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New Sources of Resistance in Winter Squash (*Cucurbita moschata*) to Phytophthora Crown Rot and Their Relationship to Cultivated Squash

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Abstract

Butternut squash (*Cucurbita moschata*) is an important vegetable crop grown and consumed in most states in the United States. *C. moschata* lines and interspecific hybrids between *Cucurbita* species are also used as rootstocks for grafting watermelon and melon. However, currently most commercially available *C. moschata* squash varieties are highly susceptible to crown and root rot caused by the oomycete pathogen *Phytophthora capsici*, especially in the southeastern United States. All available plant introductions (PIs) of *C. moschata* (319 PIs) were evaluated for resistance to crown rot. Four-week-old plants were inoculated with 10^4 zoospores from a local South Carolina isolate of *P. capsici*. Plants were rated for disease severity 3 weeks after inoculation using a 0 to 5 rating scale (0 = no symptoms and 5 = plant dead). The majority (87%) of the *C. moschata* PIs were highly susceptible

to crown rot in the first evaluation and were rated as 5. Reevaluation of the promising PIs identified several potential new sources of resistance (e.g., Grif 935, PI 442272, PI 442264, PI 512142, PI 438724, PI 438778, and PI 442280). Variability in resistance reaction among plants within a PI was also observed, and not all plants exhibited resistance. Further evaluation of S_1 generation from the most resistant plants (rated ≤ 1) demonstrated that highly resistant plants could be selected from these PIs to develop lines for use in breeding programs. These new sources of resistance can be utilized for developing new crown and root rot resistant rootstocks for watermelon grafting and for developing resistant varieties for human consumption.

Keywords: winter squash, *Cucurbita*, *Phytophthora capsici*, crown rot

The oomycete plant pathogen *Phytophthora capsici* causes major diseases that limit production of many vegetable crops in several different plant families throughout the United States (Babadoost 2004; Erwin and Riberio 1996; Granke et al. 2012; Hausbeck and Lamour 2004; Ristaino and Johnston 1999; Sanogo and Ji 2012). Diseases caused by *P. capsici* are particularly severe in the

southeastern United States, where hot and humid conditions favorable for disease development are prevalent during the vegetable growing season year after year. Cucurbits, including pumpkin, squash, watermelon, melon, and cucumbers, are some of the most susceptible vegetable crops to *P. capsici* infection (Babadoost 2004; Donahoo et al. 2013; Gevens et al. 2008; Granke et al. 2012; Hausbeck and Quesada-Ocampo 2017; Kousik et al. 2016, 2018). On cucurbits, *P. capsici* can cause various symptoms including root and crown rot, foliar blight, and fruit rot (Babadoost 2004; Donahoo et al. 2013; Granke et al. 2012; Hausbeck and Lamour 2004; Kousik et al. 2018). In many instances, destruction caused by the oomycete pathogen *P. capsici* has resulted in complete loss of cucurbit crops and abandonment of infested fields for many years (Babadoost 2004; Granke et al. 2012; Kreutzer et al. 1940).

Numerous techniques are recommended for managing diseases caused by *P. capsici* on vegetable crops, and these include crop rotation, avoidance of overhead irrigation, selection of well-drained and pathogen-free fields, use of soil amendments, application of fungicides, and the use of resistant varieties when available. Fungicides are the most commonly used methods on cucurbits for managing *P. capsici* (Babadoost 2004; Granke et al. 2012; Hausbeck and Lamour 2004; Ji et al. 2012; Kousik et al. 2017; Ristaino and Johnston 1999; Sanogo and Ji 2012). Under extreme disease pressure, fungicides do not provide adequate control (Granke et al. 2012; Kousik et al. 2017; McGrath 1994), and therefore host resistance becomes the most reliable component of disease management programs. That said, it has been extremely challenging to develop varieties with resistance

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to *P. capsici* in cucurbit crops. Sources of resistance to diseases caused by *P. capsici* have been identified in many cucurbit crops including squashes, watermelon, and cucumber, and some solanaceous crops like pepper, tomato, and eggplant (Chavez et al. 2011; Foster and Hausbeck 2010; Foster et al. 2013; Kousik et al. 2014, 2018; Krasnow and Hausbeck 2016; LaPlant et al. 2020; Naegele et al. 2014; Padley et al. 2008, 2009; Quesada-Ocampo and Hausbeck 2010; Quesada-Ocampo et al. 2016). A few commercial varieties of pepper with resistance to some isolates of *P. capsici* are available (Barksdale et al. 1984; Foster and Hausbeck 2010; Krasnow et al. 2017). Commercial varieties of other vegetable crops with resistance to *P. capsici* are rare and generally not available.

Because of the ease of cultivation, abundant yields, and plentiful returns, crops in the genus *Cucurbita* (e.g., pumpkin, butternut squash, yellow summer squash, green zucchini squash, etc.) are grown widely in many countries throughout the world. The crops are mainly cultivated for their delicious and nutritious fruit. In some parts of the world the tender shoots are boiled as greens for consumption (Kousik et al. 2015). Zucchini and other summer squash are harvested when the fruits are immature and are boiled or steamed or in some cases eaten raw. Squash harvested at the mature fruit stage are often called winter squash for their storage ability and are a rich source of vitamin A. Among these, butternut squash in particular is an important vegetable crop grown and consumed in most states in the United States. The leading producers of *Cucurbita* spp., particularly squashes and pumpkins, are in Asia, primarily China and India (Kousik et al. 2015).

Apart from their nutritional and edible characteristics, *Cucurbita* species are also used to develop interspecific hybrid rootstocks for grafting watermelon in parts of Asia and more recently in the United States (Keinath and Hassell 2014a; King et al. 2008, 2010; Kousik et al. 2012). *Cucurbita* interspecific rootstock hybrids are generally a cross of *C. moschata* with *Cucurbita maxima*. *Cucurbita* interspecific rootstocks are naturally resistant to Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (Keinath and Hassell 2014 a, b; Kousik et al. 2012) and hence preferred as material for developing rootstocks in Asia. When watermelon grafting was introduced to the United States, most of the rootstocks evaluated were those that were developed in Asia (King et al. 2010). However, most commercially available *Cucurbita* interspecific hybrid rootstocks were highly susceptible to crown rot caused by *P. capsici* (Kousik et al. 2012). As watermelon grafting is becoming popular in the United States, especially in the southeastern regions, rootstocks with resistance to *P. capsici* would be desirable. A few sources of resistance to crown and root rot have been identified among *C. moschata* plant introductions (PIs) by evaluating a limited number of accessions using a *P. capsici* isolate from Florida (Chavez et al. 2011). The objectives of the current study were to phenotype the entire collection of *C. moschata* available from the U.S. PI collection in Griffin, GA, for resistance to crown rot using a local South Carolina isolate of *P. capsici*, with the ultimate goal of identifying new sources for use in rootstock and cultivar breeding programs. In addition, diversity analysis was conducted using available data to determine the genetic distance (relatedness) among resistant PIs identified in this study and cultivated susceptible accessions to aid breeders in selecting appropriate sources for use in their breeding programs.

Materials and Methods

Seeds of *C. moschata*. Seeds from the U.S. *C. moschata* PI collection were requested from the Plant Genetic Resources Conservation Unit, Griffin, GA, U.S.A. (<https://npgsweb.ars-grin.gov/gringlobal>). A total of 319 accessions (PIs) of *C. moschata* were available from the Germplasm Resource Information Network (GRIN) station in Griffin, GA, for evaluation for resistance to *P. capsici*.

***P. capsici* isolate and inoculum.** A local isolate of *P. capsici*, RCZ-11 collected from zucchini squash in South Carolina in 2003 and kindly provided by Anthony Keinath, Clemson University, was used for inoculations. Details of the isolate have been previously described (Kousik et al. 2014, 2018). The isolate collected in 2003 and belonging to the A2 mating type was selected for this study because it was shown to be highly aggressive in previous studies of crown and fruit rot (Donahoo et al. 2013; Kousik et al. 2018). The *P. capsici* isolate was stored for the long term on autoclaved hemp seed placed in sterile water as described before (Kousik et al. 2018; Lamour and Hausbeck 2002). The isolate was routinely maintained and cultured on V8 juice agar amended with antibiotics as described by others prior to use (Keinath 2007; Quesada-Ocampo et al. 2011). For preparing the zoospore inoculum suspension, the protocols described previously (Donahoo et al. 2013; Kousik et al. 2012) were followed. Briefly, a 7-mm plug of actively growing mycelia was transferred onto V8 agar medium Petri plates that were incubated at room temperature ($25 \pm 2^\circ\text{C}$) under fluorescent lights with a 12-h photoperiod for 5 to 6 days. The plates were not wrapped with parafilm, so as to allow air exchange and the development of sporangia inside the plate. After 5 to 6 days of incubation, abundant development of sporangia could be observed on the surface of the medium. At this stage, the plates were flooded with 10 to 15 ml of refrigerated ($4 \pm 1^\circ\text{C}$) sterile deionized water. The plates were then placed in the refrigerator for 30 min and then brought out and placed on laboratory benches to get to room temperature. After 20 to 30 min, zoospores were released from the sporangia in the water on the surface of the medium, and swimming zoospores could be easily observed under a microscope and the water got cloudy in appearance. The suspension was collected in 1-liter beakers by passing through two layers of cheesecloth. The suspension was adjusted to 1×10^4 zoospores/ml by diluting with sterile deionized water after determining the initial concentration as described by others (Donahoo et al. 2013).

Initial screening. Each PI was seeded in 6.3-cm square pots and had two replications with four plants per replication. Because of the limited number of seeds available from GRIN for each PI and space needed for evaluations, only two replications were used for the initial screening to primarily identify potential sources of resistance. The seeds were allowed to germinate and grow on greenhouse benches with regular watering as needed for 4 weeks prior to inoculations. Four-week-old plants of each PI were inoculated by pipetting 1 ml of zoospore suspension (10^4 zoospores/ml) on top of the soil surface adjacent to each plant using a repeating pipettor (50 ml total volume) set to dispense 1 ml as described before (Kousik et al. 2012). The trays were then kept in water-saturated conditions for 3 days before resuming regular watering as described before (Donahoo et al. 2013; Kousik et al. 2012). The watermelon rootstock ‘Carnivore’ and butternut squash cultivar ‘Waltham’ were used as susceptible controls.

Plants were rated on a 0 to 5 scale described previously by Chavez et al. (2011). Briefly, 0 = no visible symptoms, 1 = small brown lesion at base of crown, 2 = water-soaked lesion progressing up to cotyledons, 3 = plant partially collapsed with some wilting of leaves, 4 = plant completely collapsed with severe wilting of leaves, and 5 = plant death. Plants not dead, and rated 0 or 1, at 4 weeks after inoculation were considered resistant. Plants were observed on a weekly basis, and ratings were recorded 2 weeks and 4 weeks after inoculation.

Reevaluation of potential resistant lines. Based on the results of the initial screening of 319 PIs, we selected 39 PIs for reevaluation in the second screen. Any PI with a live plant in the initial

screen 30 to 40 days after inoculation was considered and selected for the re-evaluation. The 39 PIs were seeded and grown as described above. Three crown rot susceptible rootstocks ('Shintosa Camel', Carnivore, and WR15006) were used as controls (Donahoo et al. 2013; Kousik et al. 2012). Each PI or control had two replications with four plants per replication because of the limited quantity of available seeds. The experiment was arranged as a randomized complete block design with replications being the block and the PIs randomized within the block. Plants were inoculated and rated as described above.

Evaluating advanced generation. Plants that survived in the second screen and some from the initial screen were transferred to large 11.3-liter (3 gal) plastic pots filled with Metro Mix and allowed to grow on greenhouse benches under standard light and temperature. Individual surviving plants from the various PIs were self-pollinated to generate seeds for further evaluation in

the next generation (S_1). A total of 31 individual surviving resistant plants (some from the same PI) were advanced and seeds were generated. These 31 lines were planted in a third experiment with four replications and four plants per replication for each line. This third experiment was arranged as a randomized complete block design with replications being the block. The individual selections from PIs were randomized within the block. Inoculations and rating were conducted as described above. Waltham and 'Metro F1' butternut squash as well as 'White Bush Scallop' squash were used as susceptible controls.

Based on the results of the third evaluation, five lines developed from individual PIs (PI 442264d, PI 512142b, PI 442272a, Grif 935a, and PI 438729a) were evaluated again (fourth screen) to confirm resistance in the individual selections of the advanced generation and the ability to develop resistant sources. There were four replications with four plants per replication for each line developed

TABLE 1
Reaction (mean rating on a 0 to 5 scale) of U.S. *Cucurbita moschata* plant introductions (PIs) to *Phytophthora* crown rot caused by *Phytophthora capsici* in the initial screening of 319 PIs

PI	Mean ^z
PI 442264	3.0 l
PI 512142	3.3 kl
PI 442258	3.6 j-l
PI 442263	3.7 h-k
PI 438781, PI 442271, Grif 935	3.8 g-k
PI 482527	3.9 f-k
PI 438785, PI 438830	4.0 e-i
PI 442280, PI 164312	4.1 c-i
PI 442272, PI 442265, PI 442252	4.2 b-h
PI 634692, PI 326320, PI 458728, PI 438827	4.3 a-h
PI 451848, PI 438779, PI 442283, PI 482564, PI 511952	4.4 a-g
PI 182197, PI 249565, PI 438724, PI 438782, PI 438797, PI 482488	4.5 a-f
Grif 1738, PI 200822, PI 438778, PI 482585, PI 442284, PI 482528, PI 482534, PI 525174, PI 634702, PI 634706	4.6 a-e
PI 438787, PI 442267, PI 442273, PI 449346, PI 482516	4.7 a-d
PI 201858, PI 438730, PI 438758, PI 438768, PI 438801, PI 442248, PI 442262, PI 442275, PI 449347, PI 482541, PI 482542, PI 482554, PI 500555, PI 511948, PI 512169, PI 634693, PI 634698	4.8 a-c
PI 165575, PI 165995, PI 165995, PI 199014, PI 222760, PI 381807, PI 381818, PI 438739, PI 438788, PI 438791, PI 438800, PI 442257, PI 482513, PI 482548, PI 524427, PI 526247, PI 532373, PI 634705	4.9 ab
Grif 14225, Grif 14488, Grif 15151, Grif 17270, Grif 5603, Grif 5606, Grif 9457, PI 135371, PI 163230, PI 164168, PI 165033, PI 165561, PI 169413, PI 169415, PI 169441, PI 172344, PI 174194, PI 174817, PI 174819, PI 183258, PI 194570, PI 195312, PI 197154, PI 200737, PI 201772, PI 211993, PI 211996, PI 211998, PI 211999, PI 212011, PI 212899, PI 214156, PI 257532, PI 262890, PI 267752, PI 267753, PI 271338, PI 271341, PI 288239, PI 288239, PI 312125, PI 312165, PI 357918, PI 358507, PI 369346, PI 381808, PI 381812, PI 381815, PI 381817, PI 381819, PI 381820, PI 418965, PI 419083, PI 419137, PI 419202, PI 427212, PI 427213, PI 427214, PI 438548, PI 438549, PI 438551, PI 438553, PI 438576, PI 438578, PI 438579, PI 438579, PI 438708, PI 438722, PI 438723, PI 438725, PI 438731, PI 438732, PI 438733, PI 438735, PI 438736, PI 438737, PI 438740, PI 438741, PI 438743, PI 438747, PI 438748, PI 438750, PI 438751, PI 438752, PI 438753, PI 438756, PI 438759, PI 438760, PI 438767, PI 438769, PI 438770, PI 438772, PI 438773, PI 438775, PI 438776, PI 438777, PI 438780, PI 438784, PI 438786, PI 438790, PI 438792, PI 438793, PI 438794, PI 438795, PI 438796, PI 438798, PI 438799, PI 438802, PI 438803, PI 438806, PI 438808, PI 438815, PI 438828, PI 441723, PI 441726, PI 442247, PI 442249, PI 442250, PI 442251, PI 442254, PI 442255, PI 442266, PI 442268, PI 442274, PI 442277, PI 442278, PI 442286, PI 442287, PI 442288, PI 442289, PI 449345, PI 449348, PI 449349, PI 451835, PI 451839, PI 458650, PI 458745, PI 458746, PI 475750, PI 482487, PI 482491, PI 482492, PI 482501, PI 482506, PI 482524, PI 482525, PI 482526, PI 482535, PI 482545, PI 482546, PI 482549, PI 482556, PI 482560, PI 482565, PI 482569, PI 482573, PI 482577, PI 482586, PI 482588, PI 483345, PI 483346, PI 483347, PI 489694, PI 489695, PI 490354, PI 494832, PI 498429, PI 500488, PI 500536, PI 500557, PI 500558, PI 500559, PI 500562, PI 510631, PI 511946, PI 511949, PI 511951, PI 511955, PI 512146, PI 512150, PI 512151, PI 512161, PI 512177, PI 512675, PI 512766, PI 512819, PI 512846, PI 512866, PI 512872, PI 512876, PI 524428, PI 526243, PI 526244, PI 526245, PI 526249, PI 526251, PI 532377, PI 532378, PI 532381, PI 536494, PI 550689, PI 550690, PI 550692, PI 550693, PI 550694, PI 560946, PI 560952, PI 600843, PI 604505, PI 604506, PI 634699, PI 634700, PI 634701, PI 634703, PI 634704, PI 634982, PI 653064	5.0 a
'Shintosa Camel', 'Carnivore'	5.0 a

^z Plants were rated on a 0 to 5 scale. Means followed by the same letter are not significantly different.

from individual PIs. The experiment was arranged as a randomized complete block design with replications being the block and the individual selections from PIs randomized within the block. Inoculations and rating were conducted as described above. Waltham and Metro F1 butternut squash as well as the interspecific hybrid rootstock Carnivore were used as susceptible controls.

Statistical analysis. For each of the four screenings, the disease rating data were rank transformed. Except for the first-generation screen, the PI and cultivars (= “treatment”) were arranged in a randomized complete block design; the first generation was arranged as a completely randomized design. A general linear mixed model was fit to the data using the GLIMMIX procedure in SAS (version 9.4, Cary, NC) with the default identity link function and Gaussian error structure, the maximum likelihood estimation method, and Kenward and Rogers degrees of freedom as options. The variable “treatment” was modeled as a fixed effect and “block” as a random effect. Standardized residual and normality plots were used to determine the adequacy of model fit. Pairwise treatment differences for the fixed effect were obtained using the LSMEANS statement and LINES option ($P = 0.05$). When estimable, significance of the random effect was tested using the COVTEST statement and WALD option ($P = 0.05$).

Diversity among resistant *C. moschata* accessions. A neighbor-joining (NJ) tree of select accessions was created using *C. moschata* single nucleotide polymorphism (SNP) genotype data obtained from the Cucurbit Genomics Database (Zheng et al. 2019). Raw genotyping-by-sequencing derived SNPs (Elshire et al. 2011) in variant call format were downloaded and filtered using VCFtools (Danecek et al. 2011) to retain only individuals with <60% missing data and SNPs with (i) mean read depth >5 and <44; (ii) missing data <60%; and (iii) minor allele frequency >0.05. Genotypes supported by fewer than five reads were set to missing. Sites were then pruned to include no more than one SNP every 10 kilobases, resulting in a total of 3,701 SNPs. This dataset was filtered to retain only those accessions identified as resistant in our screen. Several accessions that we identified as resistant (PI 176531, PI 442266, PI 458740, PI 500537, PI 512142, and PI 634693) could not be included in this analysis because genotype data were not available. Five susceptible cultivated squash types and controls with genotype data in the SNP set were included for visualization in the tree as well: PI 358507 (‘Golden Cushaw’), PI 550692 (‘Ponca BN 87-4’), PI 604505 (‘Kentucky Field’), PI 634982 (Waltham butternut), and Burpee ‘Butterbush’. The NJ tree was then created using SplitsTree version 4.16.2 (Huson 1998), using the “Uncorrected P” distance metric, and visualized using the package ‘ape’ version 5.4-1 (Paradis et al. 2004) in R version 4.0.2 (R Core Team 2019). In total, 23 PIs were included in the tree. This included 18 resistant and five susceptible accessions.

Results and Discussion

Initial screen. Of the 319 PIs that were planted, seeds of 25 PIs did not germinate. Hence, ratings were recorded for 294 PIs. Symptoms of crown rot were visible within a week after inoculation on the susceptible checks. On some susceptible check plants, water-soaked lesions were observed on the hypocotyls 4 to 5 days after inoculation. Two weeks after inoculation, many plants were dead, and by 3 weeks, a majority (215 or 87%) of the *C. moschata* PIs succumbed to crown rot caused by *P. capsici* (Table 1, Fig. 1). Some of the PIs (e.g., PI 442264, PI 512142, PI 442258, PI 442263, PI 438781, PI 442271, and Grif 935) were significantly less susceptible to crown rot compared with the majority of the PIs. Significant variability in the response of plants within a PI was observed in the initial screen. This was especially true among the

PIs that had a mean rating between 3.0 and 4.2, where some plants had died and were rated as 5 and others expressed few if any symptoms within the replication. This indicated a need for further evaluations and selection of resistant plants within a PI to develop resistant sources for use in breeding programs. Such variability in reaction to various plant pathogens has been commonly observed within PIs of various cucurbit crops (Chavez et al. 2011; Donahoo et al. 2013; Kousik et al. 2014; Padley et al. 2008).

Reevaluation of potential resistance sources. Thirty-nine PIs that had at least one surviving plant in the initial screening of 319 PIs, were considered as potential sources of resistance, and were reevaluated in the second screen. Disease development was slower in the second test compared with the first. All the plants of the susceptible checks Carnivore and Shintosa Camel were dead within 2 weeks after inoculation. Significant variability among plants within a PI to crown rot was observed in this trial as well (Fig. 2). For example, PI 442264 had some plants rated 0 and showing no signs of infection, whereas some were dead and rated as 5. Figure 2 clearly depicts the variability among plants within a PI, with many having plants rated 0 and 5. For example PI 442262, PI 442271, PI 438274, and PI 442280 each included plants rated 0 as well as plants rated 5. However, overall, PI 442265, PI 634706, PI 512142, and PI 634692 had significantly lower mean crown rot rating compared with the susceptible checks (Fig. 2). PI 512142 performed consistently across the two screens.

Performance of advanced generation. Twenty-six single-plant potential resistant selections that were advanced from the initial and the second screens were evaluated for resistance to

FIGURE 1

Reaction of *Cucurbita moschata* plant introductions (PIs) to *Phytophthora* crown rot incited by the oomycete plant pathogen *Phytophthora capsici* (South Carolina isolate RCZ-11). The top panel shows the plants prior to inoculation and the bottom panel 3 weeks after inoculation. Each plant was inoculated with 10^4 zoospores/ml. Most (87%) of the plants of the 294 PIs evaluated succumbed to *P. capsici* infection. PIs with any surviving plants were identified and evaluated a second time.

crown rot in the third screen. Seeds from five of the 31 single-plant selections did not germinate in this trial. Several of the single-plant resistant selections from the previous evaluations were highly resistant, and plants within the line were rated as 0. For example, the selections made from PI 438724, Grif 935a, PI 442272, and PI 512142b were highly resistant compared with susceptible checks and some other selections (Fig. 3). However, some of the single-plant selections from the same PI continued to display a significant level of variability in the second generation. For example, all plants within PI 442264d were rated 0, whereas plants within the selection PI 442264b ranged from 0 to 5, even though the overall mean was <1 (Fig. 3).

Plants of the susceptible check Waltham butternut squash had severe crown rot in the fourth screen (Figs. 4 and 5). In comparison, the resistant selections from PI 442272 and PI 442264 did not exhibit any crown rot symptoms. Similarly, resistant selections from PI 512142, Grif 935, and PI 438724 were also resistant in the fourth screen and did not succumb to crown rot, and most plants were rated as 0. This further confirmed that resistance sources can be developed from the PIs available with GRIN. None of the plants from the resistant selections died from crown rot during the fourth evaluation, and several of these were transferred to advance the lines further. Metro F1, which was also used as a

susceptible check, had a few plants that were rated 0 and some rated 5; however, based on the overall mean rating it was significantly more susceptible than the five resistant selections.

New sources of resistance to *Phytophthora* crown rot in *C. moschata*. At least 12 potential new sources of resistance (e.g., Grif 935, PI 442272, PI 442264, PI 512142, PI 438724, PI 438778, and PI 442280) to crown and root rot caused by the local South Carolina isolate of *P. capsici* were identified. A majority of the new sources of resistance identified in this study were originally collected in Mexico (e.g., PI 442272, PI 442264, and PI 512142) according to the GRIN database. Similarly, two (PI 442262 and PI 442266) of the resistance sources identified in a previous study against a *P. capsici* isolate from Florida were from Mexico (Chavez et al. 2011). Variability in resistance reaction among plants within a PI was observed in the current study, and not all plants of any particular PI were resistant in this study. Similar results were seen in the study by Chavez et al. (2011). The variability associated with the accessions of *C. moschata* requires that additional selection and screening of individual resistant plants from germplasm collections are needed to develop resistant sources. Further evaluation of the S₁ and S₂ generations from the most resistant plants leads to the conclusion that highly resistant plants could be selected from these sources of resistance to develop

FIGURE 2

Box plots showing reaction of U.S. plant introductions (PIs) of *Cucurbita moschata* to *Phytophthora* crown rot (*Phytophthora capsici* South Carolina isolate RCZ-11) in a second greenhouse inoculated trial. PIs for inclusion in the second trial were based on the reactions in the initial screening. Each plant was inoculated with 10^4 zoospores/ml. The solid diamonds in the graph represent the mean for each PI, the bars span the interquartile range (Q1 to Q3), the short horizontal line within the bars is the median, and the whiskers extend to the minimum and maximum ratings for each PI. The horizontal lines below the x axis represent the LSD_{0.05} lines showing mean separation. The commercial interspecific hybrid rootstocks 'Carnivore' and 'Shintosa Camel' were used as susceptible controls.

germplasm lines for use in breeding programs and that the resistance is inherited.

In the previous study done in Florida, five sources of resistance to *P. capsici* were identified in *C. moschata* by evaluating 119 accessions (Chavez et al. 2011). In the current study, additional new sources of resistance were identified. Interestingly, Griff 935 and PI 442272, which were tolerant or resistant to the isolate from South Carolina in the current evaluation, were highly susceptible to a Florida isolate (Chavez et al. 2011). Resistant lines were selected from these PIs, and these selections were highly resistant compared with Waltham butternut squash and the interspecific hybrid (*C. moschata* × *C. maxima*) rootstock Carnivore. The variability among isolates of *P. capsici* in terms of virulence and the presence of race structure have been well documented on many vegetable crops (Barchenger et al. 2018; Foster and Hausbeck 2010; Granke et al. 2012b; Kim and Hwang 1992; Lee et al. 2001; Naegele et al. 2014; Oelke et al. 2003; Quesada-Ocampo and Hausbeck 2010). Therefore, when new sources of resistance are developed, they should be evaluated against multiple isolates from other states and regions within the state prior to release for use in breeding programs. The new sources of resistance can be utilized for developing new crown and root rot resistant rootstocks for watermelon grafting. Most of the commercial watermelon and melon rootstocks are hybrids of *C. moschata* × *C. maxima*. Hence, it may be necessary to develop sources of resistance to *Phytophthora* crown rot in *C. maxima* depending upon the genetics of

resistance in the resistant *C. moschata* selections. Regardless, the *C. moschata*-resistant lines developed as a part of this study can be used in breeding squashes for consumption.

Many of the sources of resistance to *P. capsici* identified in these experiments were not evaluated in the previous screen (Chavez et al. 2011). Conversely, some of the resistant sources described by Chavez et al. (2011) were not included in the initial screening of 319 PIs, because they were not available from the GRIN station in Griffin, GA, as limited seeds of these PIs are in high demand. This demand suggests that there is a need for identifying and developing additional sources of resistance as described in the current study. However, because of the variability in reaction to crown rot within potential resistance sources, an aggressive screening and selection approach will be necessary to develop resistant lines for use in breeding programs.

Diversity among resistance sources and cultivated squash. The NJ tree revealed four clusters of accessions (Fig. 6, Supplementary Table S1). One of these (cluster 1) was composed entirely of the five susceptible cultivars, which formed a single monophyletic group. Of the three clusters of resistant accessions, two exclusively featured accessions collected in Mexico (cluster 2: PI 442265, PI 442264, PI 442267, and PI 442262; and cluster 3: PI 442280, PI 438785, PI 438779, PI 442271, PI 438724, PI 438778, and PI 442272). The last cluster (cluster 4) contained the accessions most distantly related to the susceptible checks, featuring a mixture of accessions from Nepal (Griff 1738 and

FIGURE 3

Box plots showing reaction of selections made from select U.S. plant introductions (PIs) of *Cucurbita moschata* to *Phytophthora* crown rot (*Phytophthora capsici*, South Carolina isolate RCZ-11) in the third inoculated screen. The most resistant plants from the second evaluation of the PIs were saved and self-pollinated to generate seed (S_1). These were then evaluated by inoculating each plant with 10^4 zoospores/ml. The alphabets after each PI number indicates a single-plant selection made after the second screening and advanced. The solid diamonds in the graph represent the mean for each PI selection, the bars span the interquartile range (Q1 to Q3), the short horizontal line within the bars is the median, and the whiskers extend to the minimum and maximum ratings for each PI. The horizontal lines below the x axis represent the $LSD_{0.05}$ lines showing mean separation. Commercial cultivars 'White Bush Scallop' squash, 'Waltham' butternut, and 'Metro F1' butternut squash were used as susceptible controls.

FIGURE 4

Box plots showing reaction of selections (S_1 , advanced generation) made from select U.S. plant introductions (PIs) of *Cucurbita moschata* to *Phytophthora* crown rot (*Phytophthora capsici*, South Carolina isolate RCZ-11) in the fourth inoculated screen. The most resistant plants from the second evaluation of the PIs were saved and self-pollinated to generate seed. These were then evaluated by inoculating each plant with 10^4 zoospores/ml. Selections of PI 442264d, PI 512142b, Griff 935a, PI 442272, and PI 438724 based on reactions in the third screen were evaluated again. Commercial butternut squash cultivars 'Waltham' and 'Metro F1' and the *Cucurbita* interspecific hybrid rootstock 'Carnivore' were used as susceptible controls. The solid diamonds in the graph represent the mean for each PI selection, the bars span the interquartile range (Q1 to Q3), the short horizontal line within the bars is the median, and the whiskers extend to the minimum and maximum ratings for each PI. The horizontal lines below the x axis represent the $LSD_{0.05}$ lines showing mean separation.

Grif 935), Zimbabwe (PI 482488, PI 482527, and PI 482528), Yemen (PI 634692), and Sri Lanka (PI 634706).

The NJ tree also indicated that none of the resistant accessions identified in this study were genetically similar to the susceptible butternut-type squash. Pairwise genetic distances between accessions ranged between 0.03 and 0.35. The median pairwise genetic distance within cluster 1 (consisting of the susceptible butternut types) was 0.11, whereas the median genetic distance between accessions in cluster 1 and those in other clusters was 0.25, 0.24, and 0.28 for clusters 2, 3, and 4, respectively. Despite the relatively wide genetic distance between resistant and susceptible accessions, compatibility barriers within *C. moschata* are not expected. However, the wide genetic distances among resistant *C. moschata* accessions and cultivated butternut squash types may increase the possibility of linkage drag and, hence, require more extensive breeding to introgress resistance genes using conventional methods. A similar genetic diversity study using simple-sequence repeat markers with *P. capsici*-resistant *C. pepo* accessions helped identify appropriate PIs for use in breeding programs (Michael et al. 2019). One of the resistance sources identified in this study, PI 512142, was not a part of the NJ tree analysis because genotype data were not available. PI 512142 was collected from a vendor in a Mexico City market and was an old named cultivar, ‘Clabaza de Castilla’ (https://www.ars-grin.gov/npgs/pi_books/scans/196pt2/pi196pt2_203.pdf). It would be interesting to generate genotype data for such resistant sources to include in this analysis.

However, the fruit of PI 512142 appear different from the traditional butternut squash types sold in the United States and may only have a use in niche markets.

Conclusions and Management Implications

Phytophthora root and crown rot caused by *P. capsici* is an important factor limiting production wherever squashes are cultivated. The availability of resistant squash varieties to manage Phytophthora crown rot can play a major role for growers in realizing increased yields. Many new sources of resistance to Phytophthora crown rot were identified in this study that will be useful for plant breeders to incorporate resistance into rootstocks and commercial squash varieties. The genetic distance between these resistance sources and the cultivated-type squash was also determined, and this will prove useful to breeders to select appropriate sources of resistance to make crosses to cultivated-type squash. Despite the relatively wide genetic distance between the cultivated squash types and the potential sources of resistance, some sources described in this study can likely be used for developing commercial rootstocks for grafting watermelon and melons. Appropriate *C. maxima* lines will have to be developed to make crown rot resistant hybrid rootstocks with the resistance sources of *C. moschata* that were identified. Some PI that were considered susceptible to isolates from Florida were resistant to isolates from South Carolina, indicating the potential

FIGURE 5

A *Phytophthora* crown rot (*Phytophthora capsici*, South Carolina isolate RCZ-11) resistant *Cucurbita moschata* selection on the right compared with a susceptible butternut squash cultivar ‘Waltham’ on the left 3 weeks after inoculation with 10^4 zoospores/ml of *P. capsici* per plant.

FIGURE 6

Neighbor-joining (NJ) tree depicting potential sources of resistance to *Phytophthora* crown rot (*Phytophthora capsici*, South Carolina isolate RCZ-11) and select *Cucurbita moschata* cultivars. The cultivated *C. moschata* cultivars indicated in the tree—PI 358507 (‘Golden Cushaw’), PI 550692 (‘Ponca BN 87-4’), PI 604505 (‘Kentucky Field’), PI 634982 (‘Waltham’ butternut), and Burpee ‘Butterbush’—were all susceptible to crown rot. The NJ tree was developed based on genotyping-by-sequencing data (Elshire et al. 2011) for *C. moschata* from the Cucurbit Genomics Database (Zheng et al. 2019). Data were not available for some of the identified sources of resistance, and hence they were not included in the tree.

for existence of races, and hence once a commercial variety is developed it should be evaluated against the local isolates of *P. capsici* prior to being cultivated on a large scale. Resistant cultivars will ultimately have to become part of an integrated disease management program that includes well-drained fields, crop rotation, and judicious use of fungicides to manage *P. capsici*.

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