Resistance to *Phytophthora* Fruit Rot of Watermelon Caused by *Phytophthora capsici* in U.S. Plant Introductions

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Abstract. *Phytophthora* fruit rot, caused by *Phytophthora capsici*, is prevalent in most watermelon-producing regions of southeastern United States and is known to cause pre- and post-harvest yield losses. A non-wound inoculation technique was developed to evaluate detached mature fruit belonging to U.S. watermelon PIs for resistance to fruit rot caused by *P. capsici*. Mature fruit were harvested and placed on wire shelves in a walk-in humid chamber [greater than 95% relative humidity (RH), temperature $26 \pm 2^\circ\text{C}$] and inoculated with a 7-mm agar plug from an actively growing colony of *P. capsici*. Twenty-four PIs that exhibited resistance in a preliminary evaluation of 205 PIs belonging to the watermelon core collection in 2009 were grown in the field and greenhouse in 2010 and 2011 and evaluated in the walk-in humid chamber. Fruit rot development was rapid on fruit of susceptible controls ‘Black Diamond’, ‘Sugar Baby’, and PI 536464. Several accessions including PI 560020, PI 306782, PI 186489, and PI 595203 (all *Citrullus lanatus* var. *lanatus*) were highly resistant to fruit rot. One *C. colocynthis* (PI 388770) and a *C. lanatus* var. *citroides* PI (PI 189225) also showed fruit rot resistance. Fruit from PIs that were resistant also had significantly lower amounts of *P. capsici* DNA/gram of fruit tissue compared with the susceptible commercial cultivars Sugar Baby and Black Diamond. The sources of resistance to *Phytophthora* fruit rot identified in this study may prove useful in watermelon breeding programs aimed at enhancing disease resistance.

Phytophthora capsici has been documented as a pathogen on a wide variety of vegetable crops in the families Solanaceae (tomato, pepper, eggplant), Cucurbitaceae (cucumber, watermelon, squash, pumpkin, melon), Fabaceae (lima bean) (Babadoost and Zitter, 2009; Erwin and Riberio, 1996; Gubler and Davis, 1996; Hausbeck and Lamour, 2004), frasier fir (Quesada-Ocampo et al., 2009), and plants belonging to 23 other families (Granke et al., 2012b). The pathogen has also been reported to have caused severe

losses in cucurbit crop production since the 1940s resulting in cessation of production in severely infested fields (Babadoost, 2004; Babadoost and Zitter, 2009; Hausbeck and Lamour, 2004; Kreutzer et al., 1940).

Phytophthora fruit rot of watermelons caused by *P. capsici* was first reported in 1940 (Wiant and Tucker, 1940) and is prevalent in many watermelon-growing regions of the United States (Gevens et al., 2008; Hausbeck et al., 2012; Kousik et al., 2011a; McGrath, 1996). The disease is particularly severe in the southeastern United States where $\approx 50\%$ of the watermelons are grown (Florida, Georgia, Alabama, South Carolina, North Carolina, and Virginia) and optimal conditions for development of *Phytophthora* fruit rot are prevalent. Between 2003 and 2008 many watermelon growers in Georgia, South Carolina, and North Carolina did not harvest their crop as a result of severe fruit rot. In some instances fruits rotted after shipping, resulting in rejection of entire loads and loss of revenue (Jester and Holmes, 2003; Kousik et al., 2011a). Such instances led the watermelon growers’ consortium, National Watermelon Association, to list *Phytophthora* fruit rot as one of their most important research priorities (Morrissey, 2006).

The current recommended strategy for managing *P. capsici* includes a combination of several control methods, cultural practices

that ensure well-drained soils, crop rotation, soil solarization, reducing splash dispersed soil, and application of fungicides (Babadoost, 2004; Granke et al., 2012b; Hausbeck and Lamour, 2004; Kousik et al., 2011a; McGrath, 1996). Several commercial fungicides that are effective in managing *Phytophthora* fruit rot of watermelon have been identified (Kousik et al., 2011a, 2011b). However, the prevalence of *P. capsici* isolates insensitive to fungicides such as cyazofamid and mefenoxam also has been well documented (Granke et al., 2012a, 2012b; Hausbeck and Lamour, 2004; Kousik and Keinath, 2008; Jackson et al., 2012). Furthermore, the application of fungicides is not very effective when disease pressure is high (Granke et al., 2012b; Kousik et al., 2011a; McGrath, 1994). Therefore, alternative strategies such as host resistance for managing *Phytophthora* fruit rot are needed.

Host plant resistance can be considered the cornerstone of an integrated disease management program, and watermelon cultivars with resistance to *P. capsici* would be extremely useful in management of *Phytophthora* fruit rot. Host resistance to *P. capsici* in peppers was first reported by Kimble and Grogan (1960) and additional sources of resistance were subsequently reported by Barksdale et al. (1984) and Peter et al. (1984). Recently, commercial bell pepper hybrids with resistance to *P. capsici* have become available (Candole et al., 2010; Foster and Hausbeck, 2010). However, there are few reports on resistance of cucurbit crops to *P. capsici*. Resistance to crown rot caused by *P. capsici* was reported in a Korean pumpkin cultivar (Lee et al., 2001) and squash (*Cucurbita pepo*) accessions (Padley et al., 2008). Gevens et al. (2006) identified cucumber varieties that limit development of *P. capsici*; however, none of the varieties tested had complete fruit rot resistance. Resistance in cucumber was related to the developmental stage of the fruit, increasing with increasing age and size of the fruit (Ando et al., 2009; Gevens et al., 2006). Ando et al. (2009) also noted that there have been no studies to identify sources of resistance to *P. capsici* in watermelon or melon.

A majority of the evaluations to identify sources of resistance to *P. capsici* in pepper, tomato, and some cucurbits has been performed on seedlings. Over four months are usually needed to grow watermelon fruit for evaluations and because of larger fruit, more resources are required to complete the evaluations. Furthermore, resistance at the seedling stage is generally not correlated with resistance in the fruit (Gevens et al., 2006). In pepper, it was reported that resistance is controlled by different genes in the leaves and the roots (Sy et al., 2005; Walker and Bosland, 1999). In cucumbers, the plants are generally resistant to *P. capsici*; however, the fruit are very susceptible and it is possible that more than one mechanism of resistance may be involved (Gevens et al., 2006). We evaluated seedlings of over 1800 U.S. watermelon PIs for resistance to crown rot (Kousik et al., unpublished data) caused by *P. capsici*

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and 205 PIs for resistance to fruit rot (Kousik, 2011) and there appears to be no clear correlation between resistance in fruit and resistance in seedling to the same pathogen.

Because fruit rot is a major problem in watermelon, identifying sources of resistance that can be useful for developing resistant cultivars is important. In 2009, we conducted a preliminary unreplicated evaluation of 205 PIs in the core collection of U.S. watermelon PI available with the Plant Genetic Resources and Conservation unit (PGRCU), Griffin, GA (<<http://www.ars-grin.gov>>) and reported the results as an abstract (Kousik, 2011). The present studies were conducted to identify and confirm resistance in watermelon PI to *Phytophthora* fruit rot. In addition, we quantified the development of *P. capsici* in inoculated fruit tissue using real-time quantitative polymerase chain reaction to further confirm resistance.

Materials and Methods

Seeds of U.S. watermelon PIs. Seeds of U.S. watermelon PIs belonging to the core collection were obtained from PGRCU, Griffin, GA. The details of the various PIs evaluated in this study are available on the germplasm resources information network (GRIN) web site (<<http://www.ars-grin.gov/cgi-bin/npgs/html/desc.pl?151021>>). Seeds of commercial watermelon cultivars Black Diamond, Sugar Baby, Mickey Lee, and Charleston Gray used as controls were purchased from Willhite seeds (Willhite Seeds, Pooleville, TX).

***Phytophthora capsici* isolate.** *Phytophthora capsici* isolate RCZ-11, which was highly aggressive on watermelon fruit and caused severe rot, was used as the inoculum. The isolate was collected in 2003 from zucchini (*Cucurbita pepo*) plants in South Carolina and belongs to mating type A2. The isolate was kindly provided by Dr. A.P. Keinath, Clemson University. The isolate was routinely maintained on V8 juice agar amended with antibiotics (PARP) as described by others (Keinath, 2007; Quesada-Ocampo et al., 2009). The isolate also was routinely inoculated and then re-isolated from watermelon fruit to maintain its virulence.

Evaluation of watermelon core collection for resistance. The first experiment was conducted in the Summer of 2009 to identify potential sources of resistance among the watermelon PIs. Five plants each of 205 PIs from the watermelon core collection were seeded in 50-cell Jiffy trays (Jiffy Products of America, Norwalk, OH) filled with Metro Mix (Sun Gro Horticulture, Bellevue, WA) and allowed to germinate and grow in a greenhouse for 4 weeks. Four-week-old plants were transplanted onto 96-cm wide raised beds covered with white plastic mulch. Beds were spaced 6.4 m apart. Each PI plot was a single row of five plants spaced 46 cm apart with 2.7-m spacing between plots and was not replicated. Plants of susceptible commercial cultivars Mickey Lee and Black Diamond were used as controls. Irrigation was provided by drip tape placed ≈ 2.5 cm below the soil

surface along the center of the raised beds. After bedding but before planting, row middles were sprayed with glyphosate at $1.1 \text{ kg}\cdot\text{ha}^{-1}$ and Strategy [ethalfuralin ($0.45 \text{ kg}\cdot\text{ha}^{-1}$) + clomazone ($0.14 \text{ kg}\cdot\text{ha}^{-1}$)] for weed management. Weeds between beds were controlled during the season with spot application of glyphosate. Vines of the watermelon plants were turned every week so as to keep the plants from growing into neighboring plots. Standard watermelon production practices with respect to irrigation and weed management were followed (Kemble, 2010; Sanders, 2006). Fruit were harvested when mature as determined by the drying of tendril on the node bearing the fruit. Because the fruit from different PIs matured at different times in this trial, we included fruit from the susceptible cultivars in every evaluation. An average of five fruit were marked and harvested from each plot for inoculations.

Inoculation and fruit rot assessment. To handle the large number of fruit of varying sizes to be evaluated, a walk-in humidity chamber ($4 \times 3 \times 3.7$ m height) with wire shelves was used. Fruit harvested from each plot were numbered and then randomly placed on the wire shelves. Fruit were surface-disinfested with 10% sodium hypochlorite (Gevens et al., 2006; Granke et al., 2012a) before inoculation. Each fruit was inoculated by placing a 7-mm agar plug from a 4-d-old actively growing isolate of *P. capsici* in the middle (Fig. 1). The agar plug was placed on top of the fruit such that the mycelium and sporangia touched the fruit surface without injuring the fruit. After inoculation, high RH (greater than 95%) was maintained in the room using a humidifier (Charley's portable fogger, Model M6253; Charley's Greenhouse, Mt. Vernon, WA). The temperature in the room was maintained at $26 \pm 2^\circ\text{C}$. The room was continuously illuminated with fluorescent light. Five days after inoculation, the lesion diameter (Fig. 1) on each fruit was measured in two directions perpendicular to each other

as described before by others (Gevens et al., 2006; Granke et al., 2012a). The agar plug was considered the center of the lesion. Similarly, the diameter of the area with visible pathogen growth and sporulation was measured (sporulation diameter). The intensity of sporulation within the lesion was recorded on a 0 to 5 scale, where 0 = no visible sporulation; 1 = very sparse sporulation, few seen next to the agar plug; 2 = some sporulation and covering less than $\frac{1}{2}$ the lesion area and not very dense; 3 = medium sporulation covering $\frac{1}{2}$ the lesion area; 4 = heavy sporulation covering $\frac{3}{4}$ of the lesion area; and 5 = abundant sporangia, very dense and covering most (greater than 85%) of the lesion area. The length and width of each fruit was also recorded to determine the area of each fruit covered by lesion (%) because fruit size varied greatly among the PI.

Evaluation of selected watermelon PIs grown in the field. The first evaluation of the most resistant PI was conducted in the summer of 2010. Twenty-four PIs from the experiment conducted in 2009 that had less than 1.0 sporulation intensity (Table 1) were included in this trial. Two susceptible PIs identified in the 2009 trial were also included in this field experiment. The experiment was arranged in a randomized complete block design with two replications for each PI. Only two replications were used because of limited availability of seeds. Four-week-old seedlings of select PI were grown in 50-cell jiffy trays as described previously and transplanted on 4 May onto 96-cm wide raised beds covered with white plastic mulch as described before. Each PI plot was a single row of five plants spaced 46 cm apart with 2.7-m spacing between plots. Plants of susceptible commercial cultivars Mickey Lee, Black Diamond, and Sugar Baby were used as controls. Field plots were managed as described previously. Five fruits were marked (given plot numbers) and harvested from each plot for inoculations as described previously.

Fig. 1. *Phytophthora* fruit rot development on watermelon fruit of a susceptible PI. Agar plug from an actively growing colony of *Phytophthora capsici* was placed on top of the fruit as the inoculum (center). Large lesion (solid arrow, brown areas) covered with pathogen growth and sporulation (dashed arrow, whitish growth) is observed on the fruit surface. The diameter of the lesion and diameter of the area within the lesion covered with pathogen growth and sporulation (sporulation diameter) and intensity of sporulation were measured.

Table 1. Phytophthora fruit rot development on detached watermelon fruit in a preliminary evaluation of the watermelon core collection of U.S. PIs and controls grown in a field in Summer 2009, Charleston, SC.^z

PI ^y	<i>Citrullus</i> species ^x	Country where collected ^w	Fruit area covered (%) ^v	Lesion diam (cm) ^u	Sporulation diam (cm) ^t	Sporulation intensity (0–5) ^s
Black Diamond	CL	US	52.47	10.63	8.25	5.00
PI 169274	CLL	Turkey	60.47	13.00	9.25	5.00
PI 435991	CLL	China	66.24	10.40	8.40	5.00
PI 536464	CLL	Maldives	94.44	8.50	8.00	5.00
Mickey Lee	CL	US	41.65	7.71	3.94	2.06
PI 172804	CLL	Turkey	41.18	7.00	1.00	1.00
PI 418762	CLL	Afghanistan	20.00	4.00	2.00	1.00
PI 535948	CLL	Cameroon	15.24	2.67	1.67	1.00
PI 560002	CLL	Nigeria	15.13	2.30	1.20	0.70
PI 186489	CLL	Nigeria	8.29	1.19	0.62	0.54
PI 595203	CLL	US	14.25	2.10	1.12	0.41
PI 189317	CLL	Nigeria	8.65	1.29	0.43	0.29
PI 457916	CLL	Liberia	10.84	1.38	0.38	0.25
PI 560020	CLL	Nigeria	13.31	1.95	0.50	0.20
PI 494527	CLL	Nigeria	6.41	0.83	0.33	0.17
PI 532723	CLL	Zaire	6.65	1.00	0.25	0.17
PI 388770	CO	Morocco	15.79	1.24	0.13	0.13
PI 306782	CLL	Nigeria	3.81	0.53	0.07	0.07
PI 185635	CLL	Ghana	4.85	0.69	0.00	0.00
PI 189225	CLC	Zaire	0.00	0.00	0.00	0.00
PI 249008	CLL	Nigeria	4.90	0.63	0.00	0.00
PI 482283	CLC	Zimbabwe	0.00	0.00	0.00	0.00
PI 494530	CLL	Nigeria	0.00	0.00	0.00	0.00

^zData from PIs that were potential sources of resistance along with controls and most susceptible PI are presented.

^yDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^xCL = *Citrullus lanatus*; CLL = *C. lanatus* var. *lanatus*; CLC = *C. lanatus* var. *citroides*; CO = *C. colocynthis*.

^wCountry where the PI was originally collected.

^vFruit area covered is based on mean of length and width of fruit covered by the mean lesion diameter.

^uLesion diameter is the mean of two readings taken perpendicular to each other with the 7-mm agar plug being the center. The data were recorded 5 d after inoculation of the fruits. Only the accessions for which more than four fruits were evaluated in this preliminary trial are presented.

^tSporulation diameter is the diameter of the area within the lesion where visible pathogen growth and sporulation occurred on the fruit surface.

^sSporulation intensity was the amount of sporulation within the lesion based on a 0 to 5 scale.

Five d after inoculation, data on the lesion diameter, sporulation diameter, and sporulation intensity were recorded.

A second trial was conducted in Fall 2010 with 10 of the most resistant and two highly susceptible PIs. The commercial cultivars, Black Diamond, Sugar Baby, and Mickey Lee, were used as susceptible controls. The experimental design was a randomized complete block with four replications for each PI or the controls. Transplants were grown in the greenhouse and transplanted on 6 Aug. 2010. Mature fruit were harvested and inoculated on 22 Sept. 2010 and rated 5 d later as previously described.

In Summer 2011, eight of the most resistant, two highly susceptible PIs, and the controls ‘Sugar Baby’ and ‘Black Diamond’ were seeded in Jiffy trays on 12 May and then seedlings were transplanted to the field on 7 June 2011. The experimental design, similar to the second test in 2010, was a randomized complete block with four replications per plot. Mature fruit were harvested, inoculated, and rated as described previously.

Evaluation of selected PIs grown in the greenhouse. In Spring 2010, 24 PIs selected on the basis of the 2009 evaluation of the core collection were grown in a greenhouse in Charleston, SC. The greenhouse was maintained at 25 ± 2 °C. Plants were grown in 50-cell Jiffy trays and then transplanted to 11-L (3-gallon) plastic pots. Five plants of

each PI were grown. One plant was grown in each pot and was considered as a replication. Each plant was hand-pollinated to produce a self-pollinated fruit. Mature fruit were harvested and placed randomly on shelves in the walk-in humid chamber as described before. Fruit were inoculated and rated for disease development as described previously. After fruit rot ratings were completed, seeds were extracted from the fruit for use in 2011 greenhouse studies. Similar to 2010 evaluations, eight resistant PIs, two susceptible PIs, and the controls ‘Sugar Baby’ and ‘Black Diamond’ were grown and evaluated in the Spring and Fall of 2011.

Assessing *P. capsici* development in fruit tissue using quantitative polymerase chain reaction. After completing data recording, fruit tissue (10 mm length × 7 mm diameter) from highly resistant and susceptible PIs were collected from either side of the agar plug using a 7-mm cork borer for the fruit collected from the field in Summer 2010. Samples were collected 7 mm away from the agar plug. Similarly, fruit tissue samples were also collected from the field and greenhouse experiments conducted in 2011. The two fruit tissue samples from either side of the agar plug from each fruit were pooled together in one 2-mL microcentrifuge tube. Approximately 0.8 g of fresh tissue of each sample was lyophilized in a 2-mL microcentrifuge tube and ground using glass beads in

a FastPrep-24 sample preparation system (M.P. Biomedicals, Irvine, CA). DNA was extracted using techniques described by Lamour and Finley (2006) in 96-well plates. Total DNA extracted from each of the samples was determined using the Qubit fluorometer and Quant-it DNA assay kit (Invitrogen Corporation, Carlsbad, CA), as per the manufacturer’s instructions. Real-time quantitative polymerase chain reaction (qPCR) assay to quantify the amount of pathogen DNA in the fruit tissue was conducted using *P. capsici*-specific β-tubulin-based primers designed using *P. capsici*-specific sequences described by Donahoo and Lamour (2008) and primers described by Kousik et al. (2012). The primer sequences were PCBT-F: TAA CTG CCG CGT GTA TGT TC and PCBT-R: GCT CAT CTT CAG ACC CTT GG. Real-time qPCR was conducted using a SYBR green-based assay in a Roche Diagnostics LightCycler 480 (Roche Diagnostics, Indianapolis, IN) following the manufacturer’s protocol (Roche Diagnostics LightCycler 480 Version 1.5.0). The reaction mixture (10 µL) consisted of 2.5 µL of DNA extract and 7.5 µL LightCycler 480 SYBR green master mix and 0.7 µM of each primer. The thermal cycling conditions were as follows: initial denaturation at 95 °C for 5 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, 72 °C for 30 s, and a final extension step at 72 °C for 5 min. Each sample was run in duplicate to validate the reproducibility of the qPCR method. A standard curve was constructed by plotting known concentrations (0.0001 to 10 ng) of *P. capsici* DNA against the Ct values obtained from real-time PCR using software provided with the LightCycler 480 system. The amplification reactions had a high efficiency ($R^2 = 0.99$). After every qPCR run, we also examined the melting curve analysis of all the samples at the end of PCR runs to confirm that only one amplicon was being quantified. The quantity of *P. capsici* DNA present in unknown samples was calculated using the standard curve and the software provided with the LightCycler480.

Statistical analysis. All fruit rot data from the field and greenhouse experiments were analyzed using the PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC) and means were separated using Fisher’s protected least significant difference (LSD) ($\alpha = 0.05$). Correlations between the fruit rot parameters that were measured (lesion diameter, sporulation diameter, and sporulation intensity) were conducted using the PROC CORR procedure of SAS. Data on *P. capsici* DNA (ng·g⁻¹ fruit tissue) were log-transformed and then analyzed using the PROC GLM procedure of SAS. Transformed means were separated using Fisher’s protected LSD ($\alpha = 0.05$); however, actual means are presented in the tables.

Results

Phytophthora fruit rot lesion development was observed on the susceptible checks 24 h after inoculation after which the lesion rapidly

expanded for the next 3 to 4 d in all the experiments. The preliminary evaluation of 205 PIs helped identify potential sources of resistance in the watermelon core collection available with GRIN. Of the 205 PIs evaluated, the majority were highly susceptible and extensive sporulation was observed on most fruit. Overall we identified and considered 25 PIs (12%) with sporulation intensity less than 1.0 as potential sources of resistance. Twenty-two (12%) of the 159 *Citrullus lanatus* var. *lanatus* PIs we evaluated, one *C. colocynthis* (PI 388770), and two *C. lanatus* var. *citroides* PI (PI 189225) showed varying levels of resistance. Details of the reaction of some of the most resistant and susceptible PIs for which four to five fruit were evaluated is presented in Table 1. A complete list of the reactions of the 205 PIs can be obtained from the author and will also be entered into the GRIN database.

Heavy pathogen sporulation on the lesions was observed on the susceptible genotypes in 2010 (Table 2). Significant differences ($P \leq 0.0001$) in the response of the various PIs to *Phytophthora* fruit rot was observed with respect to all the disease parameters measured (lesion diameter, percent fruit area covered by lesion, sporulation diameter, and

sporulation intensity) in the first field experiment conducted in Summer 2010. Highly significant correlations ($r \geq 0.91$, $P < 0.0001$) among all the disease parameters measured were observed in this trial. Because of such highly significant correlations, we have presented data on lesion diameter and sporulation intensity in Table 2, leaving out data on sporulation diameter. Significant disease development was observed on the susceptible commercial cultivars, Sugar Baby and Black Diamond, indicating that the fruit rot test had worked effectively (Table 2). PI 435991 was the most susceptible with respect to fruit area covered by lesion and heavy sporulation intensity. Several *C. lanatus* var. *lanatus* PIs had significantly smaller lesions and reduced sporulation compared with the susceptible checks. These included PI 457916, PI 595203, 560020, and 560002. Similarly, PI 388770 (*Citrullus colocynthis*) and PI 189225 (*C. lanatus* var. *citroides*) were also significantly more resistant to fruit rot compared with the susceptible cultivars.

The 24 PIs used in the field study in Summer 2010 also were grown in the greenhouse and evaluated in 2010. Results between the first 2010 field and greenhouse evaluations were similar. Significant differences

($P \leq 0.0001$) among the PIs with respect to lesion development were observed. Significant correlations ($r > 0.90$, $P = 0.0001$) among the various fruit rot parameters (fruit area covered by lesion, lesion diameter, sporulation diameter, and sporulation intensity) measured were observed. The sporulation intensity on 10 of the PIs was less than 1.0 on the 0 to 5 scale and these PIs were significantly more resistant to fruit rot compared with the susceptible controls (Table 2). PI 306782, PI 494527, PI 186489, PI 560020, and PI 560002 collected in Nigeria and PI 595203 from the United States were observed to be highly resistant in this 2010 greenhouse trial.

Highly significant correlations ($r > 0.90$, $P < 0.0001$) were observed among the fruit rot parameters measured in the second field trial conducted in Fall 2010. Fruit of 'Sugar Baby', PI 435991, 'Mickey Lee', and 'Black Diamond' had severe rot (Table 3). Disease ratings for several *C. lanatus* var. *lanatus* PIs from Africa, including PI 560002, PI 494527, PI 457916, PI 186489, and PI 560020, were significantly lower than the susceptible controls. PI 189225 (*C. lanatus* var. *citroides*) was also resistant as observed in the first 2010 experiment.

Table 2. *Phytophthora* fruit rot development on detached watermelon fruit of U.S. PIs and controls grown in a field in Summer 2010 and in a greenhouse in 2010, Charleston, SC.

PI ^z	<i>Citrullus</i> species ^y	Country where collected	Field, Summer 2010			Greenhouse 2010		
			Fruit area covered (%)	Lesion diam (cm) ^x	Sporulation intensity (0–5) ^w	Fruit area covered (%)	Lesion diam (cm)	Sporulation intensity (0–5)
PI 435991	CLL	China	82	13.0	5.00	68	12.5	4.5
Sugar Baby	CL	Canada	76	11.8	4.70	—	—	—
PI 538888	CLL	Russia	— ^v	—	—	73	10.7	4.7
PI 512367	CLL	Spain	69	13.0	4.63	70	13.1	4.6
PI 212983	CLL	India	68	10.3	5.00	65	12.0	4.4
PI 482283	CLC	Zimbabwe	65	7.0	2.67	15	1.8	0.5
PI 379237	CLL	Yugoslavia	62	10.7	3.70	66	11.5	4.5
PI 536464	CLL	Maldives	—	—	—	61	11.5	4.7
PI 169274	CLL	Turkey	60	11.6	3.97	77	12.7	4.6
Mickey Lee	CL	US	55	9.1	3.55	76	13.1	4.3
PI 176916	CLL	Turkey	54	9.9	3.80	69	10.9	4.0
PI 186489	CLL	Nigeria	53	7.5	2.77	7	1.0	0.3
PI 306782	CLL	Nigeria	51	7.2	2.96	2	0.3	0.0
Black Diamond	CL	US	49	9.9	5.00	42	9.9	3.8
PI 306367	CLL	Angola	49	10.1	3.84	39	6.9	2.0
PI 482291	CLL	Zimbabwe	42	9.1	3.90	38	7.6	2.7
PI 378612	CLL	Zaire	40	8.6	4.00	47	10.9	3.3
PI 532723	CLL	Zaire	36	5.5	2.07	24	3.4	1.2
PI 185635	CLL	Ghana	35	5.0	1.42	19	3.6	1.3
PI 189317	CLL	Nigeria	35	6.5	2.29	56	9.3	3.8
PI 494530	CLL	Nigeria	28	3.0	0.95	12	2.4	0.9
PI 494527	CLL	Nigeria	20	3.0	0.95	7	0.9	0.3
PI 249008	CLL	Nigeria	19	3.0	1.04	12	1.9	0.8
PI 189225	CLC	Zaire	14	2.0	0.77	17	2.3	0.4
PI 560002	CLL	Nigeria	13	1.9	0.57	8	1.2	0.0
PI 595203	CLL	US	12	1.9	0.19	7	1.3	0.2
PI 560020	CLL	Nigeria	9	1.4	0.43	8	1.3	0.0
PI 388770	CO	Morocco	5	0.8	0.25	58	9.9	2.3
PI 457916	CLL	Liberia	5	1.0	0.15	—	—	—
LSD _{0.05}			22.9	3.5	1.46	20.6	3.2	1.3

^zDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^yCL = *Citrullus lanatus*; CLL = *C. lanatus* var. *lanatus*; CLC = *C. lanatus* var. *citroides*; CO = *C. colocynthis*.

^xLesion diameter is the mean of two readings taken perpendicular to each other with the 7-mm agar plug being the center. The data were recorded 5 d after inoculation of the fruits. Fruit area covered is based on mean of length and width of fruit covered by the mean lesion diameter. Data on sporulation diameter are not presented in Table 2 because all the disease parameters were highly correlated ($r \geq 0.91$, $P < 0.0001$).

^wSporulation intensity was the amount of sporulation within the lesion based on a 0 to 5 scale.

^vNot tested.

LSD = least significant difference.

The field experiment in Summer 2011 was conducted on eight PIs that were most resistant in the 2010 trials. The susceptible controls were severely rotted and significant differences ($P \leq 0.0001$) were observed among the reaction of the PI to fruit rot. These eight PIs were significantly more resistant than the susceptible controls PI 536464, 'Black Diamond', and 'Sugar Baby' (Table 4). Minimal to no sporulation was observed on the inoculated fruit of these PIs (PI 560020,

PI 457916, PI 494530, PI 595203, PI 186489, PI 306782, PI 560002, and PI 185635).

Greenhouse evaluations in the spring and fall of 2011 confirmed the resistance in the selected PIs. Like in previous evaluations, highly significant differences between the controls and the select resistant PIs were observed. The controls PI 536464 and 'Sugar Baby' had severe fruit rot with large lesions and heavy sporulation (Table 5). The resistant PIs (PI 306782, PI 560020, PI 595203,

PI 186489, PI 185635, and PI 560002) had very minimal to no sporulation.

Significantly lower amounts of *P. capsici* DNA were detected in fruit tissue of PI 388770, PI 560020, PI 457916, PI 595203, and PI 560002 compared with the susceptible controls 'Sugar Baby' and 'Black Diamond' (Table 6) in the Summer 2010 field trial. The susceptible PI had greater than 2400 ng of pathogen DNA per gram of fruit tissue compared with less than 713 ng·g⁻¹ of fruit tissue in the resistant PIs. Highly significant correlations between the amount of pathogen DNA in the fruit tissue and the fruit rot parameters, lesion diameter ($r = 0.82$, $P \leq 0.0001$), sporulation diameter ($r = 0.79$, $P \leq 0.0001$), and sporulation intensity ($r = 0.72$, $P = 0.0003$) were observed. Similar results were observed on the fruit tissue samples collected from the field-grown watermelon in Summer 2011 (Table 6).

'Black Diamond' had significantly more pathogen DNA (12,855 ng·g⁻¹ fruit tissue) followed by the other susceptible checks: PI 435991 (6,882 ng·g⁻¹), 'Sugar Baby' (3,601 ng·g⁻¹), and PI 536464 (3,337 ng·g⁻¹) compared with the resistant PIs in the greenhouse experiment in 2011. PI 185635 (296 ng·g⁻¹), PI 560002 (258 ng·g⁻¹), PI 494527 (181 ng·g⁻¹), PI 560020 (41 ng·g⁻¹), and PI 186489 (15 ng·g⁻¹) all had significantly ($P = 0.05$) lower amounts of pathogen DNA in the fruit tissue confirming their resistance to fruit rot. No *P. capsici* DNA was detected in PI 306782 and PI 595203 in this greenhouse experiment. Significant correlations ($r = 0.73$, $P \leq 0.0001$) between the levels of pathogen DNA present in fruit tissues and the visual ratings of fruit rot parameters were observed.

Discussion

An effective technique to evaluate detached watermelon fruit for resistance to Phytophthora fruit rot was developed. This technique should be useful for investigating inheritance of resistance to fruit rot, where a large number of fruit will need to be evaluated. In a preliminary study in 2008 we evaluated the watermelon core collection in the field. However, because of environmental conditions, fruit rot development in susceptible controls was not adequate to confidently assess resistance. Hence, we developed the detached fruit assay to evaluate watermelon fruit for resistance. A similar detached fruit assay was developed to evaluate cucumber for resistance to Phytophthora fruit rot (Gevens et al., 2006). Mature watermelon fruit were used in the current study because in commercial fields, fruit rot is usually observed when fruits are nearing harvest. Furthermore, fruit rot is also an important post-harvest problem in watermelon (Jester and Holmes, 2003; Kousik et al., 2011a). Mature watermelon fruit vary greatly in size among the PIs (especially during evaluation of the core collection), from very large to small fruit, and hence we used several different measurements to assess fruit rot severity. Therefore, we also calculated the

Table 3. Phytophthora fruit rot development on detached watermelon fruit of U.S. PIs and controls grown in a second field experiment, Charleston, SC, Fall 2010.

PI ^z	<i>Citrullus</i> species ^y	Country where collected ^x	Fruit area covered (%) ^w	Lesion diam (cm) ^v	Sporulation diam (cm) ^u	Sporulation intensity (0–5) ^t
PI 435991	CLL	China	99	14.2	12.7	4.56
PI 212983	CLL	India	97	13.2	13.3	5.00
Mickey Lee	CL	U.S.A	95	12.4	11.3	4.41
Sugar Baby	CL	Canada	94	12.9	11.6	5.00
Black Diamond	CL	US	69	8.1	7.8	3.75
PI 595203	CLL	US	61	8.7	4.5	1.84
PI 388770	CO	Morocco	53	3.7	3.4	2.48
PI 494530	CLL	Nigeria	36	5.1	2.3	0.76
PI 457916	CLL	Liberia	30	3.5	1.8	0.91
PI 189225	CLC	Zaire	24	2.8	2.5	0.63
PI 560020	CLL	Nigeria	22	2.8	1.2	0.40
PI 306782	CLL	Nigeria	18	2.2	0.8	0.33
PI 186489	CLL	Nigeria	15	1.8	0.9	0.45
PI 494527	CLL	Nigeria	15	1.9	0.4	0.21
PI 560002	CLL	Nigeria	15	1.8	1.1	0.40
LSD _{0.05}			28.4	3.5	3.3	1.26

^zDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^yCL = *Citrullus lanatus*; CLL = *C. lanatus* var. *lanatus*; CLC = *C. lanatus* var. *citroides*; CO = *C. colocynthis*.

^xCountry where the PI was originally collected.

^wFruit area covered is based on mean of length and width of fruit covered by the mean lesion diameter. Because many alphabets are involved in the mean separation that would clutter up the table, LSD_{0.05} values are provided to make specific comparisons.

^vLesion diameter is the mean of two readings taken perpendicular to each other with the 7-mm agar plug being the center. The data were recorded 5 d after inoculation of the fruits.

^uSporulation diameter is the diameter of the area within the lesion where pathogen visible pathogen growth and sporulation occurred on the fruit surface.

^tSporulation intensity was the amount of sporulation within the lesion based on a 0 to 5 scale.

LSD = least significant difference.

Table 4. Phytophthora fruit rot development on detached watermelon fruit of U.S. PIs and controls grown in a field, Charleston, SC, Summer 2011.

PI ^z	<i>Citrullus</i> species ^y	Country where collected ^x	Fruit area covered (%) ^w	Lesion diam (cm) ^v	Sporulation diam (cm) ^u	Sporulation intensity (0–5) ^t
PI 536464	CLL	India	95	12.2	12.2	5.00
Sugar Baby	CL	Canada	73	10.1	8.2	4.40
Black Diamond	CL	US	49	9.6	8.9	3.40
PI 435991	CLL	China	46	8.2	7.4	3.80
PI 185635	CLC	Ghana	11	1.6	0.9	0.50
PI 560002	CLL	Nigeria	10	1.3	0.2	0.10
PI 306782	CLL	Nigeria	9	1.3	0.5	0.30
PI 186489	CLL	Nigeria	9	1.3	0.4	0.20
PI 595203	CLL	US	5	0.7	0.0	0.00
PI 494530	CLL	Nigeria	3	0.5	0.1	0.10
PI 457916	CLL	Liberia	3	0.4	0.0	0.00
PI 560020	CLL	Nigeria	3	0.4	0.0	0.00
LSD _{0.05}			15.4	3.2	3.6	1.08

^zDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^yCL = *Citrullus lanatus*; CLL = *C. lanatus* var. *lanatus*; CLC = *C. lanatus* var. *citroides*; CO = *C. colocynthis*.

^xCountry where the PI was originally collected.

^wFruit area covered is based on mean of length and width of fruit covered by the mean lesion diameter. Because many alphabets are involved in the mean separation that would clutter up the table, LSD_{0.05} values are provided to make specific comparisons.

^vLesion diameter is the mean of two readings taken perpendicular to each other with the 7-mm agar plug being the center. The data were recorded 5 d after inoculation of the fruits.

^uSporulation length was the diameter of the area within the lesion where sporulation occurred on the fruit.

^tSporulation intensity was the amount of sporulation within the lesion based on a 0 to 5 scale.

LSD = least significant difference.

Table 5. Phytophthora fruit rot development on detached watermelon fruit of U.S. PIs and controls grown in a greenhouse in the Spring and Fall of 2011.

PI ^z	Fruit area covered ^y		Lesion diam ^x		Sporulation intensity ^w	
	Spring	Fall	Spring	Fall	Spring	Fall
	(%)	(%)	(cm)	(cm)	(0–5)	(0–5)
Sugar Baby	86	82	13.8	13.0	5.00	5.00
PI 536464	77	85	12.9	13.4	4.90	4.90
Black Diamond	52	68	10.2	13.5	4.00	5.00
PI 435991	51	76	8.6	13.1	3.60	4.60
PI 494530	27	14	3.0	1.7	0.96	0.13
PI 494527	15	15	1.8	1.5	1.01	0.35
PI 560002	14	18	1.8	1.9	0.80	1.08
PI 185635	11	16	1.1	1.9	0.00	0.40
PI 186489	8	10	1.0	1.3	0.21	0.00
PI 595203	8	6	1.1	0.7	0.33	0.00
PI 560020	7	9	0.8	1.1	0.25	0.40
PI 306782	4	7	0.4	0.8	0.06	0.00
LSD _{0.05}	15.7	13.1	1.5	1.5	1.08	0.08

^zDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^yFruit area covered (%) is based on mean of length and width of fruit covered by the mean lesion diameter. Because many alphabets are involved in the mean separation that would clutter up the table, least significant difference_{0.05} values are provided to make specific comparisons.

^xLesion diameter is the mean of two reading taken perpendicular to each other with the 7-mm agar plug being the center. The data were recorded 5 d after inoculation of the fruits. Data on sporulation diameter are not presented in Table 5 because all the disease parameters were highly correlated.

^wSporulation intensity was the amount of sporulation within the lesion based on a 0 to 5 scale.

LSD = least significant difference.

Table 6. *Phytophthora capsici* DNA detected in field-grown inoculated fruit tissue of select U.S. PIs in Summer 2010 and 2011 using real-time quantitative polymerase chain reaction.

PI ^z	<i>Citrullus</i> species ^y	Country where collected ^x	<i>P. capsici</i> DNA/gram fruit tissue ^w	
			2010	2011
			ng	ng
PI 435991	CLL	China	5618 a ^v	2025 bc
Sugar Baby	CL	Canada	2833 a	1715 bc
Black Diamond	CL	US	2425 a	3992 a
PI 536464	CLL	India	—	2622 bc
PI 212983	CLL	India	2411 a	—
PI 189225	CLC	Zaire	1737 ab	—
PI 186489	CLL	Nigeria	—	1385 cd
PI 560002	CLL	Nigeria	713 b	474 e
PI 595203	CLL	US	521 b	342 e
PI 457916	CLL	Liberia	352 bc	290 e
PI 560020	CLL	Nigeria	289 bc	618 de
PI 388770	CO	Morocco	30 c	—
PI 306782	CLL	Nigeria	—	201 e

^zDetails of the PIs can be obtained from the GRIN web site (<<http://www.ars-grin.gov>>).

^yCL = *Citrullus lanatus*; CLL = *C. lanatus* var. *lanatus*; CLC = *C. lanatus* var. *citroides*; CO = *C. colocynthis*.

^xCountry where the PI was originally collected.

^wThe amount of *P. capsici* DNA/gram of fruit tissue data were log transformed for analysis. Actual means are presented in the table.

^vMeans followed by the same letter in a column are not significantly different ($\alpha = 0.05$).

approximate surface area covered by the lesion to compare the various PIs. Significant correlations were observed among the fruit rot parameters measured, indicating that use of lesion diameter and sporulation intensity may be sufficient to assess resistance. The inoculum plug was placed directly on the fruit without injuring the fruit surface because injuring the surface may break down some of the natural defense mechanisms. We did not cover the inoculum plug with eppendorf tubes and petroleum jelly (Gevens et al., 2006; Granke et al., 2012a) to prevent drying of the agar plugs; instead, we increased the RH (greater than 95%) in the walk-in humid chamber to prevent drying of the agar plugs. This fruit rot evaluation method developed to screen PIs for resistance has also been

useful in evaluating post-harvest effectiveness of fungicides against *Phytophthora* fruit rot of watermelon (Kousik et al., 2011b).

In studies with cucumbers it was observed that infection generally occurred on the blossom end in the field, which was found to be more susceptible (Ando et al., 2009). However, we observed no difference in the level of infection when watermelon fruit were inoculated at the peduncle, middle, or the blossom end with respect to lesion diameter or sporulation intensity (Kousik et al., unpublished data) on resistant or susceptible accessions. In addition, it was easier to measure the disease parameters in the middle of the fruit with the advantage that the agar plug did not slide and fall off despite the high RH in the room.

The preliminary evaluation in 2009 identified 24 PIs as potential sources of resistance (Kousik, 2011). However, because of limited availability of seeds and in some instances poor germination, few fruits of some PIs were evaluated. Hence, in 2010, we evaluated all 24 PIs in the greenhouse and field and determined that some PIs (example PI 189317) that were considered as a potential source of resistance after the initial 2009 screen were susceptible and we stopped evaluating them further. The preliminary screen helped identify several resistant PIs that were confirmed as highly resistant by repeated evaluations.

The potential sources of resistance to *Phytophthora* fruit rot including PI 560002, PI 560020, PI 186489, PI 306782, PI 494527, and PI 494530 are all considered egusi-type watermelon and were collected in Nigeria (<<http://www.ars-grin.gov>>). These PIs are also called wild watermelon or egusi melon, because the seeds have a fleshy pericarp and these melons are widely cultivated in Nigeria for the high protein and carbohydrate content in their seeds, which are edible (Gusmini et al., 2004). They can also be easily crossed with commercial cultivated melon for breeding purposes (Gusmini et al., 2004). Many of the PIs with resistance to *Phytophthora* fruit rot were of the egusi type. However, not all egusi-type PIs we evaluated were resistant.

Gevens et al. (2006) identified cucumber varieties that limit development of *P. capsici*; however, none of the varieties tested had complete fruit rot resistance. The resistance in cucumber was related to developmental stage of the fruit and increased age and size of the fruit (Ando et al., 2009; Gevens et al., 2006). However, watermelon fruit of a susceptible cultivar, Crimson Sweet, in the study by Ando et al. (2009) were susceptible at all developmental stages, similar to observations in our preliminary studies on ‘Sugar Baby’ and PI 536464 (Kousik and Ikerd, 2012). However, the resistant fruit were resistant at all developmental stages (Kousik and Ikerd, 2012).

Some of the resistance sources identified in this study have also been identified with resistance to other diseases. For example, PI 595203, resistant to *Phytophthora* fruit rot, is also reported to have resistance to the aphid transmitted *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (Guner, 2004; Strange et al., 2002). In addition, markers linked to ZYMV resistance in PI 595203 have been identified and developed (Harris et al., 2009). Several of the markers are in a resistance gene analog region identified in watermelon (Harris et al., 2009; Ling et al., 2009). It would be interesting to determine if the genes for resistance to *Phytophthora* fruit rot are linked to the same region in PI 595203 as those for resistance to ZYMV. PI 560020 and PI 560002 that were highly resistant to *Phytophthora* fruit rot in this study were reported with moderate levels of resistance to powdery mildew (Davis et al., 2007). It would be interesting to explore the possibility of developing multiple disease-resistant germplasm and varieties using such accessions.

Variability among plants within a PI for their response to fruit rot was observed in this trial. Similar variability within a PI has been observed by others during evaluations of watermelon germplasm for resistance to viral and fungal diseases (Davis et al., 2007; Gillaspie and Wright, 1993; Strange et al., 2002). One of the reasons suggested for this variability is that many of the accessions in the collections were increased by open pollination at some time, thus providing the chance for cross-pollination of some of the accessions (Gillaspie and Wright, 1993; Strange et al., 2002). In addition, these accessions were originally collected in the open from various regions of the world and may have been cross-pollinated before collection.

The potential for the existence of races of *P. capsici* based on their reaction to various pepper and tomato genotypes has been reported (Glosier et al., 2008; Quesada-Ocampo and Hausbeck, 2010; Sy et al., 2005). However, little information is available on resistance of cucurbits to *P. capsici* or the existence of races based on cucurbit cultivars. The *P. capsici* isolate RCZ-11 (mating type A2) isolated from zucchini was used to evaluate for fruit rot resistance because it was highly aggressive and produced abundant visible sporangia and pathogen growth on susceptible watermelon cultivars compared with other isolates (Fig. 1). The most resistant lines identified in this study were also resistant to an aggressive isolate (mating type A1) from watermelon (Kousik, unpublished results). The type of plant from which a *P. capsici* isolate is collected does not define the host specificity of the isolate. *Phytophthora capsici* isolates collected from bean in Michigan were reported to be more aggressive on zucchini fruit than the cucurbit isolates (Quesada-Ocampo et al., 2010). Similarly, a *P. capsici* isolate named 12889, isolated from bell pepper in Michigan, was reported to be more aggressive and caused severe root and crown rot on cucurbits compared with some isolates from similar cucurbit crops (Enzenbacher and Hausbeck, 2012). Granke et al. (2012a) compared the virulence of over 100 isolates on fruit of tomato, cucumber, zucchini, and peppers and also observed that isolates from fabaceous hosts were highly virulent on cucumber and zucchini. Granke et al. (2012a) also suggested that a highly aggressive isolate of *P. capsici* on a host to be evaluated should be used regardless of the host from which it was isolated. It will be interesting to evaluate the resistant PIs against *P. capsici* isolates from different hosts (such as bean) and states of the United States to determine the potential existence of races with respect to resistance in watermelon and to determine the breadth of resistance.

Results on quantification of *P. capsici* in susceptible and resistant fruit tissue suggest that the qPCR system can be used to detect pathogen development in the fruit tissue for confirming resistance. However, it will be essential to visually observe symptom development on the fruit in addition to quantification using real-time PCR before using

such resistance in breeding programs. Significant correlations between the amounts of pathogen DNA detected and visual fruit rot ratings were noticed confirming the effectiveness of the various methods for detecting resistance. In many of the resistant fruit samples, no rot was observed and no pathogen DNA was detected. In most cases, the fruit surface of the resistant accessions was very firm with very minimal to no fungal growth compared with the susceptible lines, which were severely rotted and were very soft. Overall we detected significantly less amounts of *P. capsici* DNA in the resistant fruit tissue.

Several other researchers also have demonstrated the use of qPCR in differentiating between resistant and susceptible genotypes of plants (Kousik et al., 2012; Silvar et al., 2005). Silvar et al. (2005) detected less *Phytophthora* DNA in resistant pepper genotypes compared with the susceptible genotypes. In this study we used β -tubulin sequence-based primers as opposed to ITS sequence-based primers to quantify *P. capsici* DNA. This choice was based on our previous work with these two primer sets where β -tubulin primers performed better than the ITS primers (Kousik et al., 2012). Quantifying the pathogen DNA helped confirm resistance to *Phytophthora* fruit rot in resistant watermelon PIs.

An integrated approach has been recommended by most researchers to manage diseases caused by *P. capsici* on vegetable crops (Granke et al., 2012b; Hausbeck and Lamour, 2004). Therefore, even when watermelon cultivars with resistance to *Phytophthora* fruit rot become available, a combination of appropriate cultural practices, fungicides, and host resistance should be used to prevent the development of races and breakdown of resistance.

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