Inheritance of Resistance to *Phytophthora* Crown Rot in *Cucurbita pepo*

Vincent Njung'e Michael, Yuqing Fu, and Geoffrey Meru¹

Horticultural Sciences Department and Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, FL 33031

Additional index words. *Phytophthora capsici*, crown rot, breeding, dominant gene, PI 181761

Abstract. *Phytophthora* crown rot, caused by *Phytophthora capsici* Leonian, is a devastating disease in commercial squash (*Cucurbita pepo* L.) production across the United States. Current management practices rely heavily on the use of chemical fungicides, but existence of fungicide-resistant pathogen populations has rendered many chemicals ineffective. Host resistance is the best strategy for managing this disease; however, no commercial cultivars resistant to the pathogen are currently available. Resistance to *Phytophthora* crown rot in PI 181761 (*C. pepo*) is an important genetic resource for squash breeders worldwide; however, the underlying genetic basis of resistance in PI 186761 that would allow designing of sound breeding strategies is currently unknown. The goal of the current study was to determine the inheritance of resistance in breeding line #186761-36P, a resistant selection of PI 181761, using phenotypic data from F₁, F₂, and backcross populations derived from a cross between #181761-36P and a susceptible acorn-type cultivar, Table Queen. The results indicated that resistance in #181761-36P is controlled by three dominant genes (R4, R5, and R6). Introgression of these genes into susceptible cultivar groups of *C. pepo* will provide an important tool in the integrated management of *Phytophthora* crown rot.

Phytophthora capsici Leonian is a major pathogen with a wide host range, including vegetable crops belonging to Solanaceae, Cucurbitaceae, Leguminosae, and Brassicaceae families (Krasnow and Hausbeck, 2015; Lamour et al., 2012). It is an oomycete that overwinters in the soil as sexually produced oospores (Babadoost and Pavon, 2013). These may infect host plants directly, or, in presence of water, develop sporangia that release swimming zoospores to cause infection. *P. capsici* is the causal agent of foliar blight, root rot, fruit rot, and crown rot disease syndromes in cucurbits (Babadoost, 2016). *Phytophthora* crown rot is particularly prevalent in fields prone to flooding, often resulting in total crop loss. Consequently, current integrated management strategies rely heavily on soil water management and chemical treatment of seed, soil, and plants (Sanogo and Ji, 2012); however, *P. capsici* isolates from major *Cucurbita* growing regions, such as Michigan, New York, and Florida, exhibit insensitivity to recommended chemical pesticides (Lamour and Hausbeck, 2000, 2003; Ploetz et al., 2002). Therefore, host resistance in *Cucurbita* crops is important to augment *P. capsici* management practices (Babadoost, 2016; Hausbeck and Lamour, 2004; Sanogo and Ji, 2012).

Cucurbita moschata Duchesne, *Cucurbita maxima* Duchesne, and *Cucurbita pepo* L. are the three main squash species cultivated in the United States, with the latter being the most economically important (Paris, 2008). All commercial *Cucurbita* cultivars are susceptible to *Phytophthora* crown rot (Babadoost and Islam, 2003); however, resistance to *Phytophthora* crown rot was identified in a wild accession of *Cucurbita lundeliana* Bailey, and subsequently transferred into *C. moschata* background (breeding line #394-1-27-12) (Padley et al., 2009). Resistance in #394-1-27-12 is conferred by three independent dominant genes (Padley et al., 2009). Resistance in *C. pepo* was identified in a set of 16 PIs (Padley et al., 2008), which were later found to be genetically similar (genetic distance = 0.31) (Michael et al., 2019). Among these, PIs 181761 and 615132 were the most resistant (disease severity = 1.3 of 5.0) (Padley et al., 2008), but the former was considered ideal for breeding due to close genetic similarity to cultivar groups of subspecies *pepo* and *texana* (Michael et al., 2019). A highly resistant selection of PI 181761 (designated #181761-36P) was developed through several generations of selection. Knowledge of the underlying genetic basis of *Phytophthora* crown rot resistance in breeding line #181761-36P is essential to design sound breeding strategies for introgressing resistance into susceptible, elite cultivars of *C. pepo*, but this information is currently lacking. The objective of this study was to determine the mode of inheritance of *Phytophthora* crown rot resistance in breeding line #181761-36P.

Plant material. Breeding line #181761-36P, which is highly resistant to *Phytophthora* crown rot, was crossed with 'Table Queen' (TQ), a susceptible elite acorn-type winter squash. Controlled pollinations were carried out in the greenhouse to generate F₁, F₂, and backcross populations.

Inoculum preparation. Inoculum for the experiment was prepared from a virulent *P. capsici* isolate #121 (provided by Dr. Pamela Roberts, University of Florida) following the protocol described by Krasnow et al. (2017), with minor modifications. Briefly, a 5-mm cornmeal agar mycelial plug was transferred to 14% V8 agar plates (140 mL V8 juice, 3 g CaCO₃, 16 g agar per liter) and grown under constant fluorescent light at 28 °C. After 7 d, the plates were flooded with cold sterile distilled water (4 °C), and chilled at 4 °C for 30 min before incubation at 21 °C for 1 h to allow synchronous release of zoospores. Zoospores were quantified with a hemocytometer and diluted to 2.0 × 10⁴ zoospores per milliliter.

Phenotyping and statistical analysis. Seeds of parents (n = 12, each), reciprocal F₁ (n = 100), F₂ (n = 200), and reciprocal backcross (n = 60–142) progenies were sown in 4-inch pots containing sterilized Proline C/B growing mix (Jolly Gardener, Quakertown PA) amended with 14N-4.2P-11.6K controlled-release fertilizer (Osmocote; Scotts, Marysville, OH). At the second to third true-leaf stage, the seedlings were inoculated by delivering 5 mL of 2.0 × 10⁴ zoospores per milliliter at the crown of each plant using a pipette. Disease severity was recorded visually every 3 days from 8 d postinoculation (dpi) to 28 dpi on a scale of 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 (Padley et al., 2008) (Fig. 1). Plants having a score of 1 or less at 28 dpi were classified as resistant, whereas those having a score ≥2 were classified as susceptible (Padley et al., 2009). A χ^2 test (McHugh, 2013) was used to compare segregation ratios for each population with hypothetical segregation patterns to determine possible number of resistant genes.

Results and Discussion

Breeding line #181761-36P exhibited high resistance to *Phytophthora* crown rot characterized by only a small water-soaked lesion at the crown that dried out within a few days forming a brown scar, indicating inability of the pathogen to colonize crown tissues (Fig. 2). All #181761-36P plants grew vigorously throughout the duration of the experiment. In contrast, water-soaked lesions on susceptible TQ plants quickly expanded around the crown and progressed

Received for publication 6 Mar. 2019. Accepted for publication 11 Apr. 2019.

This research was partially funded by the National Institute of Food and Agriculture (Hatch project FLA-TRC-005564).

¹Corresponding author. E-mail: gmeru@ufl.edu

Fig. 1. A 0 to 5 rating scale was used in the experiment, in which (A) indicates a score of 0 for an asymptomatic plant, (B) a score of 1 for a plant with a small brown lesion at the base of the crown, (C) a score of 2 for a plant with the lesion progressed to the cotyledons, (D) a score of 3 for a plant that has partially collapsed with apparent wilting of leaves, (E) a score of 4 for a plant with all leaves wilted and stem collapsed, and (F) a score of 5 for a dead plant.

Fig. 2. Response of resistant breeding line #181761-36P and susceptible acorn-type cultivar, Table Queen (TQ), to infection by a virulent isolate of *Phytophthora capsici* (isolate #121).

to the cotyledons, followed by wilting, collapsing, and plant death (Fig. 2). All TQ plants, except one, died within 6 dpi. The surviving plant may have escaped infection due to human error, such as skipped inoculation or pipetting error. Such errors are common in disease screening experiments involving manual inoculation of large plant populations (Sy et al., 2005).

The resistance reaction in reciprocal F_1 progenies (#181761-36P $\times$ TQ and TQ $\times$ #181761-36P) resembled that of #181761-36P except for a few plants that succumbed to the pathogen (Table 1). The F_2 population segregated in a 57:7 [resistant (R): susceptible (S)] ratio, whereas that of backcross to susceptible parent (TQ $\times$ F_1) segregated in 5:3 (R:S) ratio (Table 1). On the other hand, progeny from backcross to resistant parent (#181761-36P $\times$ F_1) exhibited similar reaction to that in #181761-36P, except for two

Table 1. Segregation patterns of reciprocal F_1 , F_2 , and reciprocal backcross (BC) progenies derived from a cross between resistant (R) breeding line #181761-36P and susceptible (S) acorn-type cultivar, Table Queen (TQ), at 28 d post inoculation with a virulent isolate of *Phytophthora capsici* (isolate #121).

Genotype	Total plants		Expected ratios (R:S)	χ^2
	R	S		
181761-36P	12	0	1:0	—
TQ	1	11	0:1	—
F_1 : (#181761-36P $\times$ TQ)	98	2	1:0	0.04 (NS)
F_1 : (TQ $\times$ #181761-36P)	97	3	1:0	0.09 (NS)
F_2	179	21	57:7	0.04 (NS)
BC: (#181761-36P $\times$ F_1)	58	2	1:0	0.06 (NS)
BC: (TQ $\times$ F_1)	99	43	5:3	3.15 (NS)

NS = χ^2 value not significant ($P \leq 0.05$).

plants that succumbed to the pathogen (Table 1).

Taken together, the segregation patterns support a genetic model in which resistance to *Phytophthora* crown rot in breeding line #181761-36P is conferred by three dominant genes. These genes are designated R4, R5, and R6 to distinguish them from those (R1, R2, and R3) conferring resistance in *C. moschata* (Padley et al., 2009). R4 gene can confer resistance to *Phytophthora* crown rot in homozygous or heterozygous state (R4), independent of R5 and R6. However, R5 and R6 both must be present in the homozygous or heterozygous state (R5_R6_) to confer resistance to the pathogen. Occurrence of a few susceptible individuals in the F_1 and backcross (#181761-36P $\times$ F_1) progenies did not alter segregation ratios significantly ($P \leq 0.05$) (Table 1), and may have resulted from a higher zoospore concentration due to non-homogeneity of pipetted inoculum. A high inoculum density often leads to higher disease incidence (Hammond-Kosack and Jones, 1997; Nelson et al., 2017), even for resistant genotypes (Martyn and McLaughlin, 1983).

A similar inheritance model was reported for *Phytophthora* crown rot resistance in *C. moschata* line #394-1-27-12 (Padley et al., 2009), in which three independent dominant genes (R1R2R3) are required for expression of resistance. Further genetic studies will determine whether

R1R2R3 and R4R5R6 in *C. moschata* and *C. pepo*, respectively, are syntenic. Deployment of R4R5R6 (from #181761-36P) in susceptible cultivar groups of *C. pepo* will provide oligogenic resistance against *Phytophthora* crown rot (Bowers and Mitchell, 1991; Raftoyannis and Dick, 2002), thus providing a critical tool in the integrated management of the disease. Further experiments are required to confirm field resistance to *Phytophthora* crown rot in breeding line #181761-36P.

To circumvent phenotyping challenges associated with traditional breeding for *Phytophthora* crown rot resistance, it is important to identify molecular markers linked to R4R5R6 in breeding line #181761-36P. This will allow efficient discrimination of resistant and susceptible progenies through marker-assisted selection, thus saving breeding resources. Linkage analysis of F_2 and $F_{2:3}$ populations segregating for resistance to *Phytophthora* crown rot is currently under way to identify DNA markers linked to R4R5R6 in breeding line #181761-36P.

Literature Cited

- Babadoost, M. 2016. Oomycete diseases of cucurbits: History, significance, and management, p. 279–314. In: J. Jules (ed.). Horticultural reviews. John Wiley & Sons, Inc., Hoboken, NJ.
- Babadoost, M. and C. Pavon. 2013. Survival of oospores of *Phytophthora capsici* in soil. Plant Dis. 97(11):1478–1483.

- Babadoost, M. and S.Z. Islam. 2003. Fungicide seed treatment effects on seedling damping-off of pumpkin caused by *Phytophthora capsici*. *Plant Dis.* 87:63–68.
- Bowers, J.H. and D.J. Mitchell. 1991. Relationship between inoculum level of *Phytophthora capsici* and mortality of pepper. *Phytopathology* 81(2):178–184.
- Hammond-Kosack, K.E. and J.D.G. Jones. 1997. Plant disease resistant genes. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48(1):575–607.
- Hausbeck, M.K. and K.H. Lamour. 2004. *Phytophthora capsici* on vegetable crops: Research progress and management challenges. *Plant Dis.* 88(12):1292–1303.
- Krasnow, C.S., R. Hammerschmidt, and M.K. Hausbeck. 2017. Characteristics of resistance to *Phytophthora* root and crown rot in *Cucurbita pepo*. *Plant Dis.* 101(5):659–665.
- Krasnow, C.S. and M.K. Hausbeck. 2015. Pathogenicity of *Phytophthora capsici* to brassica vegetable crops and biofumigation cover crops (*Brassica* spp.). *Plant Dis.* 99(12):1721–1726.
- Lamour, K.H. and M.K. Hausbeck. 2000. Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. *Phytopathology* 90(4):396–400.
- Lamour, K.H. and M.K. Hausbeck. 2003. Susceptibility of mefenoxam-treated cucurbits to isolates of *Phytophthora capsici* sensitive and insensitive to mefenoxam. *Plant Dis.* 87(8):920–922.
- Lamour, K.H., R. Stam, J. Jupe, and E. Huteima. 2012. The oomycete broad-host-range pathogen *Phytophthora capsici*. *Mol. Plant Pathol.* 13(4):329–337.
- Martyn, R.D. and R.J. McLaughlin. 1983. Effects of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f. sp. *niveum*. *Plant Dis.* 67:493–495.
- McHugh, M.L. 2013. The chi-square test of independence. *Biochem. Med. (Zagreb)* 23:143–149.
- Michael, V.N., P. Moon, Y. Fu, and G. Meru. 2019. Genetic diversity among accessions of *Cucurbita pepo* resistant to *Phytophthora* crown rot. *HortScience* 54:17–22.
- Nelson, R., T. Wiesner-Hanks, R. Wisser, and P. Balint-Kurti. 2017. Navigating complexity to breed disease-resistant crops. *Nat. Rev. Genet.* 19(1):21–33.
- Padley, L.D., E.A. Kabelka, and P.D. Roberts. 2009. Inheritance of resistance to crown rot caused by *Phytophthora capsici* in *Cucurbita*. *HortScience* 44:211–213.
- Padley, L.D., E.A. Kabelka, P.D. Roberts, and R. French. 2008. Evaluation of *Cucurbita pepo* accessions for crown rot resistance to isolates of *Phytophthora capsici*. *HortScience* 43:1996–1999.
- Paris, H.S. 2008. Summer squash, p. 351–379. In: J. Prohens and F. Nuez (eds.). *Handbook of plant breeding, Vegetables I*. Springer, New York.
- Ploetz, R., G. Heine, J. Haynes, and M. Watson. 2002. An investigation of biological attributes that may contribute to the importance of *Phytophthora capsici* as a vegetable pathogen in Florida. *Ann. Appl. Biol.* 140(1):61–67.
- Raftoyannis, Y. and M.W. Dick. 2002. Effects of inoculum density, plant age and temperature on disease severity caused by pythiaceae fungi on several plants. *Phytoparasitica* 30(1):67–76.
- Sanogo, S. and P. Ji. 2012. Integrated management of *Phytophthora capsici* on solanaceous and cucurbitaceous crops: Current status, gaps in knowledge and research needs. *Can. J. Plant Pathol.* 34(4):479–492.
- Sy, O., P.W. Bosland, and R. Steiner. 2005. Inheritance of *Phytophthora* stem blight resistance as compared to *Phytophthora* root rot and *Phytophthora* foliar blight resistance in *Capsicum annuum* L. *J. Amer. Soc. Hort. Sci.* 130:75–78.