Recombinant Inbred Line Differential Identifies Race-Specific Resistance to *Phytophthora* Root Rot in *Capsicum annuum*

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ABSTRACT

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A differential series is the normal method for identification of races within a plant pathogen and a host interaction. A host differential is extremely useful for phytopathological as well as breeding purposes. A set of recombinant inbred lines (RILs) were developed and characterized for race differentiation of *Phytophthora* root rot caused by *Phytophthora capsici*. The highly resistant *Capsicum annuum* accession Criollo de

Morelos-334 was hybridized to a susceptible cultivar, Early Jalapeno, to generate the RIL population. The host differential characterized 17 isolates of *P. capsici* into 13 races. The establishment of a stable host differential for the *P. capsici* and *C. annuum* interaction will assist researchers in understanding the complex inheritance of resistance to *Phytophthora* root rot and to develop resistant cultivars.

Additional keywords: chile pepper, single seed descent, host-pathogen interaction.

The oomycete *Phytophthora capsici* Leon. is the causative agent of *Phytophthora* root rot, stem blight, foliar blight, and fruit rot in chile pepper (*Capsicum annuum* L.) (16,20). *P. capsici* is responsible for devastating losses worldwide (13). Currently, no truly resistant cultivar is available to aid in chile production. Nevertheless, sources of resistance have been identified. Among these, the *C. annuum* accession Criollo de Morelos-334 (CM-334) has the highest level of resistance against all known isolates of *P. capsici* (11).

Breeding for *Phytophthora*-resistant chile pepper is difficult and complex, especially with the existence of physiological races (11). Oelke et al. (11) identified nine races for *Phytophthora* root rot and four races for *Phytophthora* foliar blight in chile pepper based on a differential reaction of selected *C. annuum* cultivars. This study was one of the very first attempts to characterize races of *P. capsici*.

Within *P. capsici*, both A1 and A2 mating types exist, and have been found in the same field in New Mexico (8). This increases the probability of sexual recombination leading to the emergence of new races. To assist with the understanding of the inheritance of resistance in chile pepper to *P. capsici*, an efficient and established system to identify races would be beneficial.

A set of host differentials is comprised of lines or cultivars of a host that have one or more resistance genes to a pathogen. Using a set of recombinant inbred lines (RILs) to achieve this goal has the advantages of combining the maximum genetic variability within the population (equal representation of genotypes) with homozygous genotypes that can be replicated permanently without risk of segregation.

The *Phytophthora infestans* ([Mont.] de Bary) and potato (*Solanum tuberosum* L.) system represents a successful example of host differentials generated from a highly resistant accession *Solanum demissum* Lindl. The "Black's differentials" developed

in 1953 are still being used successfully to characterize new races of *P. infestans* (3,7). Other examples where race-specific resistance has been characterized using RILs exist in soybean (*Glycine max* [L.] Merr.), sunflower (*Helianthus annuus* L.), and chickpea (*Cicer arietinum* L.) (1,17,18,21). The objective of this study was to develop a set of RILs that could be used as a host differential to characterize race differentiation of *P. capsici* causing root rot on chile pepper.

MATERIALS AND METHODS

Plant materials. From hybridization between the resistant accession Criollo de Morelos-334 (CM-334) and the susceptible cultivar, Early Jalapeno, a set of recombinant inbred lines (RILs) was developed. The accession, CM-334 is a landrace from Mexico that has the broadest level of resistance found in any *C. annuum* accession. In fact, there is not a known isolate of *P. capsici* that causes root rot on it. The single seed descent procedure was used to obtain the RILs (4). The F₁ generation was selfed to generate an F₂ population, and for each subsequent generation a single seed was harvested from a single self-pollinated plant and sown. This process was repeated until the F₇ generation with the development of 76 New Mexico RILs (NMRILs). This study randomly selected 26 NMRILs out of the 76 NMRILs for disease screening. The accession, CM-334, served as the resistant control, whereas a highly susceptible *C. annuum* accession NMCA 10399 served as the susceptible control.

Seedlings were grown in planting trays composed of 72 cells divided into 18 four-celled containers (TOD 1804, T.O. Plastics, Clearwater, MN). Cells were filled with a commercially prepared peat moss-vermiculite soil mixture (Sun Gro Redi-earth plug and seedling mix, Sun Gro Horticulture, WA). Seeds were sown and after germination, each cell was thinned to the two healthiest plants.

Isolates and inoculum preparation. A total of 17 isolates (Table 1) collected mostly from New Mexico were used to inoculate the seedlings, following the inoculation procedure of Bosland and Lindsey (2). Each isolate was originally maintained on water

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agar plates for long-term storage in 100 × 20 mm polystyrene petri dishes. To prepare the inoculum, the water agar was cut into 0.5 cm diameter plugs and transferred to V8 agar medium (14). The V8 plates were then placed in an incubator at 28°C. After 8 days, the medium was cut into 2 × 2 cm² pieces and placed in 150 × 15 mm petri plates, partially filled with distilled water. After 3 days, the plates were removed from the incubator, and underwent a temperature-shock to induce the release of zoospores, i.e., plates were put in a refrigerator at 10°C for 1 h and then placed at 24°C for at least 30 min. The liquid containing the zoospores was collected and the concentration of live zoospores was determined by counting in a Newbauer chamber under 10× magnification. The inoculum was diluted to obtain a final zoospore concentration of 2,000 zoospores/ml.

Root screening and scoring. Each isolate was tested on 72 plants per NMRIL with a total of three replications of eight plants each randomized within a tray, and the experiment was repeated twice. Seedlings were inoculated at the four- to six-true-leaf stage as described by Bosland and Lindsey (2). The planting trays were placed into water to saturate the root zone. Each plant cell received 5 ml of the prepared inoculum, giving a final concentration of 10,000 zoospores per plant cell. The water-saturated root zone condition was maintained for 48 h before the plant trays were removed, allowed to drain, and placed on a greenhouse bench. Propagation pads on the greenhouse benches ensured a constant soil temperature of 28°C was maintained.

Approximately 10 days after inoculation, when the susceptible check exhibited extreme root rot symptoms, plants were scored for resistance or susceptibility. Plants with no lesions in the root area were considered resistant, whereas plants with symptoms ranging from very small lesions to death were considered suscep-

tible (Fig. 1). Once a plant begins to show even a small lesion, it will eventually die.

Experimental design. Each four-celled pack represented one NMRIL, and within a tray each NMRIL was replicated three times and randomly distributed in the tray. Randomly placed in each tray was a four-celled pack each of the resistant check, CM-334, and the susceptible check, NMCA 10399. All experiments were repeated twice.

Statistical design. The data were analyzed using SAS system (14). To decide whether or not the data could be pooled, a chi-square (χ^2) test of homogeneity was performed over the three repetitions. The data were pooled if the χ^2 test was significant ($P > 0.05$). To determine if an NMRIL was resistant or susceptible, a chi-square (χ^2) test of homogeneity was performed testing each to the resistant control (CM-334) and the susceptible control (NMCA 10399). The NMRILs were considered resistant or susceptible if the χ^2 test was significant ($P > 0.05$) for a specific comparison.

Mating type determination. All isolates were individually paired with a known A1 and A2 isolate to determine mating types. The isolates were placed 3 cm apart on clarified V8 media. Plates were incubated at 25°C for 3 to 5 days and then checked for oospore formation.

RESULTS

From the 17 isolates studied, 16 were virulent on the susceptible control NMCA 10399, only PWB 84 was unable to cause disease on NMCA 10399. Three isolates (PWB 83, 87, and 96) were unable to infect any of the NMRILs, but did cause root rot symptoms on NMCA 10399 (data not shown). The resistant control, CM-334, did not show any root rot symptoms when challenged by any of the 17 isolates.

The 13 isolates that caused disease symptoms on the NMRILs showed different levels of virulence. For instance, isolates PWB 80 and PWB 99 were the most virulent, as they caused disease on more NMRILs than any other isolates, 19 and 20, respectively. On the other hand, isolate PWB 101 was the least virulent as it caused disease on only 5 NMRILs, less than any other isolate (Table 2). Congruously, NMRIL C, NMRIL F, NMRIL J, NMRIL P, and NMRIL Q were the most resistant, whereas NMRIL D, AA, AB, and AC were the least resistant (Table 2). From the 26 NMRILs that were challenged with the *Phytophthora* isolates, eight were sufficient to show a differential reaction as a host differential (Table 3).

DISCUSSION

We report here the development of a unique set of host differentials for the characterization of races of *P. capsici* causing *Phytophthora* root rot in chile pepper. This host differential was

TABLE 1. Origin of isolates of *Phytophthora capsici* isolated from *Capsicum annuum*

Isolates	Acquisition date	Origin
PWB 24	1988	New Mexico
PWB 66	2000	New Mexico
PWB 80	2000	Netherlands
PWB 83	2003	New Mexico
PWB 84	1988	New Mexico
PWB 85	2004	New Mexico
PWB 86	1988	New Mexico
PWB 87	1988	New Mexico
PWB 90	1988	New Mexico
PWB 91	1988	New Mexico
PWB 94	2004	New Mexico
PWB 96	1992	New Mexico
PWB 97	1992	New Mexico
PWB 98	1992	New Mexico
PWB 99	2002	New Mexico
PWB 101	2003	New Mexico
PWB 103	2003	California

Fig. 1. Range of susceptible *Phytophthora* root rot symptoms.

based on recombinant inbred lines (RILs) that were established by hybridizing the resistant accession ‘Criollo de Morelos-334’ to the susceptible jalapeno cultivar, Early Jalapeno. From an initial pool of 26 RILs, eight were selected as the New Mexico RILs (NMRILs), which enabled field isolates of *P. capsici* to be assigned to 13 races. The successful development of the NMRILs provides a valuable tool for researchers to investigate the genetics of the *P. capsici*-*Capsicum annuum* interaction in more detail than has previously been possible. *P. capsici* is a heterothallic organism, and mating type analysis found the presence of both A1 and A2 types within the races studied (Table 3), confirming previous findings that both A1 and A2 genotypes exist in New Mexico, and

even within the same field (8). Thus, the likelihood of new race development through sexual recombination is high. The NMRILs clearly provide a system for characterizing the race of any unknown isolate, through the unique pattern of reactions across the eight differentials. The NMRILs are available, from the Chile Pepper Institute, hotchile@nmsu.edu, to bonafide researchers.

The value of defining pathogen races is clear from other host-pathogen systems, i.e., in soybean (*Glycine max* [L.] Merr.), sunflower (*Helianthus annuus* L.), and chickpea (*Cicer arietinum* L.) (1,17,18,21). Previous attempts to define races in *P. capsici* using cultivars have not been effective (11,17). Thus, the NMRILs will contribute to defining the distribution and type of virulence

TABLE 2. Phenotypic response of the 26 New Mexico recombinant inbred lines (NMRIL) tested to the 13 virulent isolates of *Phytophthora capsici*

Accessions	PWB isolates												
	99 ^a	80	91	103	66	85	86	98	24	97	94	90	101
CM-334 ^b	R ^c	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-F ^d	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-J	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-P	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-Q	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-C	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-H	S ^e	R	R	R	R	R	S	R	R	R	S	R	R
NMRIL-Z	S	S	S	R	S	S	R	R	R	R	R	R	R
NMRIL-B	S	S	S	S	S	R	R	R	S	R	R	R	R
NMRIL-A	S	S	R	S	R	R	R	R	R	S	S	S	S
NMRIL-K	R	S	S	S	R	R	S	S	R	S	S	R	R
NMRIL-O	S	S	S	S	R	R	S	S	S	R	R	R	R
NMRIL-X	S	R	S	R	R	S	R	S	R	S	S	R	S
NMRIL-G	S	S	S	S	S	S	S	S	S	R	R	R	R
NMRIL-L	S	S	S	S	S	S	S	R	S	R	S	R	R
NMRIL-T	S	S	S	S	S	S	S	S	R	R	R	R	S
NMRIL-I	S	S	S	S	S	S	S	S	S	S	R	R	R
NMRIL-N	S	S	S	S	S	S	S	S	S	S	R	R	R
NMRIL-R	S	S	S	S	S	S	R	S	S	S	S	R	R
NMRIL-S	S	S	S	S	S	S	S	S	S	S	R	R	R
NMRIL-V	S	S	S	S	S	S	S	S	S	R	S	R	R
NMRIL-E	S	S	S	S	S	S	S	S	S	S	S	R	R
NMRIL-M	S	S	S	S	S	S	S	S	S	R	S	S	R
NMRIL-D	S	S	S	S	S	S	S	S	S	S	R	S	S
NMRIL-AA	S	S	S	S	S	S	S	S	S	S	S	S	R
NMRIL-AB	S	S	S	S	S	S	S	S	S	S	R	S	S
NMRIL-AC	S	S	S	S	S	S	S	S	S	S	S	S	R
NMCA10399 ^f	S	S	S	S	S	S	S	S	S	S	S	S	S

^a PWB isolates: From most virulent isolate (PWB 99) to least virulent (PWB 101).

^b Criollo de Morelos-334 (CM-334): resistant control.

^c Resistant phenotype: no lesions on roots.

^d From most resistant (NMRIL-F) to least resistant (NMRIL-AC).

^e Susceptible phenotype: ranging from small lesions on roots to death of plants.

^f NMCA 10399: susceptible control.

TABLE 3. Race designation of the 13 *Phytophthora capsici* isolates using the New Mexico recombinant inbred line (NMRIL) host differentials

PWB designation	24 ^a	66	80	85	86	90	91	94	97	98	99	101	103
Race designation	1 ^b	2	3	4	5	6	7	8	9	10	11	12	13
Mating types	A2 ^c	A1	A1	A2	A1	A1	A1	A1	A1	A1	A2	A1	A2
Host differentials	Differential reactions												
NMRIL-A ^d	R ^e	R	S	R	R	S	R	S	S	R	S	S	S
NMRIL-B	S ^f	S	S	R	R	R	S	R	R	R	S	R	S
NMRIL-F	R	R	R	R	R	R	R	R	R	R	R	R	R
NMRIL-G	S	S	S	S	S	R	S	R	R	S	S	R	S
NMRIL-H	R	R	R	R	S	R	R	S	R	R	S	R	R
NMRIL-N	S	S	S	S	S	R	S	R	S	S	S	R	S
NMRIL-X	R	S	R	S	R	R	S	S	S	S	S	S	R
NMRIL-Z	R	S	S	S	R	R	S	R	R	R	S	R	R

^a PWB isolates: arranged in numerical order.

^b New race designation: from race 1 to race 13.

^c Mating type determination: A1 and A2.

^d Arranged in alphabetical order.

^e Resistant phenotype: no lesions on roots.

^f Susceptible phenotype: ranging from small lesions on roots to death of plants.

of *P. capsici* in different regions, and how this changes over time, as has been shown with *P. infestans* and *P. parasitica* var. *nicotianae* (5,15). The availability of resistance to different races within the gene pool of the host (9,12), and improving the understanding of the inheritance of race-specific resistance (17,18). Ultimately this greater understanding of the genetics of the *P. capsici*-*C. annuum* interaction will support breeding for effective resistance and the development of durable strategies for exploiting resistance in the field.

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