Genetic Diversity Among Accessions of *Cucurbita pepo* Resistant to *Phytophthora* Crown Rot

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Abstract. *Phytophthora capsici* Leonian, the causal agent of *Phytophthora* crown rot in squash (*Cucurbita pepo* L.), is an economically important pathogen worldwide. Currently, no *C. pepo* cultivars immune to the pathogen are commercially available, but sources of resistance to *Phytophthora* crown rot have been identified in a set of 16 *C. pepo* plant introductions (PIs). Knowledge of the genetic relationships among these accessions and their relatedness to economically important morphotypes of *C. pepo* would inform breeders' best strategies for introgressing resistance; however, this information is currently lacking. The goal of the current study was to determine genetic diversity among the resistant accessions and their genetic relatedness to susceptible morphotypes of subspecies *pepo* (Zucchini and Pumpkin) and *texana* (Acorn, Straightneck, and Crookneck) using 39 SSR markers. The markers revealed 132 alleles averaging 4.40 alleles per locus and had a mean polymorphic information content (PIC) and gene diversity of 0.44 and 0.49, respectively. CMTp235 had the highest PIC and gene diversity of 0.80 and 0.82, respectively. Hierarchical clustering by UPGMA and principal coordinate analysis (PCOA) revealed grouping into two major *C. pepo* subspecies, *texana* and *pepo*, with all the resistant accessions grouping in the latter. In order of increasing genetic distance (GD), the resistant accessions were least distant to Zucchini (GD = 0.34), followed by Pumpkin (GD = 0.40), Crookneck (GD = 0.56), Acorn (GD = 0.60), and Straightneck (GD = 0.61) morphotypes. Mean GD among the resistant accessions was 0.31 and was highest between PIs 615142 and 615132 (0.61). Based on genetic similarity, PIs 174185 and 181761 (disease severity ≤ 1.4) would be the best sources of resistance for transfer into subspecies *texana* and *pepo*, respectively. Overall, the results presented here support a closer genetic relationship between the resistant accessions and morphotypes of subspecies *pepo* than those of subspecies *texana*.

Squash (*Cucurbita* spp.) is an economically important crop of *Cucurbitaceae* family valued at \$230 million annually in the United States (U.S. Department of Agriculture–National Agricultural Statistics Service, 2017). Among the five cultivated species of *Cucurbita* genus, *C. pepo* L. is the most popular and consists of four subspecies: *texana* (Scheele) Filov (also called *ovifera* (L.) Decker); *pepo*; *fraterna* (L. H. Bailey) Lira, Andres, and Nee; and *gumala* Teppner (Gong et al., 2012; Paris et al., 2003; Robinson and Decker-Walters, 1997; Teppner, 2000). Among these, *texana* and *pepo* are the most cultivated and are further classified into eight edible morphotypes (cultivar groups) (Paris, 2001). Crookneck, Straightneck, Acorn, and Scallop morphotypes are in subsp. *texana*, whereas Cocozelle, Vegetable Marrow, Zucchini, and Pumpkin belong to

subsp. *pepo* (Paris, 1989; Paris et al., 2012). Cultivation of *C. pepo* is significantly limited by chronic losses to *Phytophthora capsici* Leonian, the causal agent of crown rot, foliar blight, and fruit rot in many vegetable crops (Hausbeck and Lamour, 2004). Currently, there are no commercial *C. pepo* cultivars immune to *P. capsici*, but sources of resistance to *Phytophthora* crown rot have been identified in *C. moshata* Duchesne (Chavez and Kabelka, 2010) and noncultivated species of squash [*C. lundelliana* Bailey and *C. okeechobeensis* (Small) Bailey] (Padley et al., 2009). However, transfer of this resistance into *C. pepo* is difficult because of wide genetic distances between the species that require resource-intensive techniques, such as embryo rescue and bridge crossing (Rakha et al., 2012; Zhang et al., 2012).

In an effort to identify sources of resistance within *C. pepo*, Padley et al. (2008) inoculated 115 PIs from 24 countries with three virulent isolates of *P. capsici*, but none was immune to the pathogen. However, 16 of the accessions showed moderate to high resistance to *Phytophthora* crown rot [disease severity between 1.3 and 2.5 on a scale of 0 (no symptoms) to 5 (plant death)] (Padley

et al., 2008). Squash breeders can use this germplasm to transfer resistance into elite morphotypes of *C. pepo*. However, information on the genetic basis of crown rot resistance in these accessions that would inform breeders of best strategies for accumulating diverse disease resistance alleles through gene pyramiding is currently lacking. In addition, information on the genetic diversity among the resistant accessions and their relatedness to susceptible morphotypes of *C. pepo* is unknown. Although no hybridization barriers exist among various subspecies of *C. pepo*, vast segregation in fruit phenotypes resulting from such crosses requires numerous backcrosses to recover elite parent genetic background (Loy, 2011). Therefore, even for interspecific trait transfer, breeders should consider selecting parents with the least intergenotype genetic distance (Gong et al., 2013; Paris, 2016).

Measurement of genetic diversity and elucidation of phylogenetic relationships among *C. pepo* genotypes has been accomplished using a variety of marker systems including allozymes (Decker, 1985) and DNA markers [random amplified polymorphic DNA, amplified fragment length polymorphism, sequence-related amplified polymorphism, intersimple sequence repeats, high-frequency oligonucleotide-targeting active gene and simple sequence repeats (SSR)] (Decker-Walters et al., 2002; Ferriol et al., 2003; Formisano et al., 2012; Gong et al., 2012; Paris et al., 2003, 2015). Among these, SSR markers are preferred because of their abundance in the genome, reproducibility, high level of polymorphism and codominance (Hodel et al., 2016; Powell et al., 1996). Moreover, a high level of synteny among *Cucurbita* species allows transferability of SSR markers across the genus for various applications (Gong et al., 2008a, 2013; Kaźmińska et al., 2017; Sim et al., 2015; Stift et al., 2004).

The goal of the current study was to determine the genetic diversity among a set of 16 *C. pepo* accessions previously identified as resistant to *Phytophthora* crown rot (Padley et al., 2008) using SSR markers developed for *Cucurbita* (Gong et al., 2008b). Further, genetic similarity between these accessions and susceptible morphotypes of *C. pepo* (Zucchini, Acorn, Straightneck, Crookneck, and Pumpkin) was determined.

Materials and Methods

Plant material and DNA extraction. Seeds of 16 *C. pepo* PIs previously identified as resistant to *Phytophthora* crown rot (Supplemental Table 1) (Padley et al., 2008) were obtained from the germplasm collection of the U.S. Department of Agriculture, Agricultural Research Service. These accessions have a mean disease score (DS) between 1.3 and 2.5 on a scale of 0 to 5 (Padley et al., 2008). Susceptible commercial cultivars representing Zucchini (Black Beauty), Crookneck (Early Golden), Acorn (Table Queen, Delicata, and Honey Bear), Pumpkin (Beppo and Lady

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Table 1. Genetic parameters revealed by 30 simple sequence repeat (SSR) markers on 16 *Cucurbita pepo* accessions resistant to *Phytophthora* crown rot and eight susceptible cultivars.

SSR	Number of alleles expected ²	Number of alleles observed	Allele size range (bp)	Gene diversity (H_E)	Heterozygosity (H_O)	PIC
CMTp239	3	2	99–114	0.04	0.04	0.04
CMTp127	3	2	107–113	0.08	0.00	0.08
CMTp21	4	3	179–203	0.12	0.04	0.12
CMTp39	4	5	118–130	0.30	0.17	0.28
CMTp84	2	2	144–150	0.33	0.00	0.28
CMTp141	3	6	108–132	0.34	0.22	0.33
CMTp53	3	3	117–125	0.36	0.00	0.32
CMTp133	3	5	105–121	0.40	0.13	0.39
CMTp109	5	3	100–122	0.42	0.09	0.38
CMTp62	5	5	118–172	0.45	0.19	0.41
CMTp205	6	5	116–142	0.45	0.29	0.41
CMTp177	5	3	189–215	0.47	0.29	0.38
CMTp68	4	4	160–186	0.47	0.21	0.42
CMTp36	3	3	143–158	0.47	0.00	0.43
CMTp229	4	2	97–99	0.49	0.00	0.37
CMTp224	5	4	147–156	0.54	0.17	0.50
CMTp206	6	4	114–123	0.54	0.14	0.48
CMTp264	3	3	100–130	0.54	0.83	0.45
CMTp176	4	5	100–118	0.55	0.04	0.51
CMTp265	3	4	78–98	0.55	0.56	0.45
CMTp231	9	5	133–197	0.56	0.50	0.52
CMTp37	9	6	111–197	0.60	0.13	0.57
CMTp249	4	6	136–162	0.61	0.41	0.56
CMTp178	4	5	125–149	0.63	0.30	0.55
CMTp245	5	4	108–132	0.65	0.48	0.57
CMTp202	4	9	124–174	0.67	0.27	0.64
CMTp26	5	8	111–197	0.69	0.17	0.65
CMTp129	5	4	119–151	0.70	0.42	0.65
CMTp201	8	4	89–111	0.75	0.43	0.70
CMTp235	6	8	124–154	0.82	0.48	0.80
Mean	4.56	4.40	—	0.49	0.23	0.44

²Number of alleles reported in Gong et al. (2008b).
PIC = polymorphic information content.

Godiva), and Straightneck (Early Prolific) morphotypes were included to study their relationship with the resistant accessions. For each genotype, five seeds were germinated in a greenhouse maintained at 22 to 32 °C using cells (5.98 × 3.68 × 4.69 cm) filled with Fafard 3B soil, which was amended with Osmocote Classic fertilizer (1.38 g/kg N, 1.38 g/kg P, 1.38 g/kg K). At the two true-leaf stage, three leaf punches from three individuals of each accession were collected, bulked, and immediately frozen in liquid nitrogen. DNA was extracted using the E.N.Z.A. kit (Omega Biotek, Norcross, GA) according to manufacturer's instructions.

SSR amplification and allele scoring. Thirty-nine previously published SSR primer pairs distributed across 19 linkage groups of *C. pepo* were used for genetic diversity analysis (Supplemental Table 2) (Gong et al., 2008b). For each primer pair, polymerase chain reaction was performed in a 15-μL reaction containing 40 ng of template DNA, 0.32 μM of a fluorescently (either 6-FAM, VIC, or PET; Supplemental Table 2) labeled M13 forward primer (GCCTCCCTCGC-GCCA) (Blacket et al., 2012), 0.04 μM of M13-tagged forward primer, 0.4 μM unlabeled reverse primer, and 1 × PROMEGA Colorless GoTaq mastermix (Promega, Madison, WI). Amplification was performed in 96-well plates on a SimpiAmp thermal-cycler (Applied Biosystems, Foster City, CA) using an initial 3 min denaturation at 95 °C, followed by 35 cycles of 15 s at 95 °C, 20 s at 52 °C, and 30 s at 72 °C. The

Fig. 1. UPGMA dendrogram showing clustering of 16 *Cucurbita pepo* accessions resistant to *Phytophthora* crown rot (Padley et al., 2008) and 8 susceptible cultivars (with asterisk). Tree scale represents branch length. Only bootstrap values above 45% are shown.

amplification was followed by a final extension step of 10 min at 72 °C. Depending on band intensity for each primer pair on an agarose gel (1% w/v), products were diluted appropriately for capillary electrophoresis. Amplification products for three primer pairs, each labeled with a different fluorescent dye, were multiplexed and combined with a GeneScan-500 ROX internal-lane size standard and Hi-Di Formamide before analysis on a 3730 96-capillary DNA Analyzer (Applied Biosystems) at the Gene Expression

and Genotyping Core facility, Interdisciplinary Center for Biotechnology Research, University of Florida. GeneMarker software (SoftGenetics, State College, PA) was used for allele calling and size estimation.

Genetic diversity analysis. Marker diversity parameters including number of alleles, polymorphic information content (PIC) (Botstein et al., 1980) and expected heterozygosity/gene diversity (Weir, 1996) were determined using PowerMarker software (v3.25) (Liu and Muse, 2005). The allelic data were

Fig. 2. Principal coordinate analysis showing clustering according to subsp. *texana* and *pepo*. Cluster 1 contains susceptible morphotypes of subsp. *texana*; Acorn ('Bush Delicata', 'Honey Bear', and 'Table Queen'), Straightneck ('Early Prolific'), and Crookneck ('Early Golden Crookneck') morphotypes. Cluster 2 contains susceptible morphotypes of subsp. *pepo*; Pumpkin ('Beppo' and 'Lady Godiva') and Zucchini ('Black Beauty') morphotypes, as well as 16 *Cucurbita pepo* accessions resistant to *Phytophthora* crown rot (1-PI 167053, 2-PI 169417, 3-PI 169450, 4-PI 169476, 5-PI 174185, 6-PI 179267, 7-PI 181761, 8-PI 181878, 9-PI 181944, 10-PI 209783, 11-PI 266925, 12-PI 288240, 13-PI 299574, 14-PI 512709, 15-PI 615132, 16-PI 615142) (Padley et al., 2008).

then converted into a binary format (1,0 for presence or absence of alleles) and genetic similarity calculated using Dice coefficient of similarity, S_D (Dice, 1945). Genetic similarity matrix was used to construct a dendrogram by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) hierarchical clustering, replicated with 1000 bootstraps on FreeTree program (Hampel et al., 2001), and visualized on the web-based interactive Tree of Life (iTOL) tool (Letunic and Bork, 2016). Genetic distances were obtained by subtracting genetic similarity values from 1 ($1 - S_D$) (Nei and Li, 1979). PCOA (Gower, 1966) was calculated and displayed with factorial analysis option in Darwin Software (v6.0) (Perrier and Jacquemoud-Collet, 2006).

Results and Discussion

SSR analysis. Out of the initial 39 SSR markers tested, a set of 9 markers were dropped during analysis and included six that were monomorphic and three that had missing data in more than five accessions. The remaining 30 markers revealed 132 alleles, which ranged in size from 78 bp

(marker CMTp265) to 215 bp (marker CMTp177) (Table 1). The number of alleles per locus ranged from two to nine alleles, with 86.7% of the markers having more than three alleles (Table 1). The mean number of alleles per locus was 4.4 and was comparable to that observed among 88 *Cucurbita* accessions by Gong et al. (2013) (4.3 alleles/locus, 74 SSR markers). Other studies in *Cucurbita* have reported wide variation in mean number of alleles per locus, for instance, Gong et al. (2012) (3.0 alleles/locus; 104 accessions; 134 SSR markers), Gong et al. (2008b) (3.3 alleles/locus; 12 accessions; 405 SSR markers), Verdone et al. (2018) (3.6 alleles/locus; 30 accessions; 8 SSR markers), Formisano et al. (2012) (3.8 alleles/locus; 23 accessions; 30 SSR markers), Murovec (2015) (6.06 alleles/locus; 51 accessions; 18 SSR markers), Barzegar et al. (2013) (6.71 alleles/locus; 26 accessions; 14 SSR markers), and Sim et al. (2015) (7.41 alleles/locus; 160 accessions; 29 SSR markers). Variation in mean number of alleles per locus across studies can be explained by differences in the number and geographic distribution of accessions used and the number and

level of polymorphism of SSR markers assayed (Murovec, 2015).

Variation in discrimination power of the markers was evident as shown by the wide range in gene diversity (H_e ; 0.04–0.82) and polymorphic information content (PIC; 0.04–0.80) (Table 1). Among the markers assayed, discriminating power was highest in CMTp235 ($H_e = 0.82$; PIC = 0.80), and lowest in CMTp239 ($H_e = 0.04$; PIC = 0.04) (Table 1). A similar observation was made by Murovec (2015), who found marker CMTp239 ($H_e = 0.028$; PIC = 0.027) to have the least discriminating power among 18 SSR markers assayed.

Dendrogram and PCOA. The UPGMA dendrogram separated the genotypes into two distinct clusters (Fig. 1). All five cultivars of *C. pepo* subsp. *texana* grouped in Cluster 1 (bootstrap value = 96), and further separated into two subclusters, one consisting of Straightneck (Early Prolific) and Crookneck (Early Golden) morphotypes and the other Acorn (Table Queen, Bush Delicata, and Honey Bear) (Fig. 1). The close association between Straightneck and Crookneck morphotypes is supported by evidence that the former was derived from the latter through

Table 2. Genetic distance matrix showing relationships among 16 *Cucurbita pepo* accessions resistant to *Phytophthora* crown rot and eight susceptible cultivars.

Genotype	PI 167053	PI 169417	PI 169450	PI 169476	PI 174185	PI 179267	PI 181761	PI 181878	PI 181944	PI 209783	PI 266925	PI 288240	PI 299574	PI 512709	PI 615132	PI 615142	Black Beauty	Yellow Crookneck	Early Prolific	Bush Delicata	Table Queen	Honey Bear	Beppo
PI 169417	0.15																						
PI 169450	0.23	0.23																					
PI 169476	0.27	0.28	0.26																				
PI 174185	0.30	0.28	0.24	0.32																			
PI 179267	0.22	0.20	0.26	0.26	0.23																		
PI 181761	0.29	0.32	0.29	0.41	0.28	0.19																	
PI 181878	0.29	0.29	0.20	0.35	0.28	0.22	0.22																
PI 181944	0.24	0.24	0.23	0.33	0.25	0.20	0.20	0.24															
PI 209783	0.27	0.25	0.33	0.33	0.26	0.12	0.21	0.32	0.23														
PI 266925	0.25	0.26	0.27	0.29	0.22	0.13	0.22	0.30	0.19	0.14													
PI 288240	0.24	0.25	0.33	0.31	0.26	0.18	0.30	0.39	0.27	0.16	0.17												
PI 299574	0.33	0.36	0.35	0.35	0.30	0.25	0.32	0.33	0.31	0.32	0.30	0.32											
PI 512709	0.34	0.32	0.36	0.39	0.38	0.30	0.35	0.39	0.32	0.35	0.30	0.35	0.29										
PI 615132	0.47	0.46	0.49	0.44	0.55	0.48	0.53	0.54	0.49	0.49	0.49	0.48	0.47	0.40									
PI 615142	0.36	0.38	0.37	0.45	0.35	0.25	0.30	0.33	0.36	0.32	0.32	0.34	0.31	0.41	0.61								
Black Beauty	0.37	0.32	0.38	0.42	0.39	0.28	0.33	0.44	0.32	0.25	0.26	0.25	0.24	0.27	0.51	0.37							
Yellow Crookneck	0.57	0.54	0.57	0.56	0.49	0.50	0.61	0.63	0.54	0.54	0.51	0.51	0.60	0.53	0.66	0.56	0.57						
Early Prolific	0.65	0.61	0.65	0.61	0.51	0.57	0.63	0.62	0.64	0.62	0.61	0.61	0.54	0.58	0.66	0.58	0.63	0.33					
Bush Delicata	0.58	0.58	0.53	0.60	0.53	0.59	0.62	0.58	0.55	0.63	0.62	0.60	0.61	0.63	0.64	0.68	0.64	0.35	0.52				
Table Queen	0.55	0.54	0.54	0.64	0.55	0.58	0.58	0.53	0.57	0.57	0.59	0.62	0.53	0.62	0.68	0.57	0.49	0.41	0.57	0.51			
Honey Bear	0.58	0.58	0.53	0.63	0.56	0.64	0.67	0.61	0.58	0.63	0.62	0.63	0.61	0.63	0.67	0.65	0.58	0.35	0.48	0.31	0.29		
Beppo	0.31	0.36	0.41	0.41	0.41	0.34	0.42	0.47	0.38	0.37	0.37	0.34	0.41	0.42	0.51	0.46	0.40	0.48	0.53	0.56	0.63	0.53	
Lady Godiva	0.32	0.34	0.36	0.44	0.39	0.33	0.38	0.39	0.37	0.37	0.37	0.40	0.42	0.43	0.54	0.44	0.43	0.54	0.53	0.61	0.65	0.56	
																						0.17	

selection (Gong et al., 2012; Paris, 2001), a relationship corroborated in previous phylogenetic studies (Decker, 1985; Ferriol et al., 2003; Gong et al., 2012; Paris, 2001). Within the Acorn subcluster, Honey Bear and Table Queen cultivars, which have typical turbinate fruits, associated more closely, but farther from Delicata, which has oblong fruit shape (Loy, 2011). In Cluster 2 (bootstrap value = 89), Zucchini ('Black Beauty') and Pumpkin ('Beppo' and 'Lady Godiva') morphotypes of *C. pepo* subsp. *pepo* grouped together with the 16 resistant accessions. The pumpkin cultivars, used in the seed oil and snack industry (Lelley et al., 2009), formed a tight subcluster (bootstrap value = 99). Zucchini morphotype associated more closely with accessions from South Africa (PI 299574) and Spain (PI 512709). The lower section of cluster 2 (Fig. 1) was occupied by resistant accessions originating from Germany (PIs 266925 and 209783) and the Mediterranean Basin (PIs 167053, 169417, 169450, 174185, 179267, 181761, 181944, and 288240). The Mexican accession, PI 615132, assumed an outlying position in the second cluster and generated unique alleles in 10 of the polymorphic loci examined (data not shown).

A scatter plot based on PCOA supported UPGMA clustering and revealed a clear delineation between the two *C. pepo* subspecies. All the resistant accessions grouped in subsp. *pepo* (Fig. 2). The PCOA also confirmed subclustering according to fruit morphotypes within subsp. *texana*. The first three PCOA coordinates explained 58% of the variability contained in the genetic distance matrix.

Genetic distance. Genetic distance (GD) among all genotypes ranged from 0.12 to 0.68 (Table 2). Among cultivars of subsp. *texana*, the average GD was 0.41, which was largest between Acorn and Crookneck morphotypes (0.52), but lowest between Straightneck and Crookneck (0.33) (Table 3). Similarly, the average GD among cultivars of subsp. *pepo* was 0.39 and was largest between Zucchini and Pumpkin morphotypes (0.42) but lowest between the two Pumpkin cultivars (0.17). As expected, the average GD between subsp. *texana* and subsp. *pepo* (intersubspecific) (0.56) was higher than that observed among different morphotypes within each subspecies (intrasubspecific) (0.42–0.52) (Table 3). Previous studies also support a general trend in which GD between subspecies is larger than that among morphotypes within the same subspecies. For example, Gong et al. (2012) reported larger GD between subsp. *texana* and *pepo* (0.67), than among morphotypes within the same subspecies (0.27–0.41). Similarly, Paris et al. (2003) observed larger genetic separation between subsp. *texana* and *pepo* (average GD = 0.22) than among morphotypes within the same subspecies (0.09–0.11).

Overall, there was low average GD among the 16 resistant accessions (0.31), with the least GD between PIs 179267 and 209783 (0.12), and largest between PIs

Table 3. Average genetic distances between 16 *Cucurbita pepo* accessions resistant to *Phytophthora* crown rot and susceptible morphotypes of subsp. *texana* (Acorn, Straightneck, and Crookneck) and *pepo* (Pumpkin and Zucchini).

Morphotype	Resistant accessions	Acorn	Zucchini	Pumpkin	Crookneck
Acorn	0.60				
Zucchini	0.34	0.57			
Pumpkin	0.40	0.59	0.42		
Crookneck	0.56	0.37	0.57	0.51	
Straightneck	0.61	0.52	0.63	0.53	0.33

615142 and 615132 (0.61) (Table 2). In order of increasing GD, the resistant accessions were least distant to Zucchini (GD = 0.34), followed by Pumpkin (GD = 0.40), Crookneck (GD = 0.56), Acorn (GD = 0.60), and Straightneck (GD = 0.61) morphotypes (Table 3). The low average GD (0.37) between the resistant accessions and morphotypes of subsp. *pepo* implies that it may be easier to transfer *Phytophthora* crown rot resistance and recover elite genetic background through backcross breeding (Loy, 2011). On the other hand, transfer of resistance from the PIs into morphotypes of subsp. *texana*, and the ensuing recovery of elite parental background, may take longer due to a higher GD (0.59) that could result in vast segregation of fruit phenotypes as commonly observed in intersubspecific crosses involving *C. pepo* (Loy, 2011). However, breeders can use GD information to select parents with the least genotypic differences (Gong et al., 2013; Paris, 2001). Among the most resistant accessions, PI 181761 (DS = 1.3) was genetically closest to subsp. *pepo* (average GD = 0.36) and would be the valuable source of resistance for morphotypes within the subspecies. On the other hand, PI 174185 (DS = 1.4) was more genetically similar to subsp. *texana* (average GD = 0.51) and would be a valuable source of resistance for morphotypes within the subspecies.

Another important implication of GD is in the selection and use of multiple resistant accessions in the breeding program that would increase the likelihood of incorporating diverse resistance alleles. Although information on the genetic basis of crown rot resistance is currently unknown for the resistant PIs, breeders can select accessions that are least related based on GD data, thus increasing the likelihood of accumulating different alleles in the breeding program (McGregor et al., 2011). This is particularly important in cases where gene pyramiding is likely to offer broad and durable disease resistance (Nelson et al., 2017; Stuthman et al., 2007). Although PIs 181761 and 174185 would be valuable sources of resistance for subsp. *pepo* and *texana*, respectively, they are genetically very similar (GD = 0.28) and thus potentially carry the same resistance alleles (McGregor et al., 2011). In contrast, PI 615132 (DS = 1.3) is not closely related to either PI 181761 (GD = 0.53) or PI 174185 (GD = 0.55) and thus may have unique resistant alleles. Therefore, breeders should consider using PI 615132 in tandem with PIs 181761 and 174185 as sources of resistance to *Phytophthora* crown rot.

Overall, data (dendrogram, PCOA, and GD) presented here support a closer genetic relationship among the resistant accessions and morphotypes of subsp. *pepo* than those of subsp. *texana*. Further, considering intergenotype GDs, PIs 181761 and 174185 are the best sources of resistance for subsp. *pepo* and *texana*, respectively. However, PI 615132 may harbor unique resistance alleles that could augment resistance to *Phytophthora* crown rot in breeding programs. The results of the current study will be useful for future breeding and genetic studies.

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Supplemental Table 1. Mean disease severity (DS) score and country of origin of 16 *Cucurbita pepo* accessions resistant (mean disease score < 2.5) to *Phytophthora* crown rot.

Accession	Country	DS ²
PI 181761	Lebanon	1.3
PI 615132	Mexico	1.3
PI 174185	Turkey	1.4
PI 615142	Kazakhstan	1.4
PI 169417	Turkey	1.6
PI 266925	Germany	1.8
PI 209783	Germany	1.9
PI 512709	Spain	1.9
PI 169450	Turkey	2.1
PI 181944	Syria	2.1
PI 167053	Turkey	2.3
PI 169476	Turkey	2.3
PI 179267	Turkey	2.3
PI 181878	Syria	2.3
PI 288240	Egypt	2.3
PI 299574	South Africa	2.5

²Mean disease severity score determined by Padley et al. (2008) on a scale of 0 to 5.

Supplemental Table 2. Thirty-nine simple sequence repeat primers used in this study (Gong et al., 2008b).

Marker name	Forward primer	Reverse primer	Expected size (bp)	Linkage group	M13-primer fluorescent label
CMTp21	TGTCCAATTCTTCATTGCTCA	GGATTCCACCACAATTGAGA	201	LGp7	6-FAM
CMTp26	GTCTTGGTCTTGGGGTGGTT	AAACAAAGTGGCGGGTGTC	187	LGp10a	VIC
CMTp36	GAAGTGCCGTGGCTGAGAG	TTCAGCTTCCCGGATTATGG	151	LGp12	PET
CMTp37	GTCTGGTCTTGGGGTGGTTC	AGAAACAAAGTGGCGGGTGT	186	LGp10a	6-FAM
CMTp38	AGGATTTTCAGGTATGGTCGTG	AGACGTTCCCTCCCTCT	160	LGp8	VIC
CMTp39	GGCGAAAAGGAAGAACGAAT	TTTTTCTCCCCCTCCACAT	132	LGp9	PET
CMTp53	ACAACCATCACACGCTTTC	TTGGAATCAGTTCAGTTCG	120	LGp8	6-FAM
CMTp61	AAGGGTTCGTCGGAGAAACT	CAACGACTCCCAGCACCTAT	118	LGp6	VIC
CMTp62	GTGCCCCGTCAGTCGGAAT	TGTCGACGAAGATAGCAATAGCA	134	LGp4	PET
CMTp68	CAGACCCATTTCATTTGACC	ATTGATTGGGACGTGAGGAA	189	LGp13	6-FAM
CMTp74	ACCCCTCTTGTCTTCAAGG	TTACTCCCATTTTCCCATTT	184	LGp3A	VIC
CMTp75	TGGGGAAAATGGGAGTAAGAG	TGCTTGCCTTGGCTTACATA	118	LGp3A	PET
CMTp76	TGAAACAGTCATCTCTTTTGTGA	TCTCTGGGTGTGTTTCTTGC	175	LGp6	6-FAM
CMTp77	GGCCATATACGAGGTCAGGTT	TTAGCGATTCAACGGAGGTC	102	LGp20	VIC
CMTp84	GCGGTAACAGGTTGTTTGGT	CCATCAGGATACCTACAAAGG	150	LGp19	PET
CMTp106	ACCCTAAGCCAGATATTCATGC	GGGCTTCATGCATCTAGTTTG	153	LGp5	6-FAM
CMTp109	CAGAGCACCAGATCAGTGGA	GCAAAGCCTCCGCTCTATT	104	LGp7	VIC
CMTp121	CCCAACCCGTCATAAACA	TTTAGGCCCAATGTTTGTCT	217	LGp7	PET
CMTp127	TTCTCTGTTTCCGTCATG	CACAAAAGGGGTTTGAGGTT	112	LGp14	6-FAM
CMTp129	CTCTTGCTCATCTTCTTGTG	CCCACCCATTTACCCTCTAGT	147	LGp5	VIC
CMTp133	TGCTCTCTTTGAATTCAGCAT	AGGGATCTTCCATTTCAT	113	LGp17	PET
CMTp141	ATTCATGTCATACTACCGACTTC	CGGATTATATAAGATGGCAAG	135	LGp12	6-FAM
CMTp176	CCTGGACTTCCACATCAGTT	ACTACGTGTCTCTGCAGGAAG	111	LGp14	VIC
CMTp177	AGCCAAACCAGCATCGAAAT	CGACGATGTCAAACCTCCAC	194	LGp18	PET
CMTp178	GAACATTTCTGGTTCAATC	CTTTCAAAGAAGCGTAAAG	132	LGp15	6-FAM
CMTp201	AGGAGTGGTGGGCTAATACG	TGAAATTGAGGGAGGAGAG	110	LGp1	VIC
CMTp202	GTAAGGCGTGGTTGGAAGC	GTTCCCTTTCCCCCTTTGTA	156	LGp15	PET
CMTp205	CATGAAACGATATGAGTAGG	TGAGGTATTTTAGGAATAAAGA	143	LGp6	6-FAM
CMTp206	CCACCGATTGCAAAATTCAG	TTCCGAAAAGAAACCGTTACC	114	LGp16	VIC
CMTp224	CACCGACGACTCCATCATC	CTTCTTGTCCTCCCAAAATCACA	151	LGp6	PET
CMTp229	GATCCTACACGCCGTTTGTG	TCATGACCTCGTCACTCTCG	103	LGp7	6-FAM
CMTp231	TCTGGTCTTGGGGTGGTTCT	AGAAACAAAGTGGCGGGTGT	170	LGp10a	VIC
CMTp235	ATGATGGAGTCCAGTCGAG	ACCCACACACCCTCTCCTC	148	LGp5	PET
CMTp239	ATCTCGGTAAAGCTCCGTTC	TATGGTCTGCTCCACAAT	101	LGp10a	6-FAM
CMTp245	AGGTAACCTGACCCAGCTA	CCCATCTCTCAAGCTCATCC	134	LGp11	VIC
CMTp249	GCAATCATTAAGGAATGGCACT	CCGACCCATGGATCTTATCTT	175	LGp3A	PET
CMTp260	CCCTAGACCCATCATAGTCG	ACATTTGGTTACTTCCCCATT	155	LGp7	6-FAM
CMTp264	TGTCCATAGTCGAACGTCCA	CCCATTGTGATGATGAGTACG	104	LGp6	VIC
CMTp265	CCTCTCATTTCTCCCATCTC	TGATCCGGTAGGGGTCTACTC	102	LGp11	PET