Inheritance of Resistance to Phytophthora Crown Rot in Cucurbita pepo

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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). Introduction 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 zoospores (Babadoost and Islam, 2003); however, resistance to Phytophthora crown rot (Babadoost and Islam, 2003) has been reported in 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.

Materials and Methods

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 inoculation at 21 °C for 1 h to allow synchronous release of zoospores. Zoospores were quantified with a hemocytometer and diluted to 2.0 x 10^3 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 x 10^4 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, with 0 = no symptoms, 1 = small brown lesion at base of stem, 2 = lesion has progressed up to 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 χ² test (McHugh, 2013) was used to compare segregation ratios for each population with hypothesized 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...
The resistance reaction in reciprocal F1 progenies (#181761-36P × TQ and TQ × #181761-36P) resembled that of #181761-36P except for a few plants that succumbed to the pathogen (Table 1). The F2 population segregated in a 57:7 resistant (R): susceptible (S) ratio (Table 1). On the other hand, progeny from backcross to resistant parent (#181761-36P × F1) exhibited similar reaction to that in #181761-36P, except for two plants that succumbed to the pathogen (Table 1).

Taken together, the segregation patterns suggest 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 F1 and backcross (#181761-36P × F1) progenies did not alter segregation ratios significantly (P ≤ 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 F2 and F2:3 populations segregating for resistance to Phytophthora crown rot is currently under way to identify DNA markers linked to R4R5R6 in breeding line #181761-36P.

Table 1. Segregation patterns of reciprocal F1, F2, 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) | χ² |
|----------|--------------|----------------------|-----|
| #181761-36P | 12 | 0 | 1:0 | — |
| TQ | 1 | 1 | 0:1 | — |
| F1: (#181761-36P × TQ) | 98 | 11 | 5:3 | 0.04 (ns) |
| F1: (TQ × #181761-36P) | 97 | 3 | 5:3 | 0.09 (ns) |
| F2 | 179 | 21 | 57:7 | 0.04 (ns) |
| BC: (#181761-36P × F1) | 58 | 2 | 5:3 | 0.06 (ns) |
| BC: (TQ × F1) | 99 | 43 | 5:3 | 3.15 (ns) |

ns = χ² value not significant (P ≥ 0.05).

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.
