# A Novel *Capsicum* Gene Inhibits Host-Specific Disease Resistance to *Phytophthora capsici*

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## ABSTRACT

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A novel disease resistance inhibitor gene (inhibitor of *P. capsici* resistance [*Ipcr*]), found in the chile pepper (*Capsicum annuum*) variety 'New Mexico Capsicum Accession 10399' (NMCA10399), inhibits resistance to *Phytophthora capsici* but not to other species of *Phytophthora*. When a highly *P. capsici*-resistant variety was hybridized with NMCA10399, the resultant $F_1$ populations, when screened, were completely susceptible to *P. capsici* for root rot and foliar blight disease syndromes, despite the dominance inheritance of *P. capsici* resistance in chile pepper. The $F_2$ population displayed a 3:13 resistant-to-susceptible (R:S) ratio. The testcross population displayed a 1:1 R:S ratio, and a backcross population to NMCA10399 displayed complete susceptibility. These results demonstrate the presence of a single dominant inhibitor gene affecting *P. capsici* resistance in chile pepper. Moreover, when lines carrying the *Ipcr* gene were challenged against six *Phytophthora* spp., the nonhost resistance was not overcome. Therefore, the *Ipcr* gene is interfering with host-specific resistance but not the pathogen- or microbe-associated molecular pattern nonhost responses.

*Additional keywords*: host resistance, susceptibility factor.

*Phytophthora capsici* is arguably the most destructive pathogen to chile pepper (*Capsicum* sp.) (6). Though difficult to estimate, annual damage of Phytophthora blight to chile pepper yield world-wide exceeds U.S. $100 million (3). In the shadow of such losses, control measures are needed to safeguard food production from disease outbreak. Plants have adapted the capability to perceive a danger to their health and respond defensively. Knowledge of plant defenses is important for the development of *Capsicum* varieties with enhanced resistance to Phytophthora blight.

The best strategy to prevent disease in plants is the development of resistant varieties because it is less expensive, environmentally friendly, and a sustainable alternative to chemical applications such as fungicides (11). In addition, planting disease-resistant varieties does not require alteration to cultivation practices. Disease resistance manifests itself in two forms: host and nonhost resistance. Nonhost resistance is defined as the resistance shown by a plant species toward many pathogens for which it is not considered to be a host, and it is effective against all known isolates of those given pathogens (13,26). The mechanisms of nonhost resistance are not well understood but likely vary on a case-by-case basis and rely upon one or more mechanisms, such as the strengthening of the plant cytoskeleton to provide a physical barrier or the production of secondary metabolites with antimicrobial activity that present a pre- and post-invasion defense mechanism (10,12,15,22,26).

The second form of resistance is host resistance, which is expressed when a plant that is considered a host can resist infection of a specific pathogenic strain (19). Race-specific resistance operates within host resistance and is defined as resistance to specific isolates of a pathogen but not to all. This type of resistance is associated with the gene-for-gene mechanism (7,19). Basically, a protein product (effector), produced by an avirulence gene in the pathogen is recognized by a resistance (*R*) gene in the host. A successful recognition results in the induction of a signal transduction in the host that initiates the host defense responses and the inhibition of pathogen growth (35).

In the *Capsicum annuum–P. capsici* pathosystem, resistance against *P. capsici* in *C. annuum* is reported as a dominant trait (24,31,38). The inheritance of Phytophthora blight resistance is complex. Depending on the site of infection, different disease syndromes (i.e., root rot, foliar blight, fruit rot, and stem blight) are manifested, each of which requires the presence of a specific *R* gene for the control of each particular disease syndrome (31,38). In addition, different *R* genes control the resistant phenotype against different physiological races of *P. capsici* within each disease syndrome (24).

In *Capsicum* spp., specific *R* genes for *P. capsici* resistance and their mode of action remain largely unknown (30). To better understand the mode of inheritance for host resistance, a unique *P. capsici*-susceptible accession, 'New Mexico Capsicum Accession 10399' (NMCA10399), was evaluated. When tested against several physiological races of *P. capsici*, only susceptible individuals were obtained in the $F_1$ population from the hybridization of NMCA10399 to 'Criollo de Morelos-334' (CM-334), which is the accession most resistant to *P. capsici* known. Thus far, CM-334 has displayed resistance to all *P. capsici* isolates (27,33). Because CM-334 has *P. capsici* resistance that is considered dominant (38), the presence of only susceptible individuals from this hybridization with NMCA10399 suggested inhibition of the *P. capsici* resistance mechanism present in CM-334 by a gene or genes that has been provisionally designated "inhibitor of *P. capsici* resistance" (*Ipcr*).

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*The *e*-Xtra logo stands for "electronic extra" and indicates that Figures 1, 2, and 3 appear in color online.

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Because the *Ipcr* gene has been found to cause susceptibility to all isolates of *P. capsici* evaluated to date, it was important for this study to test *Ipcr* for its influence on nonhost resistance against other *Phytophthora* spp. This study had two goals: (i) evaluate the mode of inheritance for *Ipcr* and (ii) characterize *Ipcr* for its effect on host verses nonhost resistance against different *Phytophthora* spp.

## MATERIALS AND METHODS

**Plant materials.** For this study, the *C. annuum* accession CM-334 ($P_R$) was used as a resistant parent. The inhibitor *C. annuum* accession NMCA10399 ($P_I$) and 'Camelot' ($P_S$) (Seminis Vegetable Seed, Inc., St Louis) were used as susceptible parents. To date, NMCA10399 has been susceptible to all *P. capsici* isolates tested (27,32). This accession is a landrace known as jalapeno espinalteco, collected near Vera Cruz, Mexico. The commercial bell pepper Camelot, grown in the eastern United States, has been used as a *P. capsici*-susceptible control in previous studies (40).

Three $F_1$ populations were developed from the hybridizations of CM-334 × NMCA10399 ($P_R \times P_I$), CM-334 × Camelot ($P_R \times P_S$), and NMCA10399 × Camelot ($P_I \times P_S$). Individual $F_1$ plants were self-pollinated to obtain $F_2$ populations. In addition, $F_1$ individuals from the CM-334 × NMCA10399 hybridization were backcrossed to $P_R$ and $P_I$, creating a backcross population ([CM-334 × NMCA10399] × CM-334) ($BC_1P_R$) and a testcross population ([CM-334 × NMCA10399] × NMCA10399) ($BC_1P_I$).

***C. annuum* planting.** Seed to be inoculated for root rot were planted in 72-celled plastic trays, (T. O. Plastics, Clearwater, MN) and seed to be inoculated for foliar blight were planted in larger 48-celled plastic trays (T. O. Plastics). Trays were filled with a commercial peat moss–vermiculite soil mixture (Sun Gro Redi-earth Plug & Seedling mix; Sun Gro Horticulture, Bellevue, WA). Two seeds per cell were sown. Trays were placed on propagation pads to maintain soil temperature at 28°C to promote better germination, watered twice a day, and fertilized with a slow-release fertilizer (Osmocote 14N-6.2P-11.6K; The Scott Miracle-Gro Company, Marysville, OH). Plants were grown in a climate-controlled greenhouse maintained at 28 and 18 ± 6°C day and night temperatures, respectively, with a 12-h photoperiod. When the plants reached the four- to six-true-leaf stage, they were inoculated with *Phytophthora* spp.

In order to verify the phenotype of each susceptible individual from each $F_1$ population while maintaining the plant for hybridization or self-pollination, individual $F_1$ plants were regenerated as cuttings before inoculation. The cuttings were placed under 70% relative humidity until rooting was evident. Once rooted, the selected plants were transplanted and used for hybridizations or self-pollination to obtain seed for the next generation.

***Phytophthora* isolates.** Six species of *Phytophthora* were provided by The World Phytophthora Collection, Department of Plant Pathology and Microbiology, University of California, Riverside, and included *P. cinnamomi*-P2444 (isolated from *Persea americana*), *Phytophthora citrophthora*-P1163 (isolated from *Citrus* sp.), *P. infestans*-P9175 (isolated from *Solanum lycopersicum*), *P. nicotianae*-P10116 (isolated from *Metrosideros excelsa*), *P. sojae*-P7082 (isolated from *Glycine max*), and *P. capsici* physiological race-*1* (American Type culture Collection [ATCC]: MYA-2289) (isolated from *C. annuum*). *P. capsici* race-*1* was chosen because of its well-characterized virulence toward *C. annuum* accessions (31).

To test the effect of *Ipcr* on race-specific resistance, three additional *P. capsici* races were inoculated on the CM-334 × NMCA10399 ($P_R \times P_I$) $F_1$ and $F_2$ populations. These included race-*2* (ATCC: MYA-2291), race-*12* (not in ATCC but available by request), and race-*15* (ATCC: MYA-2339). *P. capsici* physiological race-*2* was chosen because of its aggressive virulence toward *C. annuum* accessions; race-*12* and race-*15* were chosen for their mild virulence toward *C. annuum* accessions. *P. capsici* physiological races *-1*, *-2*, and *-12* were isolated from *C. annuum* in New Mexico and race-*15* was isolated from *C. annuum* in New Jersey.

**Pathogen growth and inoculation of *C. annuum* plants with *P. capsici*.** A specific growing protocol for each *Phytophthora* sp. was followed to ensure growth, virulence, and zoospore production. For *P. capsici*, the methodology used by Bosland and Lindsey (4) was followed. To confirm the pathogenicity of the *P. cinnamomi* isolate, avocado fruit were inoculated using the method of Hüberli et al. (14). For *P. citrophthora*, susceptible tomato (*S. lycopersicum*) leaves were inoculated using the technique described by Flors et al. (8). For *P. infestans*, the technique described by Appel et al. (2) for inoculating potato (*S. tuberosum*) tubers was used. The technique described by Widmer et al. (39) was followed for *P. nicotianae* to inoculate susceptible tobacco (*Nicotiana benthamiana*) plants. Soybean (*G. max*) hypocotyls were inoculated with the injection method described by Dorrance and Schmitthenner (5) to maintain the *P. sojae* isolate.

To test for the effect of *Ipcr* on host and nonhost resistance, the six *Phytophthora* spp. were inoculated following the procedure described by Bosland and Lindsey (4) for root rot screening with an inoculum concentration of 10,000 zoospores/plant, and the procedure of Monroy-Barbosa and Bosland (25) for foliar blight screening with an inoculum concentration of 2,000 zoospores/plant on *C. annuum* plants. These two inoculum concentrations provide accurate and reliable results—distinguishing susceptible and resistant phenotypes to *P. capsici* in *C. annuum*.

Evaluation of Phytophthora root rot and foliar blight disease syndromes used the disease scales described by Bosland and Lindsey (4) and Monroy-Barbosa and Bosland (25), respectively. For root rot disease, healthy plants with no symptoms (disease scale level 0 to 1) were considered resistant, while plants showing slight root darkening to death were considered susceptible (levels 2 to 9) (4). For foliar blight, healthy leaves with no symptoms (level 0) or hypersensitive reaction (HR) (level 1) were considered resistant, while inoculated leaves showing lesions larger than the inoculation disc to complete leaf necrosis (levels 2 to 5) were considered susceptible (25). The total number of resistant and susceptible plants were counted and presented as a resistant-to-susceptible (R:S) ratio. Parents ($P_R$, $P_I$, and $P_S$) were used as controls for the inoculation tests. The plants were scored 12 days after inoculation for root rot and 4 days after inoculation for foliar blight, when the susceptible controls in each test showed level 9 for root rot and level 5 for foliar blight on each respective disease scale.

**Experimental design and statistical analysis.** To test for nonhost resistance, groups of 24 plants of each parent ($P_R$, $P_I$, and $P_S$) were considered experimental units in a completely randomized design. A single group of each parent was screened for root rot and foliar blight resistance with one of each *Phytophthora* sp. Each group was inoculated with only one *Phytophthora* sp. and only for one disease syndrome. The inoculations were replicated three times.

To characterize the inheritance of the *Ipcr* gene, single plants were considered experimental units in a completely randomized design. Two replicate tests in two different years for root rot and two replicate tests in one year for foliar blight were evaluated. At least 108 individuals were evaluated in each $F_2$ population in order to ensure 99.9% probability of observing a single segregant individual (23).

Data were analyzed using SAS (version 9.3; SAS Institute, Cary, NC). A $\chi^2$ goodness-of-fit test ($\alpha = 0.05$) compared the expected versus the observed segregation (R:S) ratios in all the populations. Expected ratios were based on hypothetical Mendelian segregation ratios where the presence of a single dominant epi-

static gene is involved. The hypothesis was rejected for a specific segregation ratio for any population with $P$ value ≤ 0.05. A $\chi^2$ test of homogeneity ($P \leq 0.05$) was used to combine data from replicated tests.

## RESULTS

**Genetic characterization of a disease resistance inhibitor gene in NMCA10399.** In all inoculation trials, the *P. capsici*-resistant parent (CM-334) always displayed phenotypic resistance; no lesions were observed. The susceptible parents (NMCA10399 and Camelot) always scored 9 (plant death) for root rot and 5 (complete leaf necrosis) for foliar blight toward *P. capsici*, as would be expected (Fig. 1). For each population, a $\chi^2$ test of homogeneity ($P \leq 0.05$) indicated data from replicated tests could be combined for simplified data presentation (data not shown).

The CM-334 × Camelot $F_1$ population displayed complete resistance—showing the dominant inheritance of *P. capsici* resistance for root rot and foliar blight disease syndromes. A segregation ratio of 3:1 (R:S) in the CM334 × Camelot $F_2$ population demonstrated that Camelot is homozygous recessive for the *R* gene, meaning that it lacks any *R* genes for *P. capsici* resistance, whereas CM-334 is homozygous dominant for the *R* genes (Table 1; Fig. 2). The NMCA10399 × Camelot $F_1$ and $F_2$ populations were completely susceptible, indicating that neither of the susceptible parents (Camelot and NMCA10399) have any effective *R* genes against *P. capsici*-induced syndromes with respect to the isolate tested (Table 1; Fig. 2).

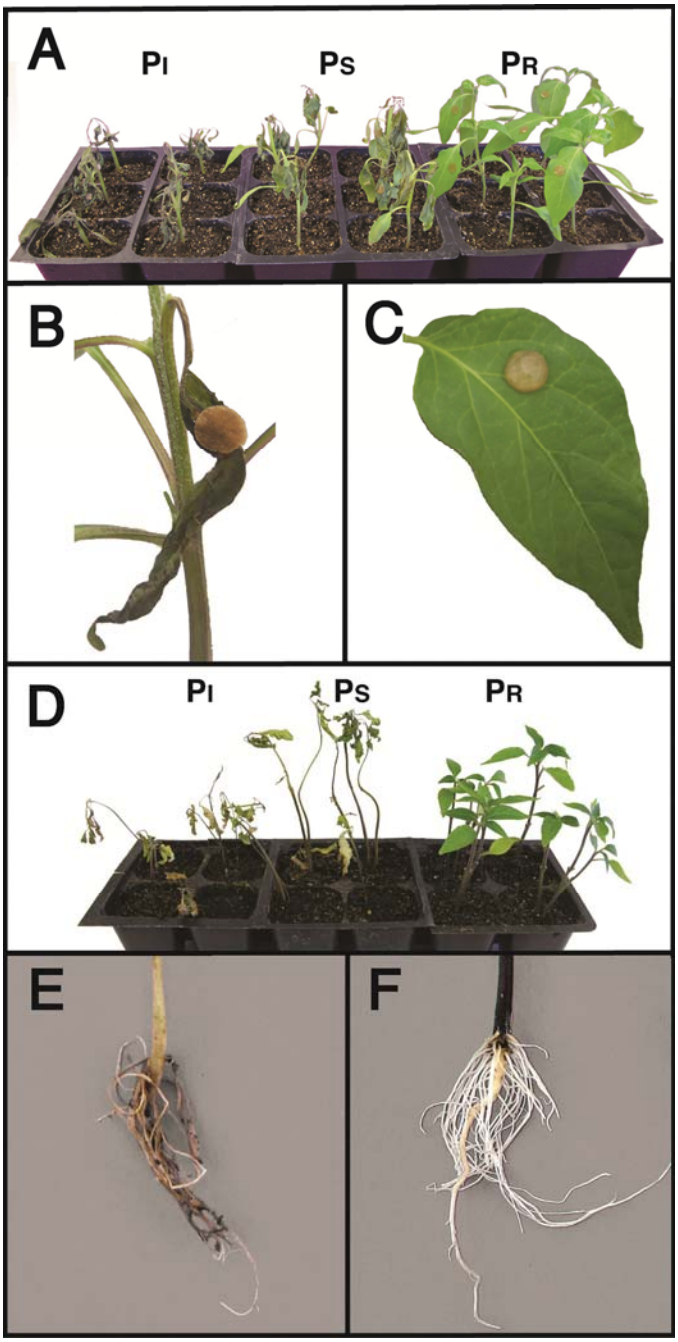


**Fig. 1.** Symptoms for *Phytophthora capsici* induced root rot and foliar blight diseases of chile pepper (*Capsicum annuum*). **A,** Resistant and susceptible foliar blight reactions for the inhibitor accession, 'New Mexico Capsicum Accession 10399' (NMCA10399) ($P_I$); the susceptible accession, 'Camelot' ($P_S$); and the *P. capsici* resistant accession, 'Criollo de Morelos-334' (CM-334) ($P_R$) 4 days after inoculation. **B,** Susceptible foliar blight. **C,** Resistant foliar blight. **D,** Resistant and susceptible root rot reactions for $P_I$, $P_S$, and $P_R$ 12 days after inoculation. **E,** Susceptible root rot. **F,** Resistant root rot.

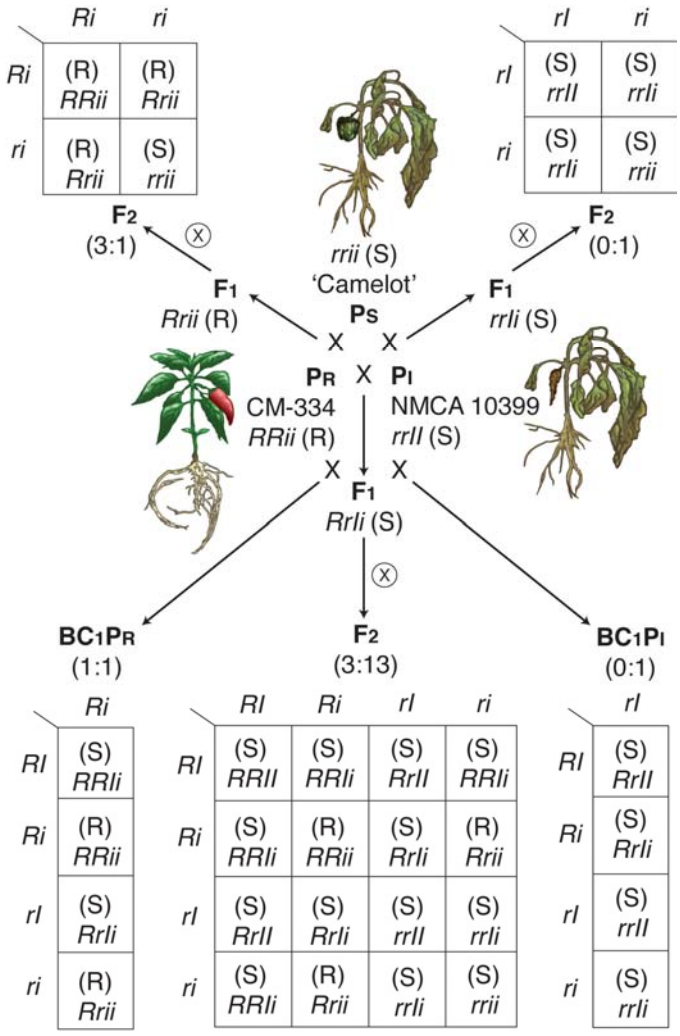


**Fig. 2.** Inheritance of an inhibitor of *Phytophthora capsici* resistance (*Ipcr*) gene in chile pepper (*Capsicum annuum*). The *Phytophthora capsici*-resistant accession, 'Criollo de Morelos-334' (CM-334) ($P_R$), was hybridized to *P. capsici*-susceptible accessions 'New Mexico Capsicum Accession 10399' (NMCA10399) ($P_I$) and 'Camelot' ($P_S$) to generate $F_1$ and $F_2$ populations, respectively. The CM-334 × NMCA10399 ($F_1$) population was backcrossed to the resistant and inhibitor parents to generate $BC_1P_R$ and $BC_1P_I$ populations. Resistant-to-susceptible (R:S) phenotypic ratios in the $F_2$ and backcross populations are shown in parentheses below. R and S represent resistant and susceptible phenotypes, respectively, for both root rot and foliar blight disease syndromes. The *R*/*r* alleles are representative of dominant/recessive *R* genes for root rot ($R_{rr}$) and foliar blight ($R_{fb}$); *I*/*i* are dominant/recessive alleles for *Ipcr*.

The CM-334 × NMCA10399 $F_1$ population displayed complete susceptibility to Phytophthora root rot and foliar blight disease syndromes. The CM-334 × NMCA10399 $F_2$ population displayed a 3:13 (R:S) segregation ratio, indicating that the *Ipcr* gene has an epistatic dominant effect over the dominant *R* genes for root rot and foliar blight (Table 1; Fig. 2). After this finding, the CM-334 × NMCA10399 $F_1$ and $F_2$ populations were separately inoculated with three additional *P. capsici* races for root rot (race-*2*, race-*12*, and race-*15*) to test whether *Ipcr* can inhibit resistance against other *P. capsici* races. In all cases, the populations behaved the same. The CM-334 × NMCA10399 $F_1$ population showed complete susceptibility and the $F_2$ population showed a 3:13 (R:S) ratio when inoculated for all three races (data not shown).

The testcross population, ([CM-334 × NMCA10399] × CM-334) ($BC_1P_R$), fit the expected ratio of 1:1 (R:S). The backcross population ([CM-334 × NMCA10399] × NMCA10399) ($BC_1P_I$) displayed complete susceptibility for foliar blight and root rot syndromes (Table 1; Fig. 2).

**Nonhost resistance evaluation in NMCA10399.** To ensure the virulence of the isolates, the six *Phytophthora* spp. were inoculated to their respective hosts. All the inoculated hosts showed disease symptoms: *P. capsici* infected the *C. annuum* accessions NMCA10399 and Camelot, *P. cinnamomi* infected avocado fruit, *P. citrophthora* infected tomato leaves, *P. infestans* infected potato leaves and tubers, *P. nicotianae* infected tobacco plants, and *P. sojae* infected soybean hypocotyls. These results indicate that each *Phytophthora* sp. was virulent.

The accession CM-334 displayed phenotypic resistance against the six *Phytophthora* spp. Only when NMCA10399 and Camelot were inoculated with *P. capsici* did they display susceptible root rot and foliar blight disease symptoms. Conversely, these accessions showed no susceptibility in roots or leaves when they were inoculated with the other five *Phytophthora* spp. (Table 2). Nonhost resistance type I (no visible symptoms) was observed in CM-334 under root rot and foliar blight inoculations with the *Phytophthora* spp. Type II nonhost resistance (associated with rapid localized necrosis) was expressed in the form of HR (Fig. 3) when leaves of NMCA10399 and Camelot plants were inoculated with *P. nicotianae* and *P. citrophthora* (Table 2). These plants had HR lesions only on the inoculated leaves using the Monroy-Barbosa and Bosland (25) paper-disc protocol. Plants with HR lesions were maintained for an additional 4 weeks, and the lesions did not expand, nor were any new lesions formed on the inoculated leaf or any other leaf.

## DISCUSSION

Inheritance and molecular analysis of race-specific resistance within *P. capsici*-induced root rot and foliar blight diseases shows that *P. capsici* resistance in *C. annuum* is polygenic. Walker and Bosland (38) inoculated cloned *C. annuum* plants for root rot and foliar blight. Their results demonstrated that both syndromes require the presence of separate *R* genes in order to be resistant for both syndromes. Ogundiwin et al. (28) supported this claim by their investigation of quantitative trait loci (QTLs) for root rot and foliar blight resistance to find that, although some QTLs were shared for both syndromes, separate QTLs accounted for resistance to root rot and foliar blight. Moreover, the presence of several race-specific *R* genes is necessary for resistance against

TABLE 2. Phenotypic reaction of the resistant parent 'Criollo de Morelos-334' (CM-334), the inhibitor parent 'New Mexico Capsicum Accession 10399' (NMCA10399), and the susceptible parent 'Camelot' under Phytophthora root rot (Rot) and foliar blight (Blight) disease screening

| | Disease syndromes[b] | | |
|---|---|---|---|
| Accession, species[a] | Rot | Blight | Obs.[c] |
| CM-334 | | | |
| *Phytophthora capsici* | R | R | … |
| *P. cinamomi* | R | R | … |
| *P. citrophthora* | R | R | … |
| *P. infestans* | R | R | … |
| *P. nicotianae* | R | R | … |
| *P. sojae* | R | R | … |
| NMCA10399 | | | |
| *P. capsici* | S | S | … |
| *P. cinamomi* | R | R | … |
| *P. citrophthora* | R | R | HR-L |
| *P. infestans* | R | R | … |
| *P. nicotianae* | R | R | HR-L |
| *P. sojae* | R | R | … |
| Camelot | | | |
| *P. capsici* | S | S | … |
| *P. cinamomi* | R | R | … |
| *P. citrophthora* | R | R | HR-L |
| *P. infestans* | R | R | … |
| *P. nicotianae* | R | R | HR-L |
| *P. sojae* | R | R | … |

[a] *Capsicum annuum* accessions and *Phytophthora* spp.
[b] R = resistant phenotype, no visible lesions and S = susceptible phenotype, visible disease symptoms.
[c] Observations: HR-L = hypersensitive response observed on leaves during foliar blight screening.

TABLE 1. Phenotypic response of the resistant parent 'Criollo de Morelos-334' (CM-334), the inhibitor parent 'New Mexico Capsicum Accession 10399' (NMCA10399), the susceptible parent 'Camelot', and the $F_1$, $F_2$, and backcross populations screened for Phytophthora root rot and foliar blight using *Phytophthora capsici* race-*1*

| | | | Disease syndromes[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Expected | | Root rot[b] | | | | Foliar blight[c] | | | |
| Entry | Genotype[d] | Ratio (R:S)[e] | R | S | $\chi^2$ | *P* value | R | S | $\chi^2$ | *P* value |
| CM-334 ($P_R$) | *RRii* | 1:0 | 24 | 0 | – | – | 30 | 0 | – | – |
| NMCA10399 ($P_I$) | *rrII* | 0:1 | 0 | 24 | – | – | 0 | 30 | – | – |
| 'Camelot' ($P_S$) | *rrii* | 0:1 | 0 | 24 | – | – | 0 | 34 | – | – |
| $P_R \times P_I$ ($F_1$) | *RrIi* | 0:1 | 0 | 127 | – | – | 0 | 35 | – | – |
| $P_R \times P_S$ ($F_1$) | *Rrii* | 1:0 | 79 | 0 | – | – | 37 | 0 | – | – |
| $P_I \times P_S$ ($F_1$) | *rrIi* | 0:1 | 0 | 72 | – | – | 0 | 32 | – | – |
| $P_R \times P_I$ ($F_2$) | *R-ii*/*--I-*/*rrii* | 3:13 | 123 | 556 | 0.04 | 0.84 | 44 | 175 | 0.26 | 0.61 |
| $P_R \times P_S$ ($F_2$) | *R-ii*/*rrii* | 3:1 | 79 | 32 | 0.87 | 0.35 | 111 | 47 | 1.9 | 0.17 |
| $P_I \times P_S$ ($F_2$) | *rr--* | 0:1 | 0 | 110 | – | – | 0 | 117 | – | – |
| ($[P_R \times P_I] \times P_R$) ($BC_1P_R$) | *R-Ii*/*R-ii* | 1:1 | 56 | 55 | 0.009 | 0.92 | 35 | 27 | 1.03 | 0.31 |
| ($[P_R \times P_I] \times P_I$) ($BC_1P_I$) | *-rI-* | 0:1 | 0 | 48 | – | – | 0 | 13 | – | – |

[a] R and S: number of resistant (R) or susceptible (S) plants. *P* value, where $\alpha = 0.05$.
[b] Plants were scored using the Bosland and Lindsey (4) protocol.
[c] Plants were scored using the Monroy-Barbosa and Bosland (25) protocol.
[d] R/r: dominant/recessive resistance gene, I/i: dominant/recessive inhibitor of *Phytophthora capsici* resistance (*Ipcr*) gene.
[e] Resistant-to-susceptible ratio.

different physiological races of *P. capsici* for both root rot and foliar blight (24,27,32). Troung et al. (34) demonstrated this gene-for-gene mechanism of resistance by showing that unique QTLs are required for *P. capsici* race-specific resistance in *C. annuum*. This study of the *Ipcr* gene did not validate whether or not race-specific resistance operating within resistance to root rot ($R_{rr}$) and foliar blight ($R_{fb}$) are controlled by separate genes but accepts the de facto conclusions of previous research that they are.

The $F_1$ hybridization of the *P. capsici*-resistant parent CM-334 to the susceptible Camelot displayed complete resistance, which is consistent with the dominant inheritance of *P. capsici* resistance with respect to the CM-334 genes (Table 1), establishing CM-334 as dominant and homozygous for resistance to root rot and foliar blight, suggesting $R_{rr}R_{rr}R_{fb}R_{fb}ipcripcr$ as the most appropriate genotype for CM-334, whereas Camelot is homozygous recessive, $r_{rr}r_{rr}r_{fb}r_{fb}ipcripcr$, for these loci. The CM-334 × Camelot $F_2$ population ratio of 3:1 (R:S) is indicative of a single *R* gene that is absent in Camelot, and explains its susceptible phenotype (Table 1). Conversely, total susceptibility obtained in the NMCA10399 × Camelot $F_1$ and $F_2$ populations establishes that neither NMCA10399 nor Camelot have any *R* genes for root rot or foliar blight resistance.

The results from the $F_1$ population of the CM-334 × NMCA10399 hybridization showed a dominant effect of the *Ipcr* gene over the host-specific *R* genes from CM-334. If the inhibitor gene (*Ipcr*) is present, then it is expected that the whole population would express susceptibility, displaying a 0:1 (R:S) ratio, with the theoretical genotype for this population being $R_{rr}r_{rr}R_{fb}r_{fb}Ipcripcr$ (Fig. 2). In the $F_1$ population, it was observed that all the individuals from the CM-334 × NMCA10399 hybridization were susceptible, which is evidence of the action of *Ipcr* inhibiting *P. capsici* resistance in *C. annuum*. These individuals have *R* genes provided by the resistant parent, CM-334; however, the susceptibility of all the individuals in the $F_1$ cannot be explained by the absence of *R* genes but, instead, is explained by the action of an inhibitor gene (*Ipcr*) interfering with the host-specific defense mechanism. Thus, a plant can be susceptible for two reasons: lack of *R* genes or presence of an inhibitor gene.

Similarly, *Ipcr* could be viewed as a dominant race-nonspecific susceptibility gene. Effectors produced by *P. capsici*, such as RXLR, *Crn*, or *PcNpp* effectors, are believed to be critical in the infection process (21). The *Ipcr* gene may be required for effector sensitivity by being a direct or indirect target, causing effector-triggered susceptibility. The *Ipcr* gene causes susceptibly for both root rot for several *P. capsici* races and foliar blight syndromes, despite the presence of *R* genes. Isolation and biochemical analysis of the *Ipcr* gene are necessary to differentiate whether it functions as a repressor or susceptibility factor.

The 3:13 (R:S) ratio in the CM-334 × NMCA10399 $F_2$ population when inoculated with four *P. capsici* races for root rot and one for foliar blight confirms the presence of *Ipcr* and its epistatic effect on *P. capsici* resistance (Fig. 2). The ratio observed in this population establishes that NMCA10399 lacks the *R* genes for both disease syndromes while being homozygous dominant for the *Ipcr*-locus, suggesting $r_{rr}r_{rr}r_{fb}r_{fb}IpcrIpcr$ as the most appropriate genotype for NMCA10399. Moreover, the 1:1 (R:S) ratio witnessed in the testcross population ([CM-334 × NMCA10399] × CM-334) and total susceptibility seen in the backcross population to the inhibitor parent, ([CM-334 × NMCA10399] × NMCA10399) further support the action of a single dominate gene affecting disease resistance to *P. capsici* (Fig. 2).

When nonhost resistance was examined in NMCA10399, it displayed a resistant phenotype against all nonhost *Phytophthora* spp. tested. This demonstrates that the pathogen- or microbe-associated molecular pattern defense is still functional in NMCA10399 against other *Phytophthora* spp., despite the presence of *Ipcr.* Based on these results, *Ipcr* interferes with the host-specific *R* genes against *P. capsici* but not nonhost *R* genes.

An HR in the leaves was observed when the two "susceptible" accessions, NMCA10399 and Camelot, were challenged for foliar blight resistance with *P. citrophthora* and *P. nicotianae* (Fig. 3), which is characteristic of type II nonhost resistance (16). An HR is a rapid plant cell death defense response induced during penetration of the epidermal cell. It is usually localized to a few plant cells causing cell death—entombing the pathogen from further progression (37). This resistant reaction provides intriguing information because the HR defense mechanism is generally triggered after penetration of the pathogen hyphae. Tobacco plants inoculated with *P. infestans* and *P. capsici* displayed HR when the epidermal and, occasionally, the mesophyll cells were penetrated (17,37). Nonhost plants such as radish (*Raphanus sativus*), parsley (*Petroselinum hortense*), and *Arabidopsis thaliana* inoculated with *Phytophthora infestans* display HR after penetration (15,37). The expression of HR lesions in NMCA10399 and Camelot suggests that *P. citrophthora* and *P. nicotianae* probably defeated barriers such as callose formation or phytoalexin secretions that are part of nonhost type I resistance. If *P. nicotianae* was able to penetrate mesophyll cells in *C. annuum* plants, this observation would agree with many of the reports that *P. nicotianae* forma specialis can infect *Capsicum* plants (1,6,36), even though, in New Mexico, isolates

**Fig. 3.** Hypersensitive response expressed in leaf when the inhibitor accession, 'New Mexico Capsicum Accession 10399' (NMCA10399) ($P_I$), was inoculated with *Phytophthora nicotianae* using the Monroy-Barbosa and Bosland (25) paper-disk technique.

of *P. nicotianae* are not pathogenic on *Capsicum* spp. but are found on onion (*Allium cepa*) (9).

Because genes that partially or completely suppress the activity of *R* genes are rare—especially dominant genes—the discovery of an inhibitor gene that can completely stop the host resistance mechanism to *P. capsici* is specifically interesting. A suppressor gene (*S*) that inhibits the expression of *R* genes to leaf curl virus has been reported in cotton (*Gossypium hirsutum*) (29). Suppressor genes of stem and leaf rust (*Puccinia graminis*) resistance in wheat (*Triticum turgidum*) have been identified, and specifically located on the long arm of chromosome 7D (18,20). Also, suppression of host defense response can occur through the production of inhibitory proteins that target host enzymes (15).

The identification of the dominant *Ipcr* gene, affecting the dominant resistance of *C. annuum* CM-334 against *Phytophthora capsici*, is the first of its kind in *Capsicum* spp. The populations with *Ipcr* produced for this study were susceptible when inoculated for root rot when treated with four races of *P. capsici* and one race for foliar blight, indicating that *Ipcr* interferes with tissue- and race-specific resistance for *P. capsici*. Because *P. capsici* resistance to the different disease syndromes is controlled by different *R* genes, these results suggest that *Ipcr* is somehow functioning at a "higher" or more fundamental level to affect host resistance.

The discovery of *Ipcr* can provide new insight into the inheritance and defense mechanisms of the *C. annuum–P. capsici* pathosystem. Exploitation of this unique interaction offers a powerful approach to understanding host–pathogen interactions and the mechanisms behind host resistance. The study of the *Ipcr* gene at the molecular level could provide new information to explain the resistance mechanism leading to a greater understanding of host resistance—including the inheritance, expression, and number of genes involved in resistance against *P. capsici* in *C. annuum*.

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