**Inheritance of Resistance to Crown Rot Caused by Phytophthora capsici in Cucurbita**

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Additional index words. breeding, Cucurbita lundelliana, Cucurbita okeechobeenesis, cucurbits, squash

**Abstract.** The various disease syndromes caused by Phytophthora capsici Leonian can be devastating to squash (Cucurbita spp.) production areas of the United States. In some growing seasons, yield loss has been reported up to 100%.

A recently developed University of Florida Cucurbita breeding line, #394-1-27-12, resistant to the crown rot syndrome of *P. capsici*, was used to determine the inheritance of resistance to this disease. Data from F$_1$, F$_2$, and backcross progeny from crosses of a *P. capsici*-susceptible butternut-type winter squash (*C. moschata*) with #394-1-27-12 indicated that resistance is conferred by three dominant genes. The introgression of *P. capsici* crown rot resistance from #394-1-27-12 into morphologically diverse domesticates within *Cucurbita* will aid in the management of this economically important pathogen.

The oomycetous pathogen, *Phytophthora capsici* Leonian, is capable of causing several disease syndromes in cucurbits, including crown rot, foliar blight, and fruit rot (Roberts et al., 2001; Zitter et al., 1996). Crown rot appears at the soil line as a dark brown, water-soaked lesion that quickly collapses the stem causing plant death. Foliar blight appears as rapidly expanding, water-soaked lesions on the leaves that eventually causes dieback of shoot tips, wilting, shoot rot, and plant death. Fruit rot appears as sunken, brown, water-soaked areas that are rapidly covered by white sporangial growth under moist environmental conditions.

The incidence of disease caused by *P. capsici* in cucurbit production areas of the United States has increased with reported yield loss as high as 100% (Hausbeck and Lamour, 2004; Tian and Babadoost, 2004). Given optimal environmental conditions, an entire cucurbit field may be destroyed by *P. capsici* in a matter of days (Roberts et al., 2001; Zitter et al., 1996). The increased occurrence of *P. capsici* has prompted research for improved fungicide control programs and an interest in breeding cucurbits for resistance to *P. capsici* (Babadoost, 2000; French-Monar et al., 2005; Hausbeck and Lamour, 2004; Keinath, 2007; McGrath, 2004; Seebold and Horton, 2003; Stevenson et al., 2000, 2001; Tian and Babadoost, 2004; Waldenmaier, 2004).

*Cucurbita* are considered to be one of the most morphologically variable genera in the plant kingdom (Robinson and Decker-Walters, 1999; Whitaker and Robinson, 1986). There are 22 wild and five cultivated species of *Cucurbita*. The cultivated species, grown around the world, include *C. pepo*, *C. moschata*, *C. maxima*, *C. argyrosperma* (formerly *C. mixta*), and *C. ficifolia*. *Cucurbita* cultivars are categorized as summer or winter squash. Summer squash is eaten immature when tender and seeds are small and soft. Winter squash is generally eaten when rind and seeds are fully mature. Summer squash cultivars belong to *C. pepo*, whereas winter squash cultivars may belong to *C. pepo*, *C. maxima*, *C. moschata*, or *C. argyrosperma*.

A search for sources of resistance within *Cucurbita* to the various syndromes of *P. capsici* had been performed at the University of Florida and included representatives from *C. maxima*, *C. moschata*, *C. pepo*, and three wild species, *C. ecuadoriensis*, *C. lundelliana*, and *C. okeechobeenesis* (Babadoost et al., 2007). From this screen, resistance to the crown rot syndrome of *P. capsici* was identified in the wild species, *C. lundelliana*, and *C. okeechobeenesis* subsp. *okeechobeenensis*. This resistance, derived from the two wild *Cucurbita* species, was introgressed through a series of hybridizations, self-pollinations, and single plant selections into a winter squash (*C. moschata*) background. One line, designated #394-1-27-12, was advanced to the F$_2$ generation and is homozygous for *P. capsici* crown rot resistance. The objective of this study was to characterize the inheritance of resistance to crown rot caused by *P. capsici* within the *Cucurbita* breeding line #394-1-27-12.

**Materials and Methods**

**Plant material.** The Cucurbita breeding line #394-1-27-12, resistant to crown rot caused by *P. capsici*, was crossed with ‘Butterbush’ (BB), a butternut-type winter squash (*C. moschata*) highly susceptible to *P. capsici*. Controlled pollinations were carried out in the greenhouse to generate F$_1$ (BB × F$_2$-1-27-12), F$_2$, and reciprocal backcross (BC) progenies. The susceptible control used in all studies was ‘Butterbush’.

*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 (University of Florida, Southwest Research and Education Center, Immokalee, FL). Inoculum was prepared using a modified procedure based on Mitchell et al. (1978), Mitchell (1978), and Mitchell and Kammwischer-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 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.

**Experimental design and data analysis.** Evaluation of #394-1-27-12, ‘Butterbush’, F$_1$, F$_2$, and BC progenies for response to *P. capsici* crown inoculation was performed in greenhouse studies using completely randomized designs. Seed were sown in 15.2-cm azalea plastic pots containing Fafard #3S potting mix (Fafard Inc., Agawam, MA). Seedlings were watered daily and greenhouse temperatures were maintained between 19 and 34 °C. At the cotyledon stage, each seedling received 2 g of slow-release fertilizer (14N–14P–14K; Grace Sier Horticulture Products, Milpitas, CA). The F$_2$ progeny test consisted of 200 individuals plus 10 replicates of both parents. The four BC progeny tests, performed separately, consisted of 100 individuals each of BB × F$_1$ and F$_1$ × BB plus 10 replicates of both parents.
The Cucurbita breeding line #394-1-27-12 exhibited no symptoms of crown rot caused by *P. capsici* under the conditions of this study (Fig. 1; Table 1). Five days postinoculation, all individual plants of the susceptible cultivar Butterbush developed tan–brown water-soaked lesions at their crown that rapidly expanded and caused stem collapse and plant death. The F₁ progeny of the cross between ‘Butterbush’ and #394-1-27-12 reacted similarly to that of the resistant parent, #394-1-27-12, remaining asymptomatic. The F₂ progeny segregated in a 27:37 [resistant (R):susceptible (S)] ratio, whereas backcrosses to the susceptible parent, ‘Butterbush’, segregated in a 1:7 (R:S) ratio. Progeny of backcrosses to the parent #394-1-27-12 were all resistant. Collectively, the segregation ratios support a genetic model in which resistance to the crown rot syndrome caused by *P. capsici* is conferred by three independent dominant genes (R₁R₁R₁).

Efforts to breed for *P. capsici* crown rot resistance in squash will require the introgression of all three dominant genes conferring resistance from #394-1-27-12. Plants will be resistant to crown rot only if the three dominant alleles are present in either a homozygous or heterozygous state (R₁R₁R₁). The identification of molecular markers associated with resistance to *P. capsici* crown rot would facilitate breeding efforts. Using molecular markers, instead of a phenotypic assay, can increase the precision and efficiency of subsequent selection steps applied in plant breeding. Codominant polymerase chain reaction-based molecular markers tightly linked (less than 5 cm) to *P. capsici* crown rot resistance would provide the most benefit allowing distinction between individuals that are homozygous or heterozygous at each of the three loci required for resistance. Molecular analysis of the segregating progeny developed in this study is currently underway to identify markers linked to the three alleles conferring resistance to *P. capsici* crown rot. Because the genes for resistance to *P. capsici* crown rot within #394-1-27-12 may be from either *C. lundelliana* or *C. okeechoboeensis*, or both, molecular analysis may also shed light as to the contributor of this resistance.

In pepper (*Capsicum annuum* L.), it has been shown that resistance to root rot, stem blight, and foliar blight, caused by *P. capsici*,...
Phytophthora capsici is present. Relevant in species. HortScience 213 V

crown rot. Euphytica 162:23–

resistance within P. capsici Cucurbita on vegetable crops: Research
to mefanoxam in southeast Florida. Phyto-

from pumpkin and species,

– Phytophthora, Capsi-
P. capsici P. infestans P. capsici P. capsici Phythium

Cucurbita moschata from South Carolina to

#394-1-27-12 is currently being tested

specific growing regions. The resistance in

#394-1-27-12 are the

same or are different. This determination is

important because either syndrome may

result in severe losses in squash production
when P. capsici is present. Relevant in pepper and potato breeding (Bonde et al.,

1940; Rudorf et al., 1950; Sy et al., 2005), if
different genes or mechanisms are

in conferring resistance to crown rot and

foliar blight in #394-1-27-12, successful

breeding for resistance to P. capsici in squash
will require introgression of all resistance genes involved.

Physiological races of P. capsici have

been identified within the P. capsici-C.

annuum interaction (Glosier et al., 2008;

Oelke et al., 2003). This plays an important
role in developing pepper cultivars with
resistance to P. capsici isolates found in

specific growing regions. The resistance in

#394-1-27-12 is currently being tested
against P. capsici isolates from different

regions of the United States and Europe to

test for specificity. If physiological races

within the P. capsici–Cucurbita interaction
are identified, it will play an important role in

breeding for P. capsici resistance within

Cucurbita.

Different screening methods have been
developed to examine plants for their
response to the various disease syndromes
caused by P. capsici. The greenhouse assay

used in this study allowed for precise

observations of plant response to crown

inoculation and for the determination of

inheritance of resistance to crown rot caused by

P. capsici. This assay provides a

standardized test environment and allows for the

screening of test material using defined P.

capsici inoculum sources. This assay will aid in

the introgression of P. capsici crown rot

resistance from #394-1-27-12 into the

morphologically diverse edible-fruited
decidescates within Cucurbita.

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