Evaluation of *Cucurbita pepo* Accessions for Crown Rot Resistance to Isolates of *Phytophthora capsici*

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Abstract. *Phytophthora capsici* causes several disease syndromes on *Cucurbita pepo* L. (squash, pumpkin, and gourd), including crown rot, foliar blight, and fruit rot, which can lead up to 100% crop loss. Currently, there are no *C. pepo* cultivars resistant or tolerant to this pathogen, which can aid in disease management strategies. The objective of this study was to evaluate a select group of *C. pepo* accessions for resistance to the crown rot syndrome of *P. capsici*. One hundred fifteen *C. pepo* accessions, from 24 countries, were evaluated for their disease response to inoculation with a suspension of three highly virulent *P. capsici* isolates from Florida. Replications of each accession, including susceptible controls, were planted in the greenhouse using a randomized complete block design. At the second to third true leaf stage, each seedling was inoculated at their crown with a 5-mL *P. capsici* suspension of 2×10^4 zoospores/mL. Fourteen days after inoculation, the plants were visually rated on a scale ranging from 0 (no symptoms) to 5 (plant death). Mean disease rating scores (DRS) and SDs were calculated for each accession and ranged from 1.3 to 5.0 and 0 to 2.0, respectively. Eight accessions with the lowest mean DRS were rescreened. Results paralleled those of the initial study with one accession, PI 181761, exhibiting the lowest mean DRS at 0.5. Further screening and selection of accessions from the *C. pepo* germplasm collection should aid in the development of breeding lines and cultivars with resistance to crown rot caused by *P. capsici*.

The oomycetous pathogen, *Phytophthora capsici* Leonian, infects a wide range of plant taxa involving more than 49 species (Erwin and Ribeiro, 1996). Oospores, the sexual stage of *P. capsici*, can survive in the soil, in crop debris, and in certain weeds for long periods of time (French-Monar et al., 2006; Hausbeck and Lamour, 2004; Zitter et al., 1996). The asexual zoospores of *P. capsici* contained in sporangia can be dispersed across a field by rain drops and irrigation water in a relatively short period of time. Given optimal conditions, an entire field of crops can be devastated by *P. capsici* in a matter of days (Roberts et al., 2001; Zitter et al., 1996).

The incidence of disease caused by *P. capsici* on cucurbits has increased in

vegetable production regions of the United States with reported yield loss as high as 100% (Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). The increased occurrence and severity of *P. capsici* has prompted research for fungicide management alternatives and interest in breeding cucurbits for resistance or tolerance (Babadoost, 2000; French-Monar et al., 2005; Hausbeck and Lamour, 2004; Keinath, 2007; McGrath, 2004; Seebold and Horten, 2003; Stevenson et al., 2000, 2001; Tian and Babadoost, 2004; Waldenmaier, 2004).

Cucurbita pepo L. (pumpkin, squash, gourd) is an economically important group of the Cucurbitaceae (Paris et al., 2003). Eight cultivar groups of edible-fruited domesticates of *C. pepo* have been described (Paris, 1986), which includes pumpkin, cocozelle, vegetable marrow, zucchini, acorn, scallop, crookneck, and straightneck. *Phytophthora capsici* can infect *C. pepo* at any growth stage and is capable of causing crown rot, foliar blight, and fruit rot (Roberts et al., 2001; Zitter et al.,

1996). Crown rot appears at the soil line causing stems to turn dark brown, become water-soaked, and quickly collapse causing plant death. Foliar symptoms appear as rapidly expanding, water-soaked lesions. Dieback of shoot tips, wilting, shoot rot, and plant death quickly follows initial infection. Fruit, which can be infected at any stage of maturity, may exhibit sunken, brown, water-soaked areas, which are rapidly covered by white sporangial growth under moist environmental conditions. Currently, there are no *C. pepo* cultivars resistant or tolerant to *P. capsici* (Hausbeck and Lamour, 2004).

Germplasm collections are valuable sources of beneficial genes, including resistance or tolerance to numerous plant pathogens. The *C. pepo* germplasm collection maintained at the USDA-ARS North Central Regional Plant Introduction Station (NCRPIS), Ames, IA, has more than 900 *C. pepo* accessions available for evaluation. Although *P. capsici* causes several disease syndromes on *C. pepo*, the objective of this study was to evaluate a select group of *C. pepo* accessions for resistance to the crown rot syndrome of *P. capsici*.

Materials and Methods

Plant material. Because no core collection representing *C. pepo* has been established to date, accessions selected for this study were based on two criteria, fruit type and geographic location. Based on NCRPIS descriptors, accessions with oblong yellow fruit were chosen. In addition, randomly chosen representatives from each geographic location of the collection with at least two accessions were selected. Overall, the 115 accessions selected represented 24 countries. Susceptible controls used in this study were *C. pepo* 'Early Prolific Straightneck' and 'Yellow Summer Squash'.

Phytophthora capsici isolates and inoculum preparation. Three highly virulent *P. capsici* mating type A1 isolates (01-1938A, RJM98-730, and RJM98-805) collected from squash were obtained from Dr. P. Roberts (SWREC, Immokalee, FL). Inoculum was prepared using a modified procedure based on Mitchell et al. (1978), Mitchell (1978), and Mitchell and Kannwischer-Mitchell (1992). For each *P. capsici* isolate, one 5-mm mycelial plug from cornmeal agar was transferred to a 20% clarified V8 agar plate. After 7 d of growth at room temperature, 10 5-mm V8 agar mycelial plugs from each plate were placed into a 20% clarified V8 broth plate to grow for an additional 7 d in a 28 °C incubator. The V8 broth was then drained and each plate was washed two times with sterilized distilled water. Sterilized distilled water was added to cover mycelial growth in all plates, which were then placed under incandescent lights at 28 to 30 °C to induce sporangial development. After 24 h, sporangia were chilled at 4 °C for 45 min to induce zoospore release. The mycelia from each plate were strained through cheesecloth and a 1-ml encysted zoospore sample was

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counted using a hemacytometer. A suspension of the three isolates, containing equal portions of each, was prepared at a concentration of 2×10^4 zoospores/mL.

Greenhouse studies, inoculation, and scoring for response to inoculation. The selected *C. pepo* accessions were evaluated in two separate studies. The first study evaluated accessions based on fruit type (71 accessions). The second study evaluated accessions based on geographic location (44 accessions). For each study, a randomized complete block design was used. Eight blocks containing a single seed of each accession and the two susceptible controls were sown in standard 18-cell flats containing Fafard #3S potting mix (Fafard Inc., Agawam, MA). Not all of the 115 accessions germinated in all eight replications. Greenhouse temperatures were maintained between 19 to 34 °C. Seedlings were watered daily and at the cotyledon stage, each received 1 g of slow-release fertilizer (Osmocote 14N-14P-14K; Grace Sierra Horticulture Products, Milpitas, CA). At the second to third true leaf stage, each seedling was inoculated at its crown with 5 mL of the 2×10^4 zoospores/mL suspension of *P. capsici*. Before inoculation, the potting mix was watered and remained saturated for 24 to 36 h to optimize the zoospore infection process. Fourteen days after inoculation, the plants were visually rated based on a scale ranging from 0 to 5 in which 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to the cotyledons causing constriction at the base, 3 = plant has partially collapsed with apparent wilting of leaves, 4 = plant has completely collapsed with severe wilting present, and 5 = plant death. A mean disease rating score (DRS), calculated as a weighted average, and SDs were calculated for each accession and the susceptible controls. A third test was performed to rescreen accessions from the first two studies exhibiting a mean DRS of less than 2. Eight replications consisting of one plant of each of these accessions and the susceptible control 'Yellow Summer Squash' were planted in a randomized complete block design, inoculated, and visually rated for their response to *P. capsici* as described previously. Mean DRSs and SDs were calculated for each of the rescreened accessions and the susceptible control.

Results and Discussion

Mean DRSs to crown inoculation with *P. capsici* among the 115 accessions ranged from 1.3 to 5 (Table 1). Average standard deviation of DRSs within accessions was 1.1 and ranged from 0 to 2. Thirteen accessions (11.3%) had at least 50% of their replicates with disease ratings of 0 or 1. Eight of these had a mean DRS of less than 2. These eight accessions were chosen for rescreening (Table 2). Results of the rescreen study paralleled those of the initial study in that the mean DRSs among the accessions remained less than 2 and the average SD was

Table 1. Response of *Cucurbita pepo* accessions to a suspension of *Phytophthora capsici* isolates from Florida^a.

Accession or cultivar ^b	Mean DRS (0 to 5 scale)	SD	Percent plants with disease rating ≤ 1	Species ^c	Seed origin
PI 181761	1.3	0.9	75	Cp	Lebanon
PI 615132	1.3	0.5	75	Cp	Mexico
PI 174185	1.4	0.5	63	Cp	Turkey
PI 615142	1.4	0.5	63	Cp	Kazakhstan
PI 169417	1.6	1.6	71	Cp	Turkey
PI 266925	1.8	1.5	75	Cp	Germany
PI 209783	1.9	1.4	50	Cp	Germany
PI 512709	1.9	2.0	75	Cp	Spain
PI 169450	2.1	1.4	38	Cp	Turkey
PI 181944	2.1	1.8	63	Cp	Syria
PI 167053	2.3	1.8	38	Cp	Turkey
PI 169476	2.3	1.9	50	Cp	Turkey
PI 179267	2.3	1.9	50	Cp	Turkey
PI 181878	2.3	1.3	25	Cp	Syria
PI 288240	2.3	1.8	50	Cp	Egypt
PI 299574	2.5	1.9	50	Cp	South Africa
PI 234252	2.6	1.7	38	Cp	Argentina
PI 173684	2.7	1.6	14	Cp	Turkey
PI 136448	2.8	1.6	25	Cp	China
PI 285611	2.8	1.4	0	Cp	Poland
PI 169469	2.9	2.0	43	Cp	Turkey
PI 177377	2.9	1.9	38	Cp	Syria
PI 193501	2.9	1.4	0	Cp	Ethiopia
PI 368592	2.9	1.5	13	Cp	Macedonia
PI 458731	2.9	1.6	25	Cp	Argentina
PI 507885	2.9	1.6	25	Cp	Hungary
PI 163232	3.0	1.9	38	Cp	India
PI 165018	3.0	1.5	25	Cp	Turkey
PI 169448	3.0	1.9	29	Cp	Turkey
PI 222721	3.0	1.7	13	Cp	Iran
PI 311102	3.0	1.2	0	Cp	Guatemala
PI 311741	3.0	1.6	25	Cp	Poland
PI 532355	3.0	1.9	20	Cpf	Mexico
PI 167199	3.1	1.5	13	Cp	Turkey
PI 193502	3.1	1.6	25	Cp	Ethiopia
PI 304061	3.1	1.7	25	Cp	Pakistan
PI 355054	3.1	1.6	13	Cp	Iran
PI 169418	3.2	2.0	33	Cp	Greece
PI 169442	3.2	1.6	0	Cp	Turkey
PI 169443	3.3	1.9	25	Cp	Turkey
PI 257287	3.3	1.3	0	Cp	Spain
PI 234615	3.3	1.7	14	Cp	South Africa
PI 169425	3.4	1.8	25	Cp	Turkey
PI 318826	3.4	1.3	13	Cp	Mexico
PI 169426	3.5	1.7	13	Cp	Turkey
PI 181760	3.5	1.9	25	Cp	Lebanon
PI 274787	3.5	1.2	0	Cp	India
PI 357940	3.5	1.7	13	Cp	Yugoslavia
PI 175705	3.6	1.9	29	Cp	Turkey
PI 169472	3.6	1.9	25	Cp	Turkey
PI 212060	3.6	1.7	13	Cp	Greece
PI 269483	3.6	1.5	13	Cp	Pakistan
PI 364241	3.6	1.9	25	Cp	Hungary
PI 379307	3.6	1.6	13	Cp	Yugoslavia
PI 176964	3.7	1.7	14	Cp	Turkey
PI 183678	3.8	1.8	13	Cp	Turkey
PI 169475	3.8	1.8	20	Cp	Turkey
PI 175704	3.8	1.8	17	Cp	Turkey
PI 532354	3.9	1.5	0	Cpf	Mexico
Ames 26619	3.9	1.2	0	Cpo	United States
PI 169461	3.9	1.6	13	Cp	Turkey
PI 172860	3.9	1.6	13	Cp	Turkey
PI 183232	3.9	1.8	25	Cp	Egypt
PI 385970	3.9	1.6	0	Cp	Kenya
PI 532356	3.9	1.8	13	Cpf	Mexico
PI 93458	3.9	1.5	14	Cp	China
PI 135394	4.0	1.3	0	Cp	Afghanistan
PI 169429	4.0	1.9	14	Cp	Turkey
PI 169453	4.0	1.5	0	Cp	Turkey
PI 357929	4.0	1.4	13	Cp	Macedonia
Ames 26871	4.1	1.6	13	Cpo	United States
PI 169477	4.1	1.2	0	Cp	Turkey

(Continued on next page)

Table 1. (Continued) Response of *Cucurbita pepo* accessions to a suspension of *Phytophthora capsici* isolates from Florida².

Accession or cultivar ³	Mean DRS (0 to 5 scale)	SD	Percent plants with disease rating ≤ 1	Species [*]	Seed origin
PI 614683	4.1	1.6	13	Cpf	Mexico
PI 167042	4.3	1.4	0	Cp	Turkey
PI 171631	4.3	1.5	13	Cp	Turkey
PI 181763	4.3	1.2	0	Cp	Lebanon
PI 234616	4.3	1.3	0	Cp	South Africa
PI 169435	4.4	1.0	0	Cp	Turkey
PI 169445	4.5	1.0	0	Cp	Turkey
PI 169458	4.5	0.9	0	Cp	Turkey
PI 169478	4.5	0.9	0	Cp	Turkey
PI 181758	4.5	1.1	0	Cp	Lebanon
Ames 26873	4.8	0.7	0	Cpo	United States
Ames 26882	4.8	0.7	0	Cpo	United States
PI 167084	4.8	0.7	0	Cp	Turkey
PI 172868	4.8	0.7	0	Cp	Turkey
PI 274336	4.8	0.7	0	Cp	Guatemala
Ames 26608	4.9	0.4	0	Cpo	United States
Ames 26622	4.9	0.4	0	Cpo	United States
PI 135398	5.0	0.0	0	Cp	Afghanistan
PI 379314	5.0	0.0	0	Cp	Macedonia
PI 615141	5.0	0.0	0	Cp	Kazakhstan
Ames 26607	5.0	0.0	0	Cpo	United States
Ames 26609	5.0	0.0	0	Cpo	United States
Ames 26610	5.0	0.0	0	Cpo	United States
Ames 26612	5.0	0.0	0	Cpo	United States
Ames 26616	5.0	0.0	0	Cpo	United States
Ames 26617	5.0	0.0	0	Cpo	United States
Ames 26620	5.0	0.0	0	Cpo	United States
Ames 26624	5.0	0.0	0	Cpo	United States
Ames 26833	5.0	0.0	0	Cpo	United States
Ames 26872	5.0	0.0	0	Cpo	United States
Ames 26875	5.0	0.0	0	Cpo	United States
Ames 26876	5.0	0.0	0	Cpo	United States
Ames 26877	5.0	0.0	0	Cpo	United States
Ames 26879	5.0	0.0	0	Cpo	United States
Ames 26883	5.0	0.0	0	Cpo	United States
Ames 26884	5.0	0.0	0	Cpo	United States
Ames 26885	5.0	0.0	0	Cpo	United States
Ames 26887	5.0	0.0	0	Cpo	United States
Ames 26888	5.0	0.0	0	Cpo	United States
Ames 26890	5.0	0.0	0	Cpo	United States
Ames 26891	5.0	0.0	0	Cpt	United States
Ames 26892	5.0	0.0	0	Cpt	United States
Ames 26893	5.0	0.0	0	Cpt	United States
EPS	5.0	0.0	0	Cp	United States
YSS	5.0	0.0	0	Cp	United States

²Accessions are ranked according to their mean disease rating score (DRS).

³Susceptible *C. pepo* controls EPS, 'Early Prolific Straightneck' and YSS, 'Yellow Summer Squash'.

^{*}Cp = *Cucurbita pepo*; Cpf = *Cucurbita pepo* subsp. *fraterna*; Cpo = *Cucurbita pepo* var. *ozarkana*; Cpt = *Cucurbita pepo* var. *texana*.

Table 2. Response of eight selected accessions of *Cucurbita pepo* to a suspension of *Phytophthora capsici* isolates from Florida.

Accession or cultivar ^a	Disease rating scale (DRS) ^y						Mean DRS	SD
	Number of plants within each category							
	0	1	2	3	4	5		
PI 181761	4	4	0	0	0	0	0.5	0.5
PI 615132	4	1	0	2	1	0	1.4	1.7
PI 174185	2	3	3	0	0	0	1.1	0.8
PI 615142	1	2	4	1	0	0	1.3	0.9
PI 169417	5	0	1	1	0	1	1.3	1.9
PI 266925	2	4	0	1	0	1	1.5	1.7
PI 209783	3	1	2	0	0	2	1.9	2.1
PI 512709	2	0	5	0	0	1	1.9	1.6
YSS	0	0	0	0	0	8	5.0	0.0

²Susceptible *C. pepo* control YSS, 'Yellow Summer Squash'.

³Disease rating based on a scale ranging from 0 (no symptoms) to 5 (plant death).

1.4. PI 181761 exhibited the lowest mean DRS at 0.5 with all plants in this accession rated as either 0 or 1. These findings suggest that accessions within the *C. pepo* collection

are worth considering as potential sources of resistance to *P. capsici*.

In this study, all accessions collected in the United States were susceptible to the

suspension of *P. capsici* isolates from Florida. Based on the origin of the eight accessions chosen for rescreening, four were obtained from Germany and Turkey. Evaluating additional accessions from Asia, Europe, and Mexico in the future might be worthwhile.

The findings from these tests suggest that heterogeneity for resistance to *P. capsici* crown rot may be present within the *C. pepo* accessions evaluated. Many cucurbit accessions are maintained by open or sib pollination; therefore, heterogeneity for a particular trait among its individuals may exist. Screening and continuous selection of individuals originating from cucurbit accessions can lead to breeding lines homogeneous for particular trait(s). This approach was used to develop the melon race one powdery mildew [*Podosphaera xanthii* (syn. *Sphaerotheca fuliginea* auct. p.p.)] resistant watermelon (*Citrullus lanatus* var. *lanatus*) line, PI 525088-PMR (Davis et al., 2006).

Phytophthora capsici can cause disease on all plant tissue of susceptible hosts. Disease on each of these tissues can be considered a separate disease syndrome, i.e., crown rot, root rot, foliar blight, and fruit rot. Different genetic mechanisms may be responsible for host resistance to the various syndromes. This is the case with root rot and foliar blight resistance in pepper (*Capsicum annuum* var. *annuum*) (Walker and Bosland, 1999). A similar situation exists in the host-pathogen interaction of *P. infestans* and potato (*Solanum tuberosum* L.). Different genes are responsible for the resistances in tubers, vines, and foliage of the potato plant (Budin et al., 1978; Howard, 1978).

Physiological races within the *P. capsici*-*C. annuum* interaction have been identified (Glosier et al., 2008; Oelke et al., 2003). Pathogen races are important in pepper breeding as cultivars resistant to *P. capsici* isolates found in specific growing regions continue to be developed. Although we have tentatively identified resistance to isolates of *P. capsici* from Florida in *C. pepo*, additional studies will be performed to evaluate the *Phytophthora* crown rot-resistant lines against isolates from around the world. If physiological races within the *P. capsici*-*C. pepo* interaction are identified, it will play an important role in breeding for *Phytophthora* resistance of the edible-fruited domesticates of *C. pepo*.

Results from this study indicate that there is potential resistance to *P. capsici* crown rot within *C. pepo* accessions. Through screening and selection, the development of *C. pepo* lines homozygous for *P. capsici* resistance will allow us to study the inheritance of resistance, evaluate the *P. capsici*-*C. pepo* interaction, and create *Phytophthora* crown rot resistant cultivars to aid in disease management of this pathogen. Further studies are also necessary to evaluate the *Phytophthora* crown rot-resistant *C. pepo* breeding lines developed from this study for their response to *Phytophthora* foliar blight and fruit rot.

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