 7 days after inoculation.

RESULTS AND DISCUSSION

The pollen taken from plant 1 of PI 271322 that was used to pollinate flowers on Keystone produced a good set and abundant seed. When the F_1 plants of this cross were inoculated by the infiltration method, we noticed that about half of the plants showed a hypersensitive response (Table 1). The original inoculation was by spraying, which does not permit hypersensitivity to be recognized. Selfed seed were saved from both hypersensitive and nonhypersensitive F_1 plants and a backcross was made between a hypersensitive F_1 plant and Keystone. The three progenies that resulted from selfing hypersensitive F_1 plants all segregated 3 hypersensitive:1 nonhypersensitive. The two progenies that resulted from selfing nonhypersensitive F_1 plants both produced only nonhypersensitive plants (Table 1). Both hypersensitive and nonhypersensitive plants in the F_2 generation were also selfed to produce F_3 progeny. Each of the F_3 progeny that arose from a hypersensitive F_2 parent segregated 3 hypersensitive:1 nonhypersensitive, and each progeny that arose from a nonhypersensitive parent had only nonhypersensitive plants.

We concluded that the hypersensitivity in PI 271322 is controlled by a dominant gene. The original plant of PI 271322 that had been used to pollinate the Keystone flowers was apparently heterozygous for the hypersensitivity gene, which is why only half of the F_1 plants were hypersensitive. All hypersensitive F_1 plants should have been heterozygous, so the F_2 progeny from these would be expected to segregate 3:1, as they did. It should be possible to find some homozygous individuals in the F_2 that would not produce segregating progenies, but the three plants chosen for growing F_3 progenies were apparently all heterozygous.

More seed of PI 271322 were grown and inoculated by infiltration to detect hypersensitivity. A second hypersensitive plant was found and also crossed with Keystone (Table 2). In this case, the F_1 was all hypersensitive and the F_2 segregated 3 hypersensitive:1 nonhypersensitive as before. Plant 2 of PI 271322 was apparently homozygous for this gene, as is confirmed by the hypersensitivity of all the selfed progeny of plant 2.

Backcrosses were made between the F_1s and the available parents. When the plant 2 F_1 was backcrossed with the hypersensitive parent, all progeny were hypersensitive as expected (Table 2). When this F_1 and the plant 1 F_1 were backcrossed with the nonhypersensitive parent, however, both backcross progenies gave a relatively poor fit to the expected 1:1 ratio (Tables 1 and 2). In one case, however, there was an excess of nonhypersensitive plants (Table 1), and in the other case, there was an excess of hypersensitive plants (Table 2). If the two backcross progenies are combined, the fit to a 1:1 ratio is very good ($X^2 = 1.0279$, $P = 0.25$–0.5).

PI 271322 had previously been reported resistant to bacterial spot by Sowell and Dempsey (15), Hibberd et al (8), and Stall (17). None of these workers reported hypersensitivity in this line. The most detailed work on inheritance was by Stall (17), who reported that resistance was expressed as a low number of lesions after infiltration with race 1 compared with a high number of lesions in Early Calwonder. Resistance seemed to be recessive to susceptibility, with perhaps two genes involved. In addition to these, other genes with quantitative inheritance have been observed in the present study (10). Thus, it seems there is a large amount of variability for resistance included in materials that are carried as PI 271322, including the single gene conferring hypersensitivity that we are reporting and several others.

We propose to name this single dominant gene in PI 271322 (*C. annuum*) that confers hypersensitivity to race 1 of *X. campestris* pv. *vesicatoria* "Bs_3." Thus, this gene for hypersensitivity joins Bs_1, which confers hypersensitivity to race 2 and was found in PI 163192 (3,18), and Bs_2, which confers hypersensitivity to both races 1 and 2, and was found in PI 260435 (*C. chacoense*) (2) following the terminology established in Lippert et al (12).

Table 1. Segregation for hypersensitivity to *Xanthomonas campestris* pv. *vesicatoria* in progenies of a cross between Keystone and PI 271322, plant 1

Generation	Observed no. H[a]	Observed no. N	Expected ratio	X^2	Probability range for X^2
P_1 (Keystone)	...	65	...	...	...
F_1	25	26	...	...	...
F_2 (hypersensitive F_1 plants)	251	87	3:1	0.063	0.75–0.90
	77	21	3:1	0.490	0.25–0.50
	216	66	3:1	0.303	0.50–0.75
Pooled	544	174	3:1	0.186	0.50–0.75
F_2 (nonhypersensitive F_1 plants)	0	204	0:1	...	...
	0	205	0:1	...	...
Pooled	0	409	0:1	...	...
F_3 (hypersensitive F_2 plants)	102	40	3:1	0.601	0.25–0.50
	102	41	3:1	0.841	0.25–0.50
	22	8	3:1	0.00	0.90
Pooled	226	89	3:1	1.610	0.10–0.25
F_3 (nonhypersensitive F_2 plants)	0	148	0:1	...	...
	0	144	0:1	...	...
Pooled	0	292	0:1	...	...
BC (Keystone × F_1)	58	84	1:1	4.40	0.025–0.05

[a] H = hypersensitive, N = nonhypersensitive, and BC = backcross.

Table 2. Segregation for hypersensitivity to *Xanthomonas campestris* pv. *vesicatoria* in progenies of a cross between Keystone and PI 271322, plant 2

Generation	Observed no. H[a]	Observed no. N	Expected ratio	X^2	Probability range for X^2
P_1 (Keystone)	0	36	...	...	...
P_2 (PI 271322, plant 2)	60	0	...	...	...
F_1	96	0	...	...	...
F_2	556	191	3:1	0.100	0.50–0.75
BC_1 (F_1 × Keystone)	167	120	1:1	7.373	0.01–0.005
BC_2 (F_1 × PI 271322, plant 2)	491	0	...	...	...

[a] H = hypersensitive, N = nonhypersensitive, and BC = backcross.

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