GENETIC DIVERSITY, POPULATION STRUCTURE AND HOST RESISTANCE TO PHYTOPHTHORA FRUIT ROT IN THE SOLANACEAE

By

Rachel Pearl Naegele

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ABSTRACT

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Production of eggplant (*Solanum melongena*) and pepper (*Capsicum annuum*), the third and fourth most important solanaceous crops worldwide, are limited by diseases caused by the oomycete pathogen *Phytophthora capsici* Leonian. In peppers, fruit rot resistance 3 and 5 days post inoculation (dpi) was mapped in an F6 recombinant inbred line population between a resistant, landrace Serrano and susceptible, cultivated Jalapeño. Isolate-specific interactions were evident and 10 quantitative trait loci were identified in the population with low to moderate effects. Diverse collections of eggplants (99) and peppers (160) were evaluated for genetic diversity, population structure, and fruit rot resistance to two isolates of *P. capsici*. In the eggplant and pepper collections, four genetic clusters were detected by Bayesian analysis. Resistance to one or both isolates was found for at least one accession in both collections. In the eggplant collection, population structure was detected when individuals were grouped by the following predefined categories: disease resistance, country of origin, continent of origin, and fruit shape. In the pepper collection, population structure was detected when individuals were grouped by disease resistance, country of origin, and continent of origin. These results provide a baseline for future work utilizing global pepper and eggplant resources, and developing Phytophthora fruit rot resistant cultivars.

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LITERATURE REVIEW

The Solanaceae is a large plant family consisting of more than 2500 species in 100 genera (81). This family has a great diversity of growth habits and fruit characteristics, but few of these species have any economic importance. Potatoes, tomatoes, eggplants and peppers are the major solanaceous food crops and they account for a variety of food, medicine, spices and ornamentals worldwide. A number of important weeds including nightshade are also included in the Solanaceae. Potatoes, the 3rd most important crop in the world, is the most important solanaceous crop and generates over $3.5 billion annually in the U.S. alone (USDA ERS, 2010.) Pepper and eggplant, the fourth and third most important solanaceous crops worldwide, generate an estimated $802 (pepper) and $42 (eggplant) million dollars annually in the U.S (USDA NASS). China, the leading producer of both eggplants and peppers, produces 27.7 and 15.5 million tons per year, respectively (FAO, 2011).

The Solanaceae encompasses both Old and New World species. Many cultivated solanaceous crops are New World species, originating from the Americas. Eggplant is one of the few cultivated species from the Old World. Centers of origin and diversity are often important sources of genetic diversity, and individuals from these areas can be used for crop improvement. Wild and landrace individuals can harbor many important agronomic traits including improved yield, drought and disease resistance.

The eggplant complex in its most simplified form consists of the wild and semi-wild species *Solanum incanum* and *Solanum melongena* each with four groups (A-D and E-H, respectively) (121). Domestication of the cultivated eggplant, *S. melongena* is thought to have occurred in Asia as early as 59 B.C. (52,75,118). Since that time, it has

been transported and cultivated around the world (93,121). Studies have indicated that the progenitors of domesticated eggplant (*S. melongena*) originated in Africa and were derived from the closely related *S. incanum* (part of the *S. melongena* complex) (24) and *S. linnaeanum* (121). Both *S. incanum* and *S. linnaeanum* can form partially fertile hybrids with *S. melongena* making them potential sources for desirable traits (24,25,46).

Pepper, similar to many New World crops, originated in Central and South America. Domestication of pepper is estimated to have occurred between 5000 and 6000 B.C. in the Americas where it was primarily used as a spice (86,88). Upon discovery of the Americas, peppers were transported to Europe, and subsequently the world. Cultivated pepper consists of the five species: *Capsicum annuum* and *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* (1,51,84). Crossability varies between the species, but it is generally accepted that all cultivated species but *C. pubescens* are relatively crossable (42). In the U.S., *C. annuum* is the primary pepper species grown, and includes both pungent chile-type and non-pungent bell-type peppers (42,84). Mexico is the center of origin and diversity for *C. annuum* (30,53). The remaining pepper species originated in various parts of Central and South America, and are now predominantly produced in South America or Asia (3,30,42,87). Aji peppers, Peruvian peppers and habanero are common names of cultivated non-*C. annuum* species. Species, geographical and market separations have lead to the distinct genetic pools among and between pepper species. Studies have shown that there is variability for agronomic traits of interest between species, but there is also variability between market classes within species (87,104,115).

Understanding the genetic diversity and population structure within a species is important for efficient utilization of germplasm resources. Historically, genetic diversity has been the traditional method for species evaluation rather than population structure. A number of marker types have been implemented to identify individuals with the greatest variability using genetic, isozyme, morphological, and most recently, metabolite markers (49,51,60,104,105,115). In addition to predicting heterosis when breeding, genetic diversity can also be used to provide an overview of the total diversity available within a population or collection to develop "core collections", subsets of individuals who together represent 80% of the variation of the collection. These collections provide useful subsets for screening individuals for particular traits of interest.

Population structure analysis has recently been gaining popularity as a method to detect and visualize spatial and temporal differences between genetic subpopulations (23,33,55,95,96,107). Information on population structure can provide insight about connections between phenotypic variation and the distribution of genetic diversity. Marker-trait evaluations, such as association mapping, rely on large populations to detect phenotypic correlations. Genetic relatedness can have a significant effect on these studies if population structure is not taken into account. False trait associations are common when populations exhibit genetic structure and mathematical models have been developed to account for this (13,58). If population structure is known prior to studies, it can improve utilization of the germplasm through the selection of individuals. Fruit shape, disease resistance, and other characteristics can all be affected by population structure. If population structure is present in materials being evaluated for association mapping, spurious associations may be made between a particular genotype and the trait

of interest (123). Combining phenotypic values with genetic population structure can yield useful results for association mapping and breeding.

In peppers and eggplants, significant genetic diversity and population structure within, and between, geographic regions exists (1,2,51,52,60,77,82,83,91). In peppers, most studies have focused on genetic diversity and population structure within the centers of origin ((1,3,49,67,83). Demonstrated population structure between wild, semi-wild and domesticated *C. annuum* in Mexico was identified (1,83). Population structure has also been evaluated in Italian, Tunisian and Turkish populations of peppers (2,10,60,91). Each of these studies evaluated genetic diversity and/or population structure. No studies have evaluated the level of diversity and population structure on a global basis or linked those results with phenotypic traits of interest. Previous studies have shown that admixture between pepper species is limited, consistent with reports of low crossability between some species (51,104). Similar to peppers, many studies have looked at the genetic diversity of eggplants within specific countries or regions, and a recent study compared genetic differentiation and structure in three countries (6,34,52,59,70,72,75,78,90,93). Most studies have looked at eggplant diversity within a single region (6,75,78), but many have also looked at variability between *Solanum spp.* to solidify the boundaries between cultivated eggplants and their relatives (6,34,70,90). Eggplants have a high degree of morphological plasticity making species designations difficult without the use of molecular markers (121).

Disease resistance is an important attribute for modern cultivars of peppers and eggplants. One disease, Phytophthora fruit rot, is caused by the destructive oomycete *Phytophthora capsici* L. This pathogen can infect multiple solanaceous species including

eggplant, pepper, and tomato (31,40,97). It can infect roots, stems, fruit and foliage of peppers or the roots, crown and fruit of eggplants at any point during development (47,53,61,100,116). In peppers, this disease causes losses worldwide. Eggplant is affected by Phytophthora root rot less frequently, and fruit symptoms are the most common in the field (37). Disease management utilizes a combination of chemical and cultural controls to reduce losses, but economic reductions in yield can still occur under conditions favorable for disease (40,47).

Phytophthora capsici is a generalist pathogen and has a large host range encompassing over 50 species including most cucurbits, beans and some brassicas in addition to the Solanaceae. This broad host range limits the effectiveness of management using crop rotations. The pathogen boasts a polycyclic disease cycle and can persist in the soil as thick walled oospores for 10 years (40). In many instances, both mating types of the pathogen have been identified in an area allowing sexual (oospore) reproduction, in addition to asexual (sporangia and zoosporangia) reproduction (20,40). Because of the genetic variability from sexual reproduction and random mutations, this pathogen also has the ability to quickly develop fungicide resistance in the field and overcome host resistance (40,47,66).

Phytophthora species, commonly called water molds, are superbly adapted to dispersal through water and water control is essential to reduce the spread and severity of the disease (36,40,47,102). While some *Phytophthoras* have evolved to utilize wind dispersion, *P. capsici* relies solely on water dispersion (40,41,57). During a rain or irrigation event, the cytoplasm of a sporangium can differentiate into 20-40 two-tailed zoospores (40,47). These zoospores can swim to a new host, attach, germinate and

penetrate the host tissue resulting in infection (11,64,122). In peppers, cultivar and germplasm testing for root rot resistance has identified multiple lines with resistance to one or more isolates, but few lines exhibit resistance to all isolates (18,62,73). The broad host range, persistent biology and genetic diversity make *P. capsici* incredibly difficult to manage.

First described by L. Leonian in 1922, *P. capsici* was identified on pepper fruit in New Mexico (69). Since this time, the more common root rot disease in pepper has upstaged fruit rot and fruit rot resistance. Most studies and breeding have emphasized the identification and implementation of genetic control through quantitative trait loci (QTL) for the root rot symptom of the disease in peppers. Studies have estimated the number of QTL for root rot resistance to vary drastically from a single dominant gene with modifiers, to 14 QTL (5,12,68,80). A major QTL for root rot resistance was identified on chromosome 5, and molecular markers have been developed for marker-assisted selection (68,98). Development and incorporation of resistant varieties into commercial production systems has been slow. A few tolerant varieties have been bred, but most are susceptible to highly virulent isolates of *P. capsici* (31) and utilize a common source of resistance. Criollo de Morelos 334 (CM334), a landrace from Mexico, is a small fruited Serrano-type pepper with resistant to all isolates evaluated to date (18,31,38,110). Most breeding for Phytophthora resistance and molecular evaluation of resistance has utilized this line (101).

Comparative studies between root rot and foliar blight in peppers suggested that there is a single dominant gene controlling much of the foliar resistance. This gene differed from the major gene contributing to root rot resistance (109,116). These results

were later confirmed by Ogundiwin et al. (80) in a separate mapping population where QTL were detected for foliar blight resistance with minimal overlap with root rot resistance. The independent associations between the foliar blight, stem blight, and root rot symptoms of the disease were also seen in a mass greenhouse evaluation by Candole et al. (18).

Root rot is less prevalent in eggplants, and mechanisms controlling resistance are less well studied than those in pepper. A study examined the resistance of two eggplant breeding lines and a single commercial cultivar to multiple isolates of *P. capsici* (32). The commercial cultivar was resistant to moderately virulent isolates, but was susceptible to highly virulent isolates. The two breeding lines had high levels of resistance to most isolates of *P. capsici* evaluated.

Fruit and root rot occur in the field, but fruit rot is more common in eggplant (37). In the field, chemical management is expensive and provides limited protection against Phytophthora fruit. Host resistance, an important part of a successful, sustainable management program, is not currently available for management of Phytophthora fruit rot and currently, no known eggplant or pepper lines or cultivars are resistant. The inheritance of fruit rot resistance in pepper has been evaluated in a single study (106). In 1978, Saini and Sharma looked at the inheritance of fruit rot in a mapping population in the field and found that it segregated in a 3:1 Mendelian fashion. They concluded that a single dominant gene controlled fruit rot resistance. Other studies have looked at morphological and physiological traits correlated with fruit rot resistance in pepper (9,111). Only cuticle thickness and reactive oxygen species production were found to be associated with ontogenic resistance in peppers (9). In eggplant, this pathogen has been

of minor importance and no studies have been done to identify resistant germplasm, QTL, or markers associated with fruit rot resistance.

Pepper and eggplant are two economically important crops with a wide range of uses worldwide. In field production, *P. capsici* can cause large losses in yield on both when conditions are suitable for disease. Current cultivated varieties are susceptible to Phytophthora fruit rot and future breeding activities should include fruit rot resistance. Identifying resistant materials using existing germplasm resources, and characterizing the population structure and genetic diversity of those resources is needed for identification and efficient implementation of Phytophthora fruit rot resistance in peppers and eggplants.

CHAPTER 1: QTL MAPPING OF FRUIT ROT RESISTANCE TO THE PLANT PATHOGEN *PHYTOPHTHORA CAPSICI* L. IN A RECOMBINANT INBRED LINE *CAPSICUM ANNUUM* L. POPULATION

ABSTRACT

Phytophthora capsici is an important pepper *(Capsicum annuum* L.) pathogen causing fruit and root rot, and foliar blight in field and greenhouse production. Determining the genetic basis for fruit rot resistance will greatly improve the efficiency of incorporating resistance into commercial cultivars. Previously, an F6 recombinant inbred line population was evaluated for fruit rot susceptibility and isolate-specific partial resistance were found among lines. In this study, Phytophthora fruit rot resistance was mapped in the same F_6 population between Criollo del Morelos 334 (CM334), a landrace from Mexico, and the cultivar 'Early Jalapeno' using a high-density genetic map. Isolate-specific resistance was mapped independently in 66 of the lines evaluated. Heritability of the resistance for each isolate at 3 days post inoculation (dpi) and 5 dpi was high h^2=0.63 to 0.68 and h^2=0.74 to 0.83, respectively. Significant additive and epistatic quantitative trait loci (QTL) were identified for resistance to *P.capsici* isolates OP97 and 13709 (3 and 5dpi) and 12889 (3dpi only). Mapping of fruit traits showed potential linkage with few disease resistance QTL. The partial fruit rot resistance from CM334 suggests that this may not be an ideal source for fruit rot resistance in pepper.

INTRODUCTION

Phytophthora capsici Leonian, is an important pathogen of pepper, *Capsicum annuum*, in the U.S and worldwide. This destructive pathogen is capable of infecting roots, stems, fruit and foliage of peppers at any point during development (47,53,61,100,116). Disease management utilizes a combination of chemical and cultural controls to reduce losses, but economic reductions in yield can still occur under conditions favorable for disease (40). The pathogen's biology, polycyclic reproduction and the development of thick walled oospores, supports long survival in the soil, rapid development of fungicide resistance and suppression of host resistance (40,47,66). Screening of pepper germplasm for root rot resistance has identified multiple lines with resistance to one or more isolates, but few lines exhibit resistance to all isolates ((18,62,73). *Phytophthora capsici*'s broad host range, persistent survival structures and isolate diversity make *P. capsici* incredibly difficult to manage.

Most studies and breeding have emphasized the identification and implementation of genetic control through the use of quantitative trait loci (QTL) for the root rot symptom of the disease. Studies have estimated the number of QTL for root rot resistance to vary drastically from a single dominant gene with modifiers, to 14 QTL (5,68,80). A major QTL for root rot resistance was identified on chromosome 5, and molecular markers have been developed for marker-assisted selection (68,98). Development and incorporation of resistant varieties into commercial production systems has been slow. A few tolerant varieties have been bred, but most are susceptible to highly virulent isolates of *P. capsici* (31). Criollo de Morelos 334 (CM334), a landrace from Mexico, is resistant to root rot for all isolates evaluated to date (17,31,32,38,110). Most breeding for

Phytophthora resistance and molecular evaluation of resistance has utilized this line (101).

Breeding for the other symptoms of the disease (fruit rot and foliar blight) has been limited. Comparative studies between root rot and foliar blight suggest that there is a single dominant gene controlling much of the foliar resistance. This gene was different from the major gene contributing to root rot resistance (109,116). This was later confirmed by Ogundiwin et al (80) in a separate mapping population where QTL were detected for foliar blight resistance with minimal overlap with root rot resistance. The independent associations between the foliar blight, stem blight, and root rot symptoms of the disease were also seen in a mass greenhouse evaluation by Candole et al. (17). Resistance to fruit rot is even less studied. Commercial cultivars are susceptible to Phytophthora fruit rot and no QTL for fruit rot resistance have been identified.

The inheritance of fruit rot resistance has been evaluated in a single study (106). In 1977, Saini and Sharma looked at the inheritance of fruit rot in a mapping population and found that it segregated in a 3:1 Mendelian fashion. They concluded that a single dominant gene controlled fruit rot resistance. Previous work by Naegele et al, in a recombinant inbred line (RIL) pepper mapping population with a different source of resistance, demonstrated partial and isolate-specific resistance to *P. capsici* (Naegele and Hausbeck (in review)).

Maintaining desirable fruit traits such as color, firmness, and gloss is important when breeding resistance into a cultivated background from landraces and wild relatives. Often, wild relatives are small fruited, pungent and have many characteristics not suitable for commercial production. Landraces, though more similar to their commercial

counterparts, often contain a number of undesirable traits. Numerous studies have looked at correlations between fruit characteristics and Phytophthora fruit rot. Pungency, pericarp thickness and fruit firmness were shown to not be associated with fruit rot (9,111). Reactive oxygen species (ROS) production had a negative correlation with disease susceptibility, and higher length to width ratio for fruit shape had a positive correlation with fruit rot (9). Linkage of fruit rot resistance with fruit-related traits would make incorporation of the resistance into a commercial background more difficult.

The objectives of this study were to map general and isolate-specific resistance in CM334 to *P. capsici*-induced fruit rot at 3 and 5 days post inoculation in an F_6 recombinant inbred line population and to identify fruit-phenotypic QTL to test for co-localization with disease resistance.

MATERIALS AND METHODS

An F_2 derived F_6 Early Jalapeno x Criollo de Morelos (CM334) recombinant inbred line population consisting of 66 individuals previously screened for fruit rot resistance ((15,94, Naegele and Hausbeck (in review)). Three isolates of *P. capsici,* 12889 (A1, I, pepper), OP97 (A1, S, cucumber) and 13709 (A2, IS, bean), from the collection of Dr. Mary Hausbeck (Michigan State University) were used for inoculations. Isolates were characterized by mating type (A1 or A2), sensitivity to mefenoxam (I=insensitive, S=sensitive and IS=intermediately sensitive) and host of origin. In brief, three detached immature green peppers per isolate from each line were surface disinfested in a 10% bleach solution for 5 min and rinsed in distilled water. Fruit were placed into a humidity chamber and inoculated with agar plugs (6 mm in diameter) from

actively growing *P. capsici* colonies placed topside down on the pepper fruit and covered with sterile, micro-centrifuge caps. Control peppers were inoculated with a V8 agar plug and covered with a sterile, micro-centrifuge cap. Peppers were kept in the humidity chambers under constant light at room temperature (25 ˚C). Peppers were evaluated at early (3dpi) and late (5dpi) responses based preliminary results of the earliest symptoms on commercial peppers and longest time to cover the fruit (data not shown). Three and five days post inoculation, lesion width and diameter was measured for each individual fruit using a hand caliper. The experiment was repeated two times for a total of 9 peppers per line per isolate.

Fruit evaluations for fruit length, width and shape were previously performed as described by Naegele and Hausbeck (in review). In brief, 20 peppers from each line were evaluated for fruit length (cm), width (cm), shape (maximum length/width in cm), and color (light green, green, dark green, purple). In addition twenty peppers from each line were evaluated for gloss (low, medium, high), firmness, (1 to 3), and pericarp thickness (in cm). Fruit length, width, shape and pericarp thickness were measured using a hand caliper. Gloss and firmness were determined as described by Chaim et al (19).

Data were analyzed using the PROC MIXED function of the SAS v9.3 software using the LSmeans statement (SAS Institute Cary, NC). Significant ($P=0.05$) interactions between line and isolate were separated using the SLICE option. Line means (LSmeans output) for isolate-specific lesion area 3 and 5 dpi were used for QTL mapping. Line means for fruit gloss, firmness and pericarp thickness were calculated in

SAS and used for QTL mapping. Narrow-sense trait heritability was estimated using the progeny mean basis method (29,118).

QTL analysis was performed using R/QTL function in the statistical software R (15,16,99) using the genetic map previously built by Hill et al (50). The map was reconstructed in R/qtl. Prior to QTL mapping, the linkage map was evaluated for recombination frequency, marker order, segregation distortion and switched genotypes. Problematic markers were removed from the map. The final map contained 3,814 markers with 222 to 450 markers per chromosome with an estimated coverage of 1267 cM (Table 1.1).

Table 1.1. Distribution and marker number for the pepper recombinant inbred line (RIL) genetic map

Chromosome	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
# of markers	450	407	415	244	222	267	140	261	513	305	363	227
cM distance	140	101	124	100	78.5	122	116	86	90	107	113	89.9
Estimated coverage: 1267.2												

Total number of markers per chromosome from P1 to P12 (# of markers), the Centimorgan (cM) distance per chromosome (cM distance) and the estimated coverage of the genetic map in cM (Estimated coverage).

Missing genotypes were identified at each marker and the genotype was estimated using the imputation method implemented in R/qtl. QTL for each trait were identified using interval mapping by the multiple imputation method. Significance of QTL was determined using 1000 replicates of the permutation test. After a single significant QTL was identified, additional additive and epistatic QTL were added using composite interval mapping. Multiple QTL, interactions and effects of individual QTL were confirmed and

estimated using a general linear model implemented in R/QTL where y equals the sum of the individual QTL and their interactions (y~Q1+Q2+Q1*Q2).

RESULTS

QTL for resistance to Phytophthora fruit rot were detected for isolates 12889, 13709 and OP97 at both 3 and 5 days post inoculation. Significant LOD thresholds at *P*=0.05 were 3.11 to 3.36 for disease responses. QTL for fruit characteristics had significant LOD thresholds at *P*=0.05 for gloss, firmness, fruit shape and pericarp thickness at 3.26, 3.32, 2.97 and 3.3, respectively.

At 3dpi, 34 lines were resistant or partially resistant to *P. capsici* (data not shown). Heritability of disease resistance to individual isolates was 0.68, 0.63 and 0.63 for isolates 12889, 13709 and OP97, respectively. All QTL were significant at *P=0.01* unless specified otherwise. Isolate-specific QTL with varying effects were detected for each of the *P. capsici* isolates tested. For isolate OP97, two QTL were detected. One QTL, located on chromosome (chr) 6 at 56 cM, explained 14.8% of the variation seen and another on chr 5 at 21 cM, explained 12% of the trait variation. The resistant allele on chr 6 was from the susceptible parent ('Early Jalapeno') (Table 1.2).

Table 1.2. Fruit rot resistance QTL

QTL with additive effects

Trait	Chr.	Pos.	Marker name	LOD Value	R^2	Source	Sig	Est. h^2
12889	2	13	CAPS_CONTIG. 10457	3.33	0.167	CM	**	0.68
3dpi	6	58.1	CAPS_CONTIG. 3200	6.66	0.381	EJ	***	
13709	5	0	CAPS_CONTIG. 10639	3.36	0.164	CM	***	0.63

Table 1.2 (cont'd)

3dpi	6	69.3	CAPS_CONTIG.10555	5.51	0.292	EJ	***	
13709	3	124	KS24039C09	4.4	0.207	CM	***	0.74
5dpi	5	71.2	CAPS_CONTIG.11780	7.49	0.330	CM	***	
OP97	5	21.1	CAPS_CONTIG.11660	5.21	0.273	CM	***	0.63
3dpi	6	56	CAPS_CONTIG.1455	3.26	0.158	EJ	***	
OP97	4	13	CAPS_CONTIG.9283	9.53	0.175	CM	***	0.83
5dpi	5	29.8	KS25046E04	4.05	0.500	CM	***	

QTL with epistatic effects

Trait	Chr.	Pos.	Marker name	LOD Value	R^2	Source	Sig
12889 3dpi	P2*P6	13*58.1	CAPS_CONTIG.10457 * CAPS_CONTIG.3200	2.76	0.136	CM*EJ	***
13709 5dpi	P3*P5	124*71.2	KS24039C09 * CAPS_CONTIG.11780	1.24	0.070	CM*CM	**
OP97 5dpi	P4*P5	13*29.8	CAPS_CONTIG.9283 * KS25046E04	3.39	0.138	CM*CM	***

Additive and epistatic effect QTL for general response resistance 3 and 5 days post inoculation (3dpi and 5dpi, respectively) and isolate specific resistance for isolates 12889, 13709 and OP97 3 and 5dpi. Chromosome (chr) and genetic map position (Pos) of the marker in cM. LOD value of the QTL, the percent of the variation explained by the QTL (R^2) and the donor parent of the positive effect allele (Source). Significance of the QTL were determined by the general linear model implemented in R/QTL at P=0.05 (*), P=0.01 (**), and P=0.001 (***). Estimated narrow sense heritability of the trait calculated using variance components (Est. h^2).

Resistance to 13709 was also correlated with two QTL. One QTL, on chr 6 at 69.3 cM, explained almost 30% of the variation and came from the susceptible parent while the other at the tip of chr 5 (0 cM) was from the resistant parent and explained 17%. Isolate 12889 also had contributions for resistance from both parents at chr 6 (58.1

cM) and chr 2 (13 cM) explaining 38 and 16.4% of the variation, respectively. An interaction between the two QTL contributed another 13%.

At 5dpi most lines evaluated were susceptible to *P. capsici*. Isolate specific responses were detected for each of the lines. The resistant parent (CM334) was the only source of resistance at 5dpi. Heritability for resistance to individual isolates was 0.76, 0.74, 0.83, for isolates 12889, 13709 and OP97, respectively. Isolate OP97 at 5 days post inoculation was the most virulent isolate and resistance to this isolate had the highest heritability. Resistant loci were detected on chr 4 (13 cM) and 5 (29.8 cM) explaining 19 and 50% of the variation observed, respectively. An interaction between the two QTL explained another 16% of the variation. Isolate 13709 had QTL located on chr 3 (124 cM), 5 (74 cM) and 6 (118 cM, *P=0.05*) explaining 14.5, 31, and 10% of the total variation observed, respectively. Interactions between the QTL on chr 5 and that on chr 6 explained an additional 7%. There were no isolate-specific QTL detected for isolate 12889 at 5 days post inoculation.

Significant QTL were detected for fruit firmness, fruit shape and pericarp thickness (Table 1.3). All QTL are significant at *P=0.01* unless otherwise specified. One QTL was detected for fruit firmness located on chr 12 at 55.4cM. This QTL explained

Table 1.3. Fruit phenotype QTL

QTL with additive effects

Trait	Chr.	Pos.	Marker	LOD	R^2	Source	Sig
Firmness	12	55.4	KS21041M02	4.486	0.298	EJ	***
Fruit shape	1	124	CAPS_CONTIG.816	1.98	0.055	CM	*
	2	71.3	CAPS_CONTIG.3755	1.83	0.051	CM	*
	4	32.2	KS20017D07	7.58	0.264	EJ	***
	5	38	KS17024G04	3.89	0.117	EJ	***
	10	2.2	KS26009E11	1.79	0.049	CM	*
Pericarp	3	114	KS17057E04	4.14	0.26	EJ	***

Table 1.3 (cont'd)

QTL with epistatic effects

Trait	Chr.	Pos.	Marker	LOD	R^2	Source	Sig
Fruit shape	P1*P4	124*32.2	KS20017D07 * CAPS_CONTIG.816	1.72	0.047	EJ*CM	*

Additive and epistatic effect QTL for fruit firmness and fruit shape. Chromosome and genetic map position (position) of the marker. LOD value of the QTL, the percent of the variation explained by the QTL (R^2) and the donor parent of the allele (Source). Significance of the QTL were determined by the general linear model implemented in R/QTL at *P*=0.05 (*), *P*=0.01 (**), and *P*=0.001 (***).

approximately 29.8% of the variation within the population. Fruit shape QTL were detected on chr 1 (*P=0.05*), 2 (*P=0.05*), 4, 5 and 10 (*P=0.05*) at positions 124, 71.3, 32.2, 38 and 2.2 cM, respectively. Together these QTL explained over 50% of the variation observed. An epistatic interaction (*P*=0.05) between the QTL located on chr 1 and 4 explained an additional 4.7%. Pericarp thickness resulted in a single QTL chr 3 (114 cM) that explained 26% of the variation. This QTL was tightly linked with a QTL for resistance to isolate 13709 5dpi. QTL for fruit firmness did not show a tight linkage (≤ 10cM) with any of the disease QTL mapped.

DISCUSSION

Host resistance to *P. capsici* is an important component for disease management in many areas of the world. Disease symptoms and the genetic factors controlling resistance to the pathogen can vary greatly depending on the site of infection. In addition to site-specific responses, isolate-specific interactions can be often host specific ((17,31,73,97)Foster and Hausbeck 2009; Quesada-Ocampo and Hausbeck 2010; McGregor 2011, Candole 2012). Even resistant commercial peppers lines could succumb

to highly virulent isolates when tested under greenhouse conditions (Foster and Hausbeck 2010). This combination of site-specific and isolate-specific responses makes breeding for resistance to *P. capsici* incredibly challenging.

Breeding for all three symptoms of *Phytophthora* infection (foliar blight, root rot and fruit rot) is important for a sustainable and effective resistance. Previously, studies have mapped root rot resistance and foliar blight in multiple pepper populations, but not fruit rot. Root rot and foliar blight are both estimated to be controlled by multiple QTL and to exhibit isolate-specific responses. Root rot is the most common symptom seen in the field, but under certain conditions, fruit rot can quickly decimate yields (8). Soil-applied systemic fungicides, a staple of any *P. capsici* management program, do not provide protection to fruit.

The idea of early and late Phytophthora resistance was first suggested on pepper roots by Pochard et al., who observed that roots exhibited an early response termed receptivity, a late response termed stability and the rate of response called induciblity (89). In our fruit study, we looked at early (3dpi) and late (5dpi) responses based preliminary results of the earliest symptoms on commercial peppers and longest time to cover the fruit. Three days post inoculation approximately half of the lines exhibited few symptoms suggesting that partial early resistance was present in a 1:1 ratio in the population, though multiple QTL were detected. Resistance at 5dpi was much less common (21 individuals) and the only QTL identified were from the resistant parent.

These results at 5dpi contrast with previous results by Saini and Sharma (106) that found resistance to be controlled by a single dominant trait. In this study, resistance to Phytophthora fruit rot at 3 and 5dpi were both controlled by multiple genes of varying

effects, with high overall heritability. In addition, the susceptible parent was also found to contribute a positive effect on disease resistance during early stages of response. It should be noted however, that as these fruit were evaluated under ideal conditions for disease that some of the lines evaluated in this study with partial resistance may be more resistant under field conditions or as the fruit matures. Studies have shown that resistance to *P. capsici* increases for fruit and plants as tissues mature (9,36,53,61). Fruit rot resistance appeared to be controlled by a single dominant gene in 'Waxy Globe', but in CM334 the trait is quantitatively inherited and highly influenced by the environment. The heritability of fruit rot resistance at 5dpi was high, and the detection of major QTL controlling resistance may make marker-assisted selection useful.

Fruit traits evaluated also showed evidence of quantitative inheritance, consistent with previous studies (19,124,126). QTL for fruit firmness did not show a tight linkage (≤ 10cM) with any of the disease QTL mapped. The fruit shape QTL on chr 5 was linked with a major effect QTL for resistance to OP97 5dpi (54% of the variation) and a moderate effect on fruit shape (~12%). The fruit shape QTL on chr 10 was a minor QTL (<10%) for both fruit rot resistance and fruit shape. This was consistent with the previous study that detected a small yet significant correlation between fruit shape and disease for at least one isolate (Naegele and Hausbeck (in review).) Pericarp thickness also had potential linkage with a disease resistance QTL with disease. This pericarp thickness QTL was within 10cM of a resistance QTL explaining 18% of the disease variation 5dpi. However, the linked QTL was only found in disease resistance to a particular isolate, and may not be useful when breeding for resistance to multiple isolates.

Most QTL identified 3 and 5dpi were located on chromosome 5, a known hot spot for *Phytophthora* resistance genes in several solanaceous species including a major gene for root rot resistance to *P. capsici* (43,112). The quantitative inheritance of fruit rot is consistent with previous studies on root rot and foliar blight in peppers (12,76,80). The resistant parent used in this study, CM334, though more tolerant than Early Jalapeno, was not immune to Phytophthora fruit rot under our conditions, and may not be the best source of fruit rot resistance. In addition, the cultivated parent (Early Jalapeno) contributed two loci on chromosome 6 to resistance 3dpi, making commercial cultivars a potential source of early resistance. In this study, the QTL effects are likely to be slightly overestimated due to the limited number of individuals evaluated in this population (Broman 2009), but provide a good first approximation. Other sources of resistance may have more complete resistance and more evaluation of lines is needed. For the QTL that were detected, further work with larger populations and more isolates will be needed to confirm the QTL in different genetic backgrounds and identify additional ones.

CHAPTER 2: GENETIC DIVERSITY, POPULATION STRUCTURE, AND RESISTANCE TO *PHYTOPHTHORA CAPSICI* OF A WORLDWIDE COLLECTION OF EGGPLANT GERMPLASM

ABSTRACT

Eggplant (*Solanum melongena* L.) is an important Solanaceous crop with high phenotypic diversity and moderate genotypic diversity. The objectives of this study were to genetically characterize a worldwide collection of eggplants evaluated for resistance to *Phytophthora capsici* fruit rot. Ninety-nine genotypes of eggplant germplasm (species, landraces and heirloom cultivars) from 32 countries and five continents were evaluated for genetic diversity, population structure, fruit shape, and disease resistance to Phytophthora fruit rot. Fruit from each line were measured for fruit shape and evaluated for resistance to two *Phytophthora capsici* isolates seven days post inoculation. One line (PI 413784) was completely resistant to both isolates evaluated, while several others exhibited isolate-specific resistance. Partial resistance to Phytophthora fruit rot was found in accessions from all three eggplant species (*S. incanum*, *S. linneanum* and *S. melongena*). Genetic diversity and population structure were assessed using 22 polymorphic simple sequence repeats (SSRs). Genetic analyses using the program STRUCTURE indicated the existence of four genetic clusters within the eggplant collection. Population structure was detected when eggplant lines were grouped by eggplant species, continent of origin, country of origin, fruit shape and disease resistance.

INTRODUCTION

Cultivated eggplant, *Solanum melongena* L., is a high-value vegetable commodity in Europe and Asia. China and India are the major producers with 27.7 and 11.9 million tons per year, respectively [2011; FAO]. Eggplant is the third most important Solanaceous crop worldwide, and fourth most important in the U.S., after potato and tomato [2011; FAO]. In the U.S., eggplants are a minor crop grown for specialty markets with an approximate production of 62 thousand tons annually [2011; FAO].

Unlike most other cultivated Solanaceous crops (tomatoes, peppers, and potatoes), eggplants are an Old World species. Domestication of the cultivated eggplant is thought to have occurred in Asia as early as 59 B.C. (52,75,118). Since that time, it has been transported and cultivated around the world (93,121). Centers of diversity for eggplant are Asia, Europe and Africa. Studies have indicated that the progenitors of domesticated eggplant (*S. melongena*) originated in Africa and were derived from the closely related *Solanum incanum* (part of the *S. melongena* complex) and *S. linnaeanum* (121). Both *S. incanum* and *Solanum linnaeanum* can form partially fertile hybrids with *S. melongena* making them potential sources for desirable traits such as abiotic and biotic disease resistance (24,25,46). Wild relatives have traditionally been a good source of disease resistance for cultivated species that exhibit lower genetic diversity (26,56,108). Heirloom varieties and landrace accessions might also harbor resistance, and are often more similar to modern cultivated varieties than wild species, making them a good source for desirable traits (78).

Phytophthora fruit rot caused by *Phytophthora capsici* L., an oomycete, is a disease that affects multiple solanaceous species including eggplant, pepper, and tomato

(31,32,40,95,97). In the field, chemical management is expensive and provides limited protection against Phytophthora fruit rot in eggplants, which is the most common *Phytophthora*-induced disease in eggplants (37). Host resistance, an important part of a successful, sustainable management program, is not available for management of Phytophthora fruit rot in eggplants and currently, no known lines or cultivars are resistant to *P. capsici*. Partial fruit rot resistance to *P. capsici* has been identified in other solanaceous species such as peppers and tomatoes (Naegele et al. unpublished; Granke et al. unpublished), but to our knowledge this has not been evaluated in eggplant.

The linkage or correlation of resistance/susceptibility to insects and pathogens with agronomic traits of interest is not uncommon in plants (22,35,74). Correlations with resistance to Phytophthora fruit rot and fruit sugar content and exocarp thickness have been found in cucurbits (74). In peppers, pericarp thickness and pungency were not correlated with resistance, but fruit shape was positively associated with increased susceptibility to Phytophthora fruit rot (44).

In addition to disease resistance, fruit shape is an important attribute for each cultivar and many studies have been performed to identify the genetic basis of fruit shape in the Solanaceae (79,94,103,113,123,126). Size, shape and color vary greatly between eggplant market classes, and it will be important to maintain this phenotypic diversity when incorporating disease resistance (90,93). This phenotypic diversity does not always translate to high levels of genetic diversity (72,93). Modern varieties of eggplant often have lower genetic diversity, and new traits are often bred into commercial varieties from landraces or wild relatives with higher genetic diversity (78). The characterization of genetic diversity is important for maintenance and utilization of germplasm resources

(wild, landrace, heirloom, breeding lines and cultivars), and the development of core collections (21,52). Genetic bottlenecks (domestication, selection of lines by market class, etc.) have limited the variability existing within cultivated lines, and eggplant has had an additional bottleneck as a result of domestication outside of its center of origin (52).

Population structure analysis has recently been gaining popularity as a method to understand and visualize spatial and temporal differences between subpopulations (23,33,55,97,107). Information on population structure can provide insight about connections between phenotypic variation and the distribution of genetic diversity. Population structure should also be taken into account when testing and incorporating desirable traits. If population structure is present in materials being evaluated for association mapping, spurious associations may be made between a particular genotype and the trait of interest (90,119). Many studies have looked at the genetic diversity of eggplants within specific countries or regions, and a recent study compared genetic differentiation and structure in three centers of diversity; however, no studies have looked at diversity and population structure in a global collection of eggplants (4,6,34,52,59,75,78,90,93).

We evaluated fruit shape, Phytophthora fruit rot resistance, genetic diversity and population structure of a diverse collection of eggplant germplasm using 22 simple sequence repeats (SSRs). Our objectives were to evaluate a worldwide collection of eggplants for population structure and genetic diversity, and to determine if the population structure is associated with fruit shape or resistance to *Phytophthora capsici*. These results provide an initial basis for understanding the worldwide population

structure of eggplants for breeding and conservation, and its relationship with disease resistance and fruit shape.

MATERIALS AND METHODS

Ninety-five accessions of eggplants (*S. melongena*), 3 accessions of *S. linnaeanum* and 1 accession of *S. incanum* were obtained from the United States Department of Agriculture Germplasm Resource Information Network (ars-grin.usda.gov), Dr. J. Prohens (Universidad de Technologia de Valencia), and the INRA (French National Institute for Agricultural Research) (Table 1.1).

Table 2.1. Eggplant germplasm used for the study of morphological and molecular variation

Species	ID	Accession	Plant ID	Country	Source
S. incanum	191	PI 500922		Zambia	USDA
S. linnaeanum	174	PI 388846	WL-74	Italy	USDA
S. linnaeanum	175	PI 388847	WL-85	Italy	USDA
S. linnaeanum	182	PI 420415	52	Colombia	USDA
S. melongena	101	C-S-16		Spain	Dr. J. Prohens
S. melongena	102	H15		Spain	Dr. J. Prohens
S. melongena	103	IVIA-371		Spain	Dr. J. Prohens
S. melongena	104	MM 108 bis		France	INRA
S. melongena	105	MM 114	Berengena larga negra	Spain	INRA
S. melongena	106	MM 1171	Large Santa Olalla	Spain	INRA
S. melongena	107	MM 1363		Costa Rica	INRA
S. melongena	108	MM 1364		Costa Rica	INRA
S. melongena	109	MM 1365		Guatemala	INRA
S. melongena	110	MM 141	Violette d'Avignon	France	INRA
S. melongena	111	MM 1750	Listada di Gandia	Spain	INRA
S. melongena	112	MM 346	Berengena redonda	Spain	INRA
S. melongena	113	MM 39	Noire de Chateaurenard	France	INRA
S. melongena	114	MM 522	Waimanolo long B1	USA	INRA
S. melongena	115	MM 56	Violette de Toulouse	France	INRA
S. melongena	116	MM 61	Zebrina	Spain	INRA
S. melongena	117	MM 64	Ronde de Valence	France	INRA
S. melongena	118	MM 69	Monstrueuse de New York	USA	INRA

Table 2.1 (cont'd)

S. melongena	119	MM 91	Black Beauty	USA	INRA
S. melongena	120	Grif 1276	46B	Thailand	USDA
S. melongena	121	Grif 14182	New Orleans Market	USA	USDA
S. melongena	122	Grif 14186	Hastings imp purple thornless	USA	USDA
S. melongena	123	PI 102727	No. 202	Uzbekistan	USDA
S. melongena	124	PI 105346	Lao Lai Hei Chieh	China	USDA
S. melongena	125	PI 115505	Giant of Benares	India	USDA
S. melongena	126	PI 140446	5917	Iran	USDA
S. melongena	127	PI 140456	7015	Iran	USDA
S. melongena	128	PI 141968	No. 1	China	USDA
S. melongena	129	PI 143410	Badenjan	Iran	USDA
S. melongena	130	PI 169641	1448	Turkey	USDA
S. melongena	131	PI 169650	2259	Turkey	USDA
S. melongena	132	PI 171851	6753	Turkey	USDA
S. melongena	133	PI 175914	9043	Turkey	USDA
S. melongena	134	PI 179500	9877	Iraq	USDA
S. melongena	135	PI 179997	10598	India	USDA
S. melongena	136	PI 181896	Aleppo 3	Syria	USDA
S. melongena	137	PI 181963	Homs 21	Syria	USDA
S. melongena	138	PI 193599	Long Violet	Ethiopia	USDA
S. melongena	140	PI 199516	M 19	Greece	USDA
S. melongena	141	PI 200881		Afghanistan	USDA
S. melongena	142	PI 204731		Turkey	USDA
S. melongena	143	PI 213193	M-57/29	Greece	USDA
S. melongena	144	PI 217962	Banjal Bemba	Pakistan	USDA
S. melongena	145	PI 223844		Philippines	USDA
S. melongena	146	PI 230333	Kairyo-onaga	Japan	USDA
S. melongena	147	PI 230334	Kitta Horyo	Japan	USDA
S. melongena	148	PI 230335	Taiwan-naga	Japan	USDA
S. melongena	149	PI 232078	Kopek	South Africa	USDA
S. melongena	150	PI 232079	Mofale	South Africa	USDA
S. melongena	151	PI 233916		El Salvador	USDA
S. melongena	152	PI 234632	Early Round Purple	South Africa	USDA
S. melongena	153	PI 241506	Badanjan	Iran	USDA
S. melongena	154	PI 249570	Makhua Proh	Thailand	USDA
S. melongena	155	PI 256077	No.1	Afghanistan	USDA
S. melongena	156	PI 263727	Rosita	Puerto Rico	USDA
S. melongena	157	PI 267104	Cylinder A-132	Former Soviet Union	USDA
S. melongena	158	PI 269600	423	Pakistan	USDA
S. melongena	159	PI 276104	Motale	South Africa	USDA
S. melongena	160	PI 286099	No. 62-46-2	USA	USDA
S. melongena	161	PI 286100	No. 62-48-2	USA	USDA

Table 2.1 (cont'd)

S. melongena	162	PI 290467	Lungi de Impant	Hungary	USDA
S. melongena	163	PI 290469	Cu-e-da-juan	Hungary	USDA
S. melongena	164	PI 304839	G2562	Brazil	USDA
S. melongena	165	PI 320501	24	Canada	USDA
S. melongena	166	PI 320504	28	Canada	USDA
S. melongena	167	PI 320509	35	Canada	USDA
S. melongena	168	PI 349612	Terongglatik	Indonesia	USDA
S. melongena	169	PI 351129	Kurume Long	Japan	USDA
S. melongena	170	PI 358232	Dolg	Macedonia	USDA
S. melongena	171	PI 358242	Morska Pata	Macedonia	USDA
S. melongena	172	PI 358244	Renski dolg	Macedonia	USDA
S. melongena	173	PI 368822	Sredno Dolg	Macedonia	USDA
S. melongena	176	PI 391646	Liu-ye-ch'ieh	China	USDA
S. melongena	178	PI 413782	22-73	Cote D'Ivoire	USDA
S. melongena	179	PI 413783	3-73	Burkina Faso	USDA
S. melongena	180	PI 413784	13-73	Burkina Faso	USDA
S. melongena	181	PI 419198	Tsu Yang	China	USDA
S. melongena	183	PI 441908	BGH 5008	Brazil	USDA
S. melongena	184	PI 452122	Lunga Violetta di Romagna	Italy	USDA
S. melongena	185	PI 452123	Tonda di Manfredonia	Italy	USDA
S. melongena	186	PI 462370	Neznyj 36	Soviet	USDA
S. melongena	187	PI 470273		Indonesia	USDA
S. melongena	189	PI 478390	O 81	China	USDA
S. melongena	190	PI 491192	Kemer	Turkey	USDA
S. melongena	192	PI 560903	Six Leaves	China	USDA
S. melongena	193	PI 561139	37	Kazakhstan	USDA
S. melongena	194	PI 561140	36	Kazakhstan	USDA
S. melongena	195	PI 593748	56A	Thaliand	USDA
S. melongena	196	PI 593806	171	Thailand	USDA
S. melongena	198	PI 593885	314	Thailand	USDA
S. melongena	199	PI 595220	Gator	USA	USDA
S. melongena	200	PI 600912	Little fingers	USA	USDA
S. melongena	201	PI 606714	Pompano market	USA	USDA
S. melongena	202	PI 639121	Puerto Rican beauty	Puerto Rico	USDA
S. melongena	203	PI 639122	Blackee	USA	USDA

Seeds were planted into 72-cell trays containing a soilless peat mixture (Suremix Michigan Grower Products, Inc. Galesburg, MI) in a polyethylene greenhouse (MSU Horticulture Teaching Farm, East Lansing, MI). Eight weeks after planting, seedlings

were transplanted to the MSU Plant Pathology Farm (East Lansing, MI). Individual lines were planted into single plots. Each individual line was established in 3 m long plot and 12 lines were planted per row. Within rows, plants were spaced 0.45 m apart. Rows were spaced 2.4 m apart, covered with black plastic mulch, and grown according to local practices. Immature eggplant fruit of marketable size were hand harvested and brought to the lab for inoculation and evaluation.

Two *P. capsici* isolates were selected from the long-term collection of Dr. Mary K. Hausbeck at MSU. Isolates were characterized by host of origin, mefenoxam sensitivity [insensitive (I) or sensitive (S)] and mating type (A1 or A2). Isolate 12889 (pepper, I, A1) and isolate OP97 (cucumber, S, A1) were maintained on unclarified V8 agar at 25 °C under constant light. Prior to inoculations, isolates were activated by inoculating and recovering each isolate from an individual pepper fruit to ensure virulence.

For inoculation, a single 6mm-diameter plug from an actively growing *P. capsici* isolate on V8 agar was placed, mycelium side down onto a non-wounded eggplant fruit surface-disinfested in 10% bleach for 5 min. Control eggplants were inoculated with a single 6mm-diameter sterile plug of agar. Plugs were covered with a sterile microcentrifuge tube and affixed into place with petroleum jelly. Eggplants were placed into a humidity chamber consisting of an aluminum pan with a ring of moistened paper towel around the edge, covered with plastic wrap, sealed with tape and kept under constant light at room temperature (25 °C). Three fruit from each eggplant line were evaluated per isolate. The experiment was performed three times. An experiment replicate included three fruit for each isolate of every line evaluated in a completely

randomized design (CRD) blocked by isolate. One line (PI 500922) was repeated only one time for a total of two experimental replicates of three fruit per isolate due to poor fruit set. Two control fruit were inoculated with a sterile plug of V8 agar for each line.

Eggplant fruit were evaluated for disease severity seven days after inoculation. Fruit were evaluated on the following progressive scale based on percentage of fruit diseased to account for differences in fruit size: 0= no visible symptoms, 1=<25% of the fruit was symptomatic, 2= 25% to <50%, 3= 50% to <75%, 4= 75% or greater percent symptomatic area (Fig. 2.1).

Figure 2.1. Eggplant Phytophthora fruit rot disease rating scale shown on various eggplant genotypes: 0= no visible symptoms, a rating of 1=<25% symptomatic area, 25%>2<50%, 50%>3<75%, and a rating of 4≥75% symptomatic area of the fruit. Visible pathogen growth was assessed as 0=absent, 1=present. (For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation).

Isolations were performed on 10% of symptomatic fruit by peeling back the external layer of the fruit and plating three small portions of fruit at the disease margin onto V8 agar plates amended with benoymyl, ampicillin, PCNB and mefenoxam [50]. *P. capsici* was identified using morphological characteristics according to Waterhouse and isolate mefenoxam sensitivity was confirmed by transferring the recovered isolates to V8 plates amended with 100ppm mefenoxam (120).

Ten immature fruit of marketable size collected from each line were measured for maximum length (cm) and midpoint width (cm) using a hand caliper. Fruit shape was calculated as the ratio of maximum length to midpoint width for each line. Fruit shape ratios were rounded to the nearest whole number. Values between 0 and 1 were considered round, 2-3 were considered oval, 4-5 were semi-elongate and greater than 5 were considered elongate.

Mean values for disease ratings for each line were estimated using the PROC MEANS function of SAS software v9.3 (SAS Institute Cary, NC). Significant differences between disease values (ratings) for lines and isolates were estimated using the PROC MIXED function of SAS software. Significant differences were detected between experiment replicates and each replicate was analyzed separately using Fisher's LSD test (P=0.05). Line-by-isolate interactions were calculated using the ANOVA slice option of PROC MIXED when $P \leq 0.05$. Lines with a consistent disease mean value of 2 or greater in each run of the experiment were considered susceptible, with a consistent mean value <2 were termed moderately susceptible, lines with a consistent mean value <1 were moderately resistant, and lines with a mean value = 0 were resistant. Significant differences for pathogen growth were estimated using the PROC GLIMMIX function of SAS at P=0.05.

Fruit shape significant differences between lines and countries were calculated using the PROC mixed function of SAS software v9.3. Countries represented by less than four lines were excluded from analyses. Unequal sample sizes among countries were accounted using the Kenward-Rogers degrees of freedom option implemented in SAS software. Line mean values for fruit shape, length and width were calculated using the

lsmeans statement of SAS software. Correlations between fruit shape parameters and disease susceptibility were estimated using the PROC CORR function of SAS. Disease susceptibility correlations were evaluated for each isolate and experimental replicate separately.

Genomic DNA was extracted from young green leaves of eggplants using the Nucleo Spin II DNA extraction kit (Machery-Nagel Germany, CAT#740770) according to the manufacturer's instructions. DNA was normalized to 5 ng/ul using the NanoDrop ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE).

One hundred ninety-two primers from previously published SSR markers (3, 52, solgenomics.org) or designed (Primer 3 http://primer3.sourceforge.net/) from putative Solanaceae defense-related genes (NCBI ncbi.nlm.nih.gov) were tested against a subset of the eggplant collection to identify polymorphic markers. Reactions were performed in 15 ul total volume and contained 1 ul DNA, and 0.15 ul GoTaq (Promega Corporation Madison, Wisconsin), 0.9 ul 25uM $MgCl_2$, 0.3 ul dNTPs, and 0.6 ul each of forward and reverse primers (Integrated DNA Technologies, Inc.), with 8.45 ul ddH_2O. PCR reactions were performed in a programmable thermal cycler (Eppendorf, Westbury, NY) using the program: initial denaturation, 94 C (3 min) followed by 35 cycles at 94 C (30s), 60 C (30s) and 72 C (1 min), with a final extension step of 10 min at 72 C. PCR products were analyzed by electrophoresis in 4% (wt/vol) agarose gel in 1x Tris-borate-EDTA buffer, stained with ethidium bromide (5 ug/ml) for visualization and compared to a 100-bp ladder (Invitrogen Life Technologies Burlington, ON Canada) to determine amplicon

sizes. SSR markers identified as polymorphic in the population were used for genetic diversity, population structure and trait associations.

Genetic diversity was estimated using Powermarker v3.25 (71) and significance at each locus was determined with 1000 permutations using the Exact test; overall genetic diversity was estimated using the Mantel test as implemented in Powermarker.

Population structure of the germplasm was analyzed using STRUCTURE v2.3.4 (92). Following preliminary analyses, burnin length, MCMC chain replication and lambda were selected to be 200,000, 500,000 and 1.52, respectively. Population number (k) was determined empirically by comparing posterior distribution likelihoods independently among 3 independent runs of K=1 to 20 as described by Evanno et al. (27). Data included 22 polymorphic SSR and were analyzed using the admixture model and correlated allele frequencies without previous population information (28,92). Fst significance between populations was determined using 1000 bootstrap replicates as implemented in Powermarker.

Visualization of the resulting Q (proportion of membership) of each individual into predefined categories (country, continent, species, disease susceptibility and fruit shape) was generated using the Population Sorting Tool (PST) in R [19,58, J.J. Morrice, unpublished]. Individuals with $Q \geq 0.6$ membership in a single subpopulation were labeled as such. Individuals with $Q < 0.6$ membership in a single subpopulation were considered admixed. Significance of population structure by continent, country, species, resistance to *P. capsici* (12889 and OP97), and fruit shape was estimated using the population differentiation test implemented in Powermarker. Significance at each locus and overall was determined using 1000 permutations. Countries represented by less than four

individuals were excluded from analyses. Significance of pairwise Fst differentiation was based on 2.5 and 97.5% confidence intervals based off of 1000 bootstrap replications.

RESULTS

Significant differences between experimental replicates indicated the effect of environmental variability on fruit disease susceptibility was high. In each repetition of the experiment, there were significant differences among plant lines (P<0.0001). No significant differences were found between isolates in any replicate of the experiment (P= 0.32, P= 0.43, and P= 0.43). The interaction between line and isolate was significant for each replicate (Replicate 1: P= 0.0008; Replicate 2: P< 0.0001; and Replicate 3 P< 0.0001) of the experiment. Differences in pathogen growth (absence/presence) and the interaction between pathogen growth and line were not significant in any replicate (approximately P=1.0 for each). The majority of the lines 89% and 87% evaluated were susceptible at 7 dpi (days post inoculation) to isolates OP97 and 12889, respectively (Table 2.2).

Table 2.2. Fruit shape parameters and mean disease ratings for each isolate overall and per individual experiment.

		Mean[a]		Fruit[b]		
Species	**Accession**	**12889**	**OP97**	**Ratio**	**Length**	**Width**
S. incanum	PI 500922	MR	MS	1.03	2.6	2.6
S. linnaeanum	PI 388846	MR	S	1.03	2.5	2.4
S. linnaeanum	PI 388847	MR	S	1.07	2.0	1.9
S. linnaeanum	PI 420415	S	S	1.06	2.1	2.1
S. melongena	C-S-16	S	S	5.59	23.0	4.1
S. melongena	Grif 1276	MS	R	1.14	4.9	4.3
S. melongena	Grif 14182	S	S	2.59	15.0	6.1
S. melongena	Grif 14186	S	S	1.75	13.5	7.8
S. melongena	H15	R	S	1.93	11.8	6.2
S. melongena	IVIA-371	S	S	2.08	15.0	7.3
S. melongena	MM 108 bis	S	S	5.23	21.5	4.2

Table 2.2 (cont'd)

S. melongena	MM 114	S	S	6.57	22.2	3.4
S. melongena	MM 1171	S	S	2.81	15.1	5.4
S. melongena	MM 1363	S	S	5.05	25.8	5.1
S. melongena	MM 1364	S	S	3.02	17.8	6.0
S. melongena	MM 1365	S	MS	1.87	14.6	7.9
S. melongena	MM 141	S	S	4.67	23.8	5.1
S. melongena	MM 1750	S	S	2.49	17.5	7.1
S. melongena	MM 346	S	S	1.31	11.4	8.7
S. melongena	MM 39	S	S	5.37	22.9	4.3
S. melongena	MM 522	S	S	8.03	26.1	3.3
S. melongena	MM 56	MS	S	2.42	16.3	6.8
S. melongena	MM 61	S	S	1.97	11.5	5.9
S. melongena	MM 64	S	S	1.16	10.0	8.7
S. melongena	MM 69	S	S	1.34	11.4	8.8
S. melongena	MM 91	S	S	1.92	14.2	7.5
S. melongena	PI 102727	S	S	2.44	14.8	6.1
S. melongena	PI 105346	S	S	1.17	10.3	8.9
S. melongena	PI 115505	S	S	1.72	11.3	6.6
S. melongena	PI 140446	S	S	1.77	12.6	7.1
S. melongena	PI 140456	S	S	3.48	21.9	6.3
S. melongena	PI 141968	S	S	4.46	19.9	4.5
S. melongena	PI 143410	S	S	1.35	10.7	7.9
S. melongena	PI 169641	S	S	3.78	19.3	5.2
S. melongena	PI 169650	S	S	4.66	19.2	4.2
S. melongena	PI 171851	S	S	4.31	17.8	4.1
S. melongena	PI 175914	S	S	2.92	14.1	4.8
S. melongena	PI 179500	S	S	3.64	16.0	4.4
S. melongena	PI 179997	S	S	3.34	15.9	4.8
S. melongena	PI 181896	MS	S	1.91	12.5	6.6
S. melongena	PI 181963	S	S	3.99	16.0	4.0
S. melongena	PI 193599	S	MS	1.84	11.6	6.5
S. melongena	PI 199516	S	S	1.74	14.2	8.8
S. melongena	PI 200881	S	S	3.82	22.1	5.9
S. melongena	PI 204731	S	S	2.73	18.1	7.6
S. melongena	PI 213193	S	S	1.07	9.8	9.2
S. melongena	PI 217962	S	S	3.26	15.4	4.8
S. melongena	PI 223844	S	S	2.89	14.8	5.3
S. melongena	PI 230333	S	S	7.15	25.6	3.6
S. melongena	PI 230334	S	S	6.84	21.2	3.1
S. melongena	PI 230335	S	S	7.43	26.2	3.5
S. melongena	PI 232078	S	S	4.01	18.2	4.6
S. melongena	PI 232079	S	S	2.36	13.1	5.6
S. melongena	PI 233916	MS	S	2.37	13.2	5.6
S. melongena	PI 234632	S	S	0.93	7.9	8.7
S. melongena	PI 241506	MS	S	2.73	16.4	6.1

Table 2.2 (cont'd)

S. melongena	PI 249570	S	S	1.53	10.0	5.8
S. melongena	PI 256077	S	S	3.07	16.1	5.3
S. melongena	PI 263727	S	MS	1.95	12.8	8.0
S. melongena	PI 267104	S	S	3.93	18.8	4.9
S. melongena	PI 269600	S	S	1.71	11.6	7.1
S. melongena	PI 276104	S	S	2.22	15.6	7.0
S. melongena	PI 286099	S	S	5.81	25.3	4.5
S. melongena	PI 286100	S	S	6.45	28.1	4.7
S. melongena	PI 290467	S	S	3.34	21.0	6.3
S. melongena	PI 290469	S	S	2.16	14.9	7.0
S. melongena	PI 304839	S	S	2.85	17.5	6.2
S. melongena	PI 320501	S	S	2.18	15.5	7.2
S. melongena	PI 320504	S	S	4.58	29.1	6.6
S. melongena	PI 320509	S	S	2.46	17.1	6.9
S. melongena	PI 349612	S	S	1.44	7.4	5.1
S. melongena	PI 351129	S	S	5.61	26.5	4.7
S. melongena	PI 358232	S	S	4.59	22.4	4.9
S. melongena	PI 358242	S	S	2.16	14.5	7.2
S. melongena	PI 358244	S	S	5.36	24.7	4.7
S. melongena	PI 368822	S	S	3.18	18.2	5.8
S. melongena	PI 391646	S	S	5.33	25.8	8.2
S. melongena	PI 413782	S	R	0.79	1.2	1.5
S. melongena	PI 413783	MS	MR	0.46	2.0	4.4
S. melongena	PI 413784	R	R	0.69	4.1	5.9
S. melongena	PI 419198	S	MS	5.63	24.3	4.4
S. melongena	PI 441908	R	MS	0.83	4.3	5.2
S. melongena	PI 452122	S	S	5.79	23.8	4.1
S. melongena	PI 452123	S	S	1.29	12.2	9.6
S. melongena	PI 462370	S	S	1.15	12.0	10.9
S. melongena	PI 470273	S	S	3.30	15.9	4.9
S. melongena	PI 478390	S	S	0.75	7.1	9.5
S. melongena	PI 491192	S	S	4.95	22.1	4.5
S. melongena	PI 560903	S	S	0.95	8.2	8.7
S. melongena	PI 561139	S	S	2.94	16.2	5.6
S. melongena	PI 561140	S	S	3.48	16.2	4.7
S. melongena	PI 593748	S	S	2.65	15.2	5.8
S. melongena	PI 593806	S	S	3.79	15.9	4.2
S. melongena	PI 593885	S	S	1.12	6.6	6.0
S. melongena	PI 595220	S	S	2.70	12.5	4.6
S. melongena	PI 600912	S	S	4.58	15.3	3.4
S. melongena	PI 606714	S	S	2.40	13.3	5.6
S. melongena	PI 639121	S	S	1.96	14.0	7.3
S. melongena	PI 639122	S	S	1.60	10.8	6.7

[a] Mean disease rating across all experimental replicates for each isolate, 12889 and OP97

[b] Mean fruit parameters for ratio (fruit length: fruit width), length (cm), and width (cm)

Symptoms included brown discoloration of the fruit and watersoaking, with occasional external mycelial growth (Fig 2.1). Eggplant accession PI 413784 was the only line completely resistant to both isolates tested. Susceptibility to one isolate did not always result in susceptibility to the other isolate. Lines PI 413782 and Grif 1276 were resistant (rating =0) to isolate OP97. PI 413783 was moderately resistant (rating <1) to isolate OP97. The single *S. incanum* line, PI 500922, and *S. melongena* lines PI 441908, MM1365, PI 193599, PI 263727 and PI 419198 were moderately susceptible (rating <2) to isolate OP97. Eggplant lines H15 and PI 441908 were resistant to isolate 12889. Two of the *S. linnaeanum* lines, PI 388847 and PI 388846, and the *S. incanum* accession PI 500922 were moderately resistant to isolate 12889. Lines PI 181896, PI 233916, MM 56, and PI 413783, and Grif 1276 were moderately susceptible to isolate 12889. *Phytophthora capsici* isolates were successfully recovered from diseased fruits and mefenoxam sensitivity was confirmed for each isolate (data not shown). Fruit shape and size varied considerably in the population (Fig 2.2).

Figure 2.2. Fruit size and shape differences between eggplants. *S. incanum* (left) and *S. linnaeanum* (right) fruit (A), *S. melongena* fruit (B) and *S. linnaeanum* and *S. melongena* fruit varying in shape, size and color (C). U.S. quarter used for size reference.

S. melongena accessions had fruit shape ratios ranging from 1 (round) to 8 (elongate). The wild species evaluated (*S. linnaeanum* and *S. incanum*) both had a fruit shape ratio of approximately 1 (round) with fruit ≤3 cm (Table 2.2). *Solanum melongena* line PI 413783 had the lowest fruit shape ratio (0.46) and line MM 522 had the highest fruit shape ratio (8). When evaluated by country, *S. melongena* fruit from Japan had the highest length:width ratio indicating fruits were slender and elongated. Fruit from Thailand had the lowest fruit shape ratio, indicating fruits were more round. Fruit length and width also varied greatly between countries. Fruit from China were the widest and Japan the narrowest. Fruit from Japan were also the longest and fruit from Thailand were the shortest (Table 2.3). No consistent correlations were detected between fruit shape ratio and width parameters and disease susceptibility for either isolate.

Table 2.3. *Solanum spp*. fruit shape, width and length variation between countries of origin.

	Fruit		
Category[a]	**Shape**[b]	**Width (cm)**[c]	**Length (cm)**[d]
China	3.0 cd	7.4 a	15.9 de
France	3.8 b	5.8 cd	18.9 b
Iran	2.3 de	6.9 ab	15.4 de
Italy	3.5 bc	6.8 abc	18.0 bcd
Japan	6.8 a	3.7 e	24.9 a
Macedonia	3.8 b	5.6 cd	20.0 b
S. Africa	2.4 de	6.5 abc	13.7 ef
Spain	3.1 c	6.0 bc	15.9 d
Thailand	2.2 d	5.6 cd	11.8 f
Turkey	3.9 b	5.1 d	18.4 bc
USA	3.6 bc	5.7 cd	16.9 cd

[a] Categories with less than five individuals representing a country were not included in analyses

[b] Mean fruit shape calculated as the ratio of fruit length to fruit width

[c] Mean fruit width at midpoint measured in cm

[d] Mean fruit length from peduncle to blossom end measured in cm

Fruit length was positively correlated with fruit susceptibility (R^2= 0.20-0.35, P<0.05) for both isolates in each experimental replicate (Table 2.4).

Table 2.4. Correlation of *Solanum spp.* fruit characteristics with disease susceptibility

	Pearson Correlation Coefficient[b]					
Category[a]	**12889 (1)**	**12889 (2)**	**12889 (3)**	**OP97 (1)**	**OP97 (2)**	**OP97 (3)**
Length	0.25*	0.22*	0.2*	0.36***	0.22*	0.27**
Width	0.32**	0.08	0.07	0.17	0.15	0.03
Shape	0.12	0.18	0.15	0.27**	0.14	0.2*

[a] Mean fruit phenotype category for each line correlated against disease susceptibility; Length = fruit length in cm from peduncle to blossom end, Width = fruit width in cm at midpoint between peduncle and blossom end, Shape = fruit shape as the length:width ratio

[b] Correlation coefficient value according the Pearson's Correlation Coefficient of each category with isolate 12889 or OP97 at biological replicates 1, 2 and 3; *, **, and *** indicate that values were significant at P=0.05, 0.01 and 0.001, respectively.

The 192 SSRs evaluated yielded 22 polymorphic markers that were used for characterizing and evaluating genetic diversity of the eggplant collection (Table 2.5). A total of 83 alleles were detected among the 22 SSRs, ranging from 2 to 7 alleles per locus with an average allele diversity of 3.8 alleles per locus. The mean genetic diversity index of the collection was 0.49 ranging from 0.03 (T0633) to 0.76 (CSM31) (Table 2.5). The mean polymorphism information content (PIC) value was 0.42 and individual markers ranged from 0.03 to 0.71 for the population. The highest PIC value was 0.35 in PI 290467 and the lowest PIC value was 0.085 in Grif 1276. Genetic diversity was equally distributed within continents (0.47-0.51), and pairwise Fsts indicated low to moderate genetic differentiation between continents (0.00 – 0.11) (Table 2.6). Genetic diversity

Table 2.5. Polymorphic primers evaluated against 99 eggplant lines

SSR	Forward sequence	Reverse sequence	AI[a]	PIC[b]	Source
BM61461	CTCATTACCACTTCATACAAAACAG	TGCAGTAGGTGTTGCTACGG	6	0.18	SolCAP
GPMS203	CACCAACACATCTTTTTCAACC	ATAATAGTGGTTGCGGCGAC	4	0.23	SolCAP
CB164833	CGGGCAGGTGCTATTATAAAAC	CGGCCGAGGTACAAGCC	3	0.49	SolCAP
T0633	GATGGGCTATGCTTGCTGTT	ACATCCCCAATGTTGTTGTG	2	0.03	SolCAP
CA516334	ACCCACCTTCATCAACAACC	ATTTGTGGCTTTTCGAAACG	6	0.55	SolCAP
GPMS178	GATTTTTGACATGTCACATTCATG	AACGTTGAAAAATAAAGTAAGCAAG	5	0.69	SolCAP
GP1102	GAACCCTTCATTCCTGTATGT	TTTGCCCGCATTATGTAAATC	2	0.35	SolCAP
C2_At5g34850	AGTGAAGTGGCTACATCCAAAATCTC	GAACAAAACATGCCCTACTGTAGGAA	7	0.51	SolCAP
C2_At1g69210	AGCTCTATTCATTTAAAACTAGTCCTCAT	TCTTTTCTTGTATTGGCGGCTAAATTC	2	0.37	SolCAP
AF348141	CCTTACGGGGAAAACCTAGC	CCATACGGACGTTGTCCTCT	5	0.62	NCBI
CAMS362	CCCCTTCTGACCTTGATTGA	TATGCCCCTCCTGTGATAGC	4	0.42	Minamiyama
GO496268.1	CGTTGCCTGTTTACCAACCT	CCTTCTTCTGCACTTCCACA	2	0.37	NCBI
C2_At5g13200	TATGGGTCCGCCTGCAGTTCCAAC	AAGTTTTCCCCATGCCGCTTCTGT	3	0.10	SolCAP
C2_At1g32410	TGTTAGTGTCTGGAGGGATTGTATTG	AGATTCGGTGTAGAGACTGGAAGTATC	4	0.57	SolCAP
CSM7F	CGACGATCACCTTGATAACG	CCTTAAATGCAGAGTTTCCAAAG	2	0.37	Hurtado
CSM27	TGTTTGGAGGTGAGGGAAAG	TCCAACTCACCGGAAAAATC	3	0.50	Hurtado
CSM30	CACTGTTCCTGGTTGCTGTG	TTTAGCTTTAGCCCATCTACCG	3	0.40	Hurtado
CSM31	CAACCGATATGCTCAGATGC	CGGGTATGGTCATGTTTTGC	6	0.71	Hurtado
CSM43	ATTTTAACCCCGGGAAAATG	ACCGCTTCTAGGTTTTGCAC	4	0.55	Hurtado
CSM44	CGTCGTTGTAACCCATCATC	TTGCCAAATTCCTTGTGTTC	3	0.36	Hurtado
CSM54	ATGTGCCTCCATTCTGCAAG	TGGGTGGGATGCTGAGTAAG	3	0.37	Hurtado
CSM73	TTCAACATAGCCTGGACCATT	AATGCAGGGTTTGGACTTCA	4	0.56	Hurtado

[a] Number of unique alleles detected in the population

[b] Polymorphism information content for each marker

Table 2.6. Genetic differentiation (pairwise Fst) estimates of SSRs for *S. melongena* grouped by continent

	Fst[b]			
Category[a]	**Africa**	**Asia**	**Europe**	**N. America**
Asia	0.00	-		
Europe	0.04*	0.02	-	
N. America	0.04*	0.00	0.03*	-
S. America	0.03	0.11*	0.05	0.06*

[a] Categories with less than four lines were excluded from analyses and are not shown

[b] Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence intervals at 1000 bootstraps

within countries was similar (0.35 – 0.48), and pairwise Fst values suggested low to great genetic differentiation among countries (Table 2.7, 2.8).

Table 2.7. Genetic diversity estimates for SSRs for *S. melongena* grouped by continent and country of origin

	Diversity estimates[b]		
Category[a]	**AlleleNo[b]**	**G_D[c]**	**PIC[d]**
Africa	2.71	0.51	0.43
Asia	3.10	0.48	0.41
Europe	2.81	0.48	0.41
N. America	3.10	0.50	0.44
S. America	2.67	0.46	0.39
China	2.18	0.39	0.32
France	2.14	0.38	0.31
Iran	2.23	0.42	0.35
Japan	1.91	0.35	0.28
Macedonia	2.18	0.40	0.33
South Africa	2.14	0.41	0.34
Spain	2.50	0.42	0.36
Thailand	2.36	0.42	0.36
Turkey	2.36	0.40	0.34
USA	2.77	0.48	0.42

[a]Categories with less than four lines were excluded from analyses and are not shown

Table 2.7 (cont'd)

[b]Mean values are presented for the average number of alleles (AlleleNo), genetic diversity (G_D) and the polymorphism information content (PIC)

Table 2.8. Genetic differentiation (pairwise Fst) estimates of SSRs for *S. melongena* grouped by country

	Fst[b]								
Category[a]	**China**	**France**	**Iran**	**Japan**	**Mace.**	**S. Africa**	**Spain**	**Thailand**	**Turkey**
France	0.00	-							
Iran	0.00	0.04	-						
Japan	0.01	0.04	0.05	-					
Macedonia	0.06	0.13*	0.09*	0.08	-				
S. Africa	0.04	0.08	0.01	0.07	0.05	-			
Spain	0.06	0.04	0.04	0.13*	0.13*	0.09*	-		
Thailand	0.05	0.04	0.07*	0.15*	0.10*	0.04	0.01	-	
Turkey	0.04	0.09	0.06	0.15*	0.03	0.05	0.09	0.04	-
USA	0.05	0.10*	0.04	0.17*	0.10*	0.05	0.04*	0.07*	0.05

[a] Categories with less than four lines were excluded from analyses and are not shown

[b] Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence intervals at 1000 bootstraps

Pairwise Fsts for disease resistance to 12889 and OP97 showed little to very great (0 to 0.52) genetic differentiation between categories (Table 9). No significant genetic

Table 2.9. Genetic differentiation (pairwise Fst) estimates of SSRs for eggplant germplasm grouped by disease resistance

	Fst[b]		
Category[a]	**MS**	**R/MR**	**S**
MS	-	0.19	0.15
R/MR	0.01	-	0.52*
S	0.03*	0.00	-

[a] 12889 appears below the diagonal and OP97 values are shaded above the diagonal; MS = moderately susceptible, R/MR = resistant/moderately resistant, S=susceptible

[b] Average values for SSRs are presented; * indicates value was outside the 95% confidence interval at 1000 bootstraps

differentiation was evident between the resistant/moderately resistant (R/MR) category and the moderately susceptible (MS) or susceptible (S) categories, but significant genetic differentiation was detected between the MS and the S category for isolate 12889. When inoculated with isolate OP97, significant differentiation was evident between the S category and the R/MR categories. There was no significant differentiation between the R/MR and MS category or the MS and S categories (Table 9). Genetic diversity of fruit shape categories was moderate to high (0.43-0.52). Pairwise Fst differentiation between fruit shape categories was low to moderate (0.0-0.1) (Table 2.10). Individuals with an elongate fruit shape were significantly differentiated from those with a round or oval fruit shape. Significant differentiation was also detected between round shaped individuals and semi-elongated individuals (Table 2.10).

Table 2.10. Genetic differentiation (pairwise Fst) estimates of SSRs for *S. melongena* germplasm grouped by fruit shape

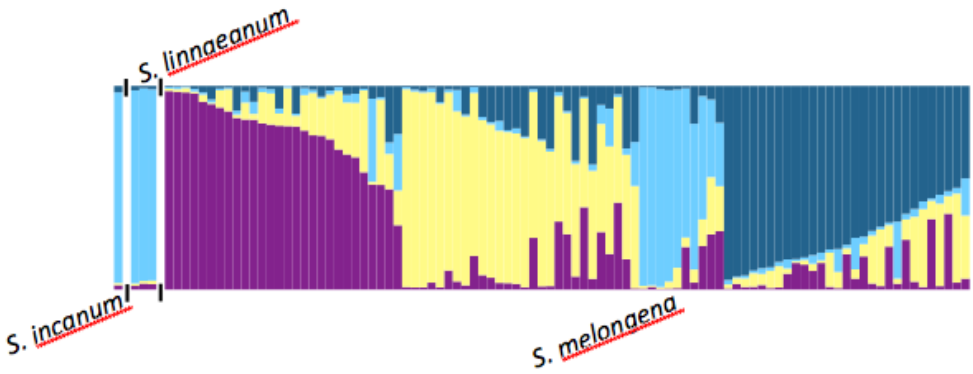
	Fst[b]		
Category[a]	**Elongate**	**Oval**	**Round**
Oval	0.10*		
Round	0.06*	0.00	
Semi-Elongate	0.04	0.02	0.03*

[a]Fruit shape category based on the ratio of mean length: mean length for each line

[b]Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence interval at 1000 bootstraps.

Population structure of the 99 accessions was estimated using the STRUCTURE software and the 22 polymorphic SSRs. Accessions were grouped into 4 genetic clusters (Ln=- 3381.8). *S. linnaeanum* and *S. incanum* accessions were placed into genetic cluster 4, while *S. melongena* individuals were distributed through each of the clusters (Fig 2.3).

Figure 2.3. Population structure grouped by species. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (steel blue).



Seventy-eight individuals could be assigned to a single cluster based on membership, while the remaining twenty-one individuals could not be assigned and were classified as admixed.

Pairwise Fsts were significant and ranged from 0.08 to 0.17, indicating 8 – 17% of the variation was explained by genetic differences between clusters. Cluster 1 had moderate differentiation from Clusters 2,3 and 4. Cluster 2 had great differentiation from Cluster 3 and Cluster 4 had moderate differentiation from Clusters 2 and 3 as according to Hartl and Clark [59]. Population structure was detected when individuals were grouped by continent of origin, country of origin, species, fruit shape and disease resistance to 12889 and OP97, as some clusters were more frequent than others in each grouping (Figures 2.4-6).

Figure 2.4. Population structure grouped by continent of origin for eggplant germplasm. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals.

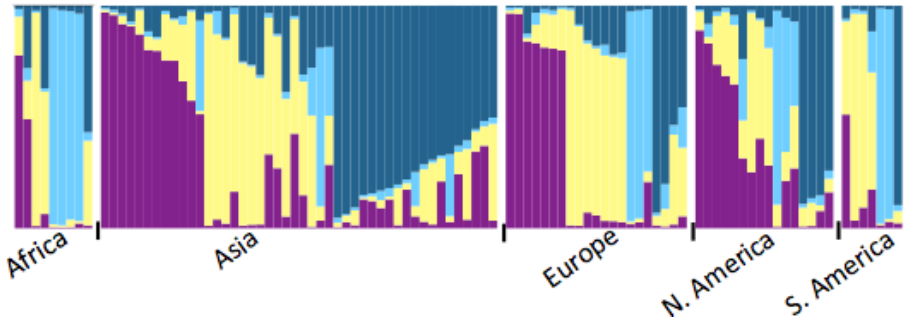


Figure 2.5. Population structure grouped by country of origin for the *S. melongena* germplasm. Only countries represented by four or more individuals were included. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals.

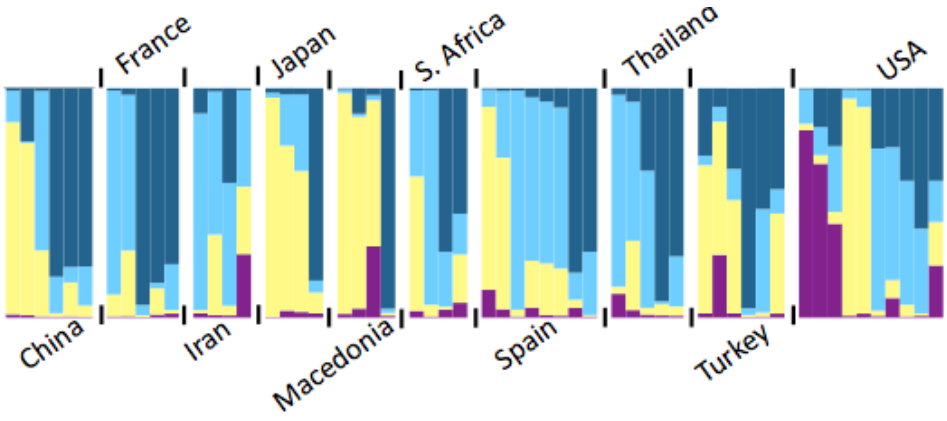
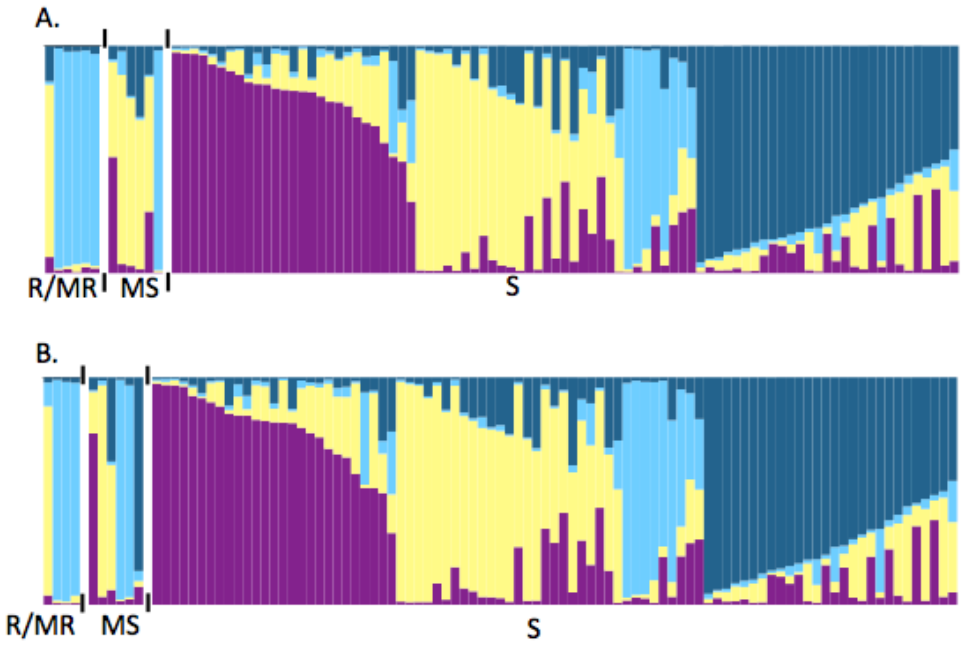


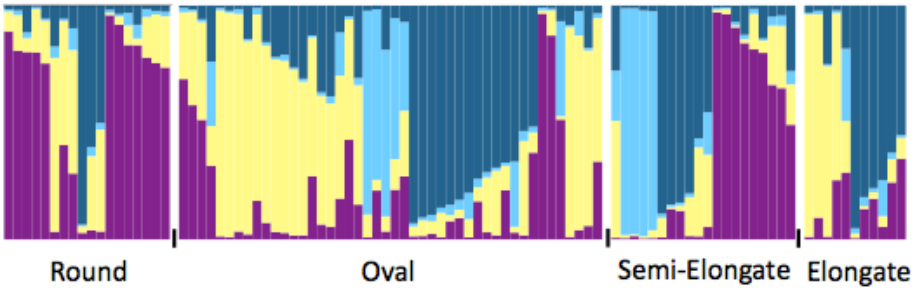
Figure 2.6. Population structure grouped by disease resistance to isolate 12889 (A) and OP97 (B). Individuals were grouped into a resistant and moderately resistant category (R/MR), a moderately susceptible category (MS), and a susceptible category (S) based on their mean disease ratings. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals**.**



Cluster 3 individuals were not represented in Asia, and Cluster 4 individuals were not represented in Africa (Figure 2.4). For both isolates, individuals from Cluster 4 were not represented in the moderately resistant/resistant categories for either isolate, had low representation in the moderately susceptible category, and were highly represented in the susceptible category. Cluster 1 individuals were highly represented in both the R/MR and S categories, but not the MS for both isolates (Figure 2.5). When grouped by fruit shape (round, oval, semi-elongate and elongate), Cluster 1 was under represented in the oval

and elongate fruit shape categories. Cluster 4 and 2 both had low representation in the round category (Figure 2.7). Cluster 3 was not represented in round or elongated individuals and had minor representation in the oval shape category.

Figure 2.7. Population structure grouped by *S. melongena* fruit shape. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue) and Cluster 4 (dark blue). A white space and black tick marks separate subgroups of individuals.



DISCUSSION

This study investigated Phytophthora fruit rot resistance, fruit shape, population structure and genetic diversity in a worldwide collection of eggplant. The overall estimate of genetic diversity of the collection was moderate (0.49) in our study, similar to a recent report on eggplant diversity (52). Bayesian clustering identified four genetic clusters in the eggplant collection. Most individuals belonged to predominantly one of the four clusters, while an additional 20% were admixed according to the inferred clustering. Admixture, an indicator of migration or interbreeding between genetic clusters, was low in our population. Inferred genetic clusters did not directly correspond with the predefined categories of continent, country, fruit shape or Phytophthora fruit rot resistance, though some clusters did appear more frequently in one category compared to another.

On eggplant, fruit rot is the most common symptom of *P. capsici* seen in the field. Symptoms start as small water-soaked lesions, turning brown and eventually covering the whole fruit. Advanced symptoms can include complete rotting of the fruit and visible mycelia on the external surface of the fruit (37). Isolate-specific interactions and partial fruit rot resistance have been identified in other solanaceous species (tomatoes and peppers) suggesting a multigenic host response, but no studies have looked at Phytophthora fruit rot in eggplant (Naegele et. al. unpublished; Granke et al. unpublished). In our study, the 99 eggplant accessions evaluated demonstrated partial and isolate-specific resistance to Phytophthora fruit rot. Most lines evaluated were completely susceptible to both isolates (~90%). Several *S. melongena* lines displayed isolate-specific resistance; these individuals were placed into genetic clusters 2 and 3, and were from Asia, Africa and Europe. These three geographic regions are known centers of eggplant diversity, and likely harbor additional sources of resistance (52,75,93,121). Only one of the ninety-nine lines evaluated, a Cluster 3 *S. melongena* landrace collected in Burkina Faso in the early 1900s, had complete resistance to both isolates evaluated. This line also showed high levels of genetic similarity to the wild eggplant relatives, *S. incanum* and *S. linnaeanum,* evaluated. While further evaluation with more isolates is necessary, PI 413784 appears to be a promising source of host resistance to Phytophthora fruit rot in eggplant.

When categorized by disease resistance (susceptible, moderately susceptible, moderately resistant and resistant) for each isolate, there was significant genetic differentiation among eggplant genotypes infected with isolates OP97 or 12889. Individuals that were resistant and moderately resistant to isolate OP97 were significantly

differentiated from individuals that were susceptible. Only susceptible individuals were significantly differentiated from the moderately susceptible individuals when inoculated with isolate 12889. These results emphasize the importance of utilizing different *P. capsici* isolates when breeding for resistance. The two wild relatives, *S. linnaeanum* and *S. incanum*, showed partial or isolate-specific resistance to the two isolates evaluated in this study.

When grouped by species, all *S. linnaeanum* or *S. incanum* individuals evaluated were predominantly genetic cluster 3. *S. incanum* is one of the progenitors of modern eggplant and has long been part of the eggplant complex (6,34). *S. linnaeanum* is a more distantly related relative and has only recently been included as a possible progenitor of the modern eggplant with limited crossability (46,121). Genetic cluster 3 individuals were also detected in the *S. melongena* category, supporting gene movement between *S. melongena* and its wild relatives, *S. incanum* and *S. linnaeanum*. These *S. melongena* individuals may have been misclassified, but are more likely the result of introgression since the wild species were small fruited and prickly.

Cultivated eggplant, similar to pepper and tomato, is a phenotypically diverse species with varying levels of genotypic diversity (6,59,70,114). *S. melongena* fruit shape, size and color is a byproduct of breeding for regional specific market classes. Phenotypic evaluation of eggplant fruit shape varied greatly among the *S. melongena* accessions evaluated, while the wild species, *S. incanum* and *S. linnaeanum*, had no variation in fruit shape. Maintaining market class variation may be difficult when incorporating traits like fruit rot resistance, which was most often observed in small-fruited varieties. Studies in peppers and tomatoes have demonstrated that fruit length and

width are correlated, making breeding for one trait, while maintaining the other, difficult (19,85). Increased fruit length in particular may be difficult to integrate with disease resistance as it was positively correlated with increased susceptibility. This was similar to previous work in peppers where fruit shape, but not length was positively correlated with fruit susceptibility (Naegele et al. unpublished).

Significant differences in fruit shape, length and width were observed among eggplant lines when grouped by country of origin, representing different market classes, in this study. Since eggplant has market classes particular to geographic areas, it was expected that population structure categorized by fruit shapes and country of origin would correspond with the inferred genetic clusters. Significant differentiation was seen between *S. melongena* individuals with elongated fruit shapes and those with round and oval fruit shapes. Individuals with a round fruit shape were also significantly differentiated from semi-elongate fruit shape individuals. These results are consistent with limited breeding among market classes. However, inferred population structure did not correspond with the fruit categories. While only genetic cluster 3 was not represented in the round or elongate shape category, all other clusters were represented by at least one individual in each category.

When grouped by country and continent, significant population structure and moderate genetic diversity was evident among the categories evaluated. The highest levels of genetic diversity were seen within the continents of Africa and N. America. The highest level of genetic diversity for countries was in the USA. The increased genetic diversity in Africa is likely due to intercrossing with related species, since Africa is the center of origin for eggplant and wild relatives. The increased genetic diversity in N.

America and the USA may be the result of breeding programs integrating wild relatives and varieties from around the world. The diversity could also be from the movement of Asian and European varieties into the US, which may be marketed under different names. Overall differentiation among countries was similar to the differentiation among continents, and future core collections should include individuals from areas with high genetic diversity and genetic differentiation. In particular, genotypes from China were not significantly differentiated from any other country, while genotypes from Thailand, Japan, Spain, Macedonia and the U.S. were frequently significantly differentiated from other countries. Similarly, Asia was not significantly differentiated from populations from Europe, Africa and N. America, while N. America, Europe and Africa were all significantly differentiated from each other. Asia, as a center of diversity and domestication, and in particular genotypes from China may be more akin to the ancestral population from which these other pools were derived.

Cultivated eggplant, compared to other solanaceous species, is an understudied crop with worldwide importance. This study provides an overview of the population structure, genetic diversity and Phytophthora fruit rot resistance of a geographically diverse set of eggplant. The estimates of genetic diversity and the four genetic clusters found in this study are likely to be lower than actual genetic diversity and structure of eggplant due to limited sampling and molecular markers. A previous study using a subset of SSRs in a smaller collection of eggplant was able to identify more allelic variation at each locus [3]. While population structure was significant for disease resistance, fruit shape, continent and country, the genetic clusters did not completely correspond with the predefined categories in our study. Future studies involving eggplant diversity, disease

resistance and other agronomic traits should aim to include individuals from around the world for maximum diversity, and will need to consider the effect of population structure on marker-trait associations.

CHAPTER 3: EVALUATION OF A DIVERSE, WORLDWIDE COLLECTION OF WILD, CULTIVATED AND LANDRACE PEPPERS (CAPSICUM ANNUUM L.) FOR RESISTANCE TO PHYTOPHTHORA FRUIT ROT, GENETIC DIVERSITY AND POPULATION STRUCTURE

ABSTRACT

Pepper is the third most important solanaceous crop in the U.S. and fourth most important worldwide. One hundred-seventy pepper genotypes representing five continents and 45 countries were evaluated for Phytophthora fruit rot resistance to two isolates of *Phytophthora capsici*. Partial resistance and isolate-specific interactions were identified in the population at both 3 and 5 days post inoculation (dpi). Most lines evaluated were susceptible or moderately susceptible at 5dpi, and no lines evaluated were completely resistant to Phytophthora fruit rot. Genetic diversity and population structure were assessed on a subset of 157 genotypes using 23 polymorphic simple sequence repeats (SSRs). Genetic diversity was moderate in the population and varied between countries, continents and disease susceptibility. The program STRUCTURE inferred four genetic clusters with moderate to very great differentiation among clusters. Significant population structure was detected when peppers were grouped by predefined categories of disease resistance, continent and country of origin.

INTRODUCTION

Pepper, a member of the Solanaceae, is an important vegetable commodity grown in temperate and tropical regions. Worldwide, pepper production is estimated at 25 million tons each year [USDA ERS]. China is the largest producer of peppers with an approximate 15.5 million tons produced in 2011 [FAO, 2011]. The U.S. is the sixth largest producer with an estimated 1 million tons (combined bell and chile peppers) [FAO, 2011]. Despite its current distribution around the world, pepper is a New World crop, domesticated and cultivated in South America since 5000-6000 B.C. (86,87). Pepper, the common term for the genus *Capsicum*, contains 25 – 30 species, of which, only five are economically important (48,117). Cultivated pepper consists of the intercrossing species: *Capsicum annuum*, *C. chinense*, *C. frutescens*, and *C. baccatum* (1,42). Another species, *C. pubescens,* is also a cultivated member of the pepper genus, but has limited fertility with the other species (77).

Mexico is the center of origin for *C. annuum*, the primary pepper species grown in the U.S. (42). The remaining species originated in other parts of Central and South America, and are predominantly grown in South America and Asia as vegetables, spices and ornamentals (3,30,42,77). Wild and landrace individuals from centers of origin and diversity can harbor genetic variability useful for enhancing fruit quality, disease resistance and other agronomically important traits (7,26,56,108). Variation in closely related pepper species, in addition to within species market variation, can provide an additional resource for breeding. Utilizing genetic diversity in closely related cultivated species and market classes within the same species instead of wild material could reduce the number of unfavorable characteristics incorporated. Understanding the genetic

diversity and underlying population structure available within populations is essential for the efficient utilization and testing of germplasm resources (21,23). Unidentified population structure can confound association mapping results creating false associations between markers and phenotypes (58,123). These types of false associations could decrease the success of marker-assisted breeding programs when incorporating traits of agricultural significance. Algorithms accounting for the population structure can result in fewer, but stronger, associations between the trait of interest and individual markers when properly applied (13,125). In peppers, population structure has been evaluated among cultivated varieties (51), and within geographic regions (77,82,83). In each of these cases, population structure was detected and could differentiate between locations (geographic regions) or market type (cultivated varieties) suggesting within species variation is available (1,3,51,83). Population structure can also provide information on the migration and interbreeding of species and populations. Population studies between *Capsicum* species have shown that admixture is limited, with few ambiguous genotypes (65,104,115).

Evaluating individuals can identify useful subsets of individuals representing much of the genetic diversity within a species. Studies on the genetic diversity within pepper are more common than population structure studies (3,30,49,54,77,82,83,104,105). Domesticated populations of peppers (landraces and cultivars) have been evaluated and compared to wild (natural occurring populations) (3,51,54,77,83,104,115). Cultivated varieties had higher heterozygosity than landraces, and only slightly lower diversity than wild populations (1,83). The authors suggested that cultivated varieties maintained high levels of diversity through hybrid generation, while

landraces, which are often maintained through inbreeding, continuously lost heterozygosity. These results were similar to a study evaluating cultivars and breeding lines compared to germplasm resources at the INRA (104,105). The authors determined that there was no significant difference between the genetic diversity within the germplasm collection and the breeding/cultivated lines, again suggesting that cultivated varieties may contain high levels of genetic diversity that can be utilized.

Phytophthora capsici Leonian is a devastating oomycete pathogen capable of causing disease on pepper (fruit and root rot and foliar blight). Sources of resistance to root rot have been identified in several *Capsicum annuum* and *C. chinense* lines (18,63). Many of the *Capsicum annuum* lines with resistance exhibit full or partial resistance to multiple isolates and are small-fruited landraces from Mexico (18,63). Few studies have looked at Phytophthora fruit rot resistance, and no correlations between resistance to *Phytophthora capsici* diseases in peppers have been identified (5,106,109,116). A single quantitative trait loci (QTL) analysis in a recombinant inbred line (RIL) population identified minor and major QTL for fruit rot resistance in immature fruit (Naegele et. al., unpublished). However, this study was limited to a single source of resistance, Criollo de Morelos. Other studies on Phytophthora fruit rot have found age-related resistance, but no correlations with fruit characteristics such as pungency or pericarp thickness. No pepper cultivars or lines evaluated to date have demonstrated a complete resistance to Phytophthora fruit rot. Since morphological traits like fruit shape, color, size, and pungency are not predictive of susceptibility to Phytophthora fruit rot, reliable molecular markers are needed to incorporate resistance. Identifying individuals with increased fruit rot resistance and molecular markers associated with that resistance will increase the

probability of identifying and incorporating Phytophthora fruit rot resistance into commercial cultivars.

The objectives of this study were to evaluate population structure, genetic diversity and Phytophthora fruit rot resistance in a worldwide collection of *C. annuum* and determine if population structure was associated with disease resistance, country or continent of origin using polymorphic simple sequence repeat (SSR) markers.

MATERIALS AND METHODS

One hundred-seventy genotypes of pepper germplasm (wild, cultivated and landrace) were requested from the USDA Germplasm Resource Information Network or commercial seed sources: Johnny's Seed, Parks Seed and Seedway (www.ars-grin.gov/) (Table 1). Twenty seeds from each line were sown into 72 cell trays containing a soilless

Table 3.1. Pepper lines used in this study.

Accession	Country	Continent	Source
CM334	Mexico	N. America	
Early Jalapeño	USA	N. America	Park's Seed
Grif 9094	Greece	Europe	USDA GRIN
Grif 9105	Former Soviet	Asia	USDA GRIN
Grif 9109	Mexico	N. America	USDA GRIN
Grif 972	China	Asia	USDA GRIN
Jn566	USA	N. America	Johnny's Seed
Jn570	USA	N. America	Johnny's Seed
Jn571	USA	N. America	Johnny's Seed
Jn574	USA	N. America	Johnny's Seed
PI 102883	China	Asia	USDA GRIN
PI 123469	India	Asia	USDA GRIN
PI 123474	India	Asia	USDA GRIN
PI 124078	India	Asia	USDA GRIN
PI 127445	Afghanistan	Asia	USDA GRIN
PI 135822	Afghanistan	Asia	USDA GRIN
PI 135826	Afghanistan	Asia	USDA GRIN
PI 135874	Pakistan	Asia	USDA GRIN

Table 3.1 (cont'd)

PI 138557	Iran	Asia	USDA GRIN
PI 138558	Iran	Asia	USDA GRIN
PI 138560	Iran	Asia	USDA GRIN
PI 138565	Iran	Asia	USDA GRIN
PI 142832	Iran	Asia	USDA GRIN
PI 148628	Iran	Asia	USDA GRIN
PI 159256	USA	N. America	USDA GRIN
PI 164311	India	Asia	USDA GRIN
PI 164560	Spain	Europe	USDA GRIN
PI 167063	Turkey	Europe	USDA GRIN
PI 169129	Turkey	Europe	USDA GRIN
PI 169140	Turkey	Europe	USDA GRIN
PI 174114	Turkey	Europe	USDA GRIN
PI 176888	Turkey	Europe	USDA GRIN
PI 177294	Turkey	Europe	USDA GRIN
PI 177301	Italy	Europe	USDA GRIN
PI 181733	Lebanon	Asia	USDA GRIN
PI 181734	Lebanon	Asia	USDA GRIN
PI 181934	Syria	Asia	USDA GRIN
PI 182646	Guatemala	S. America	USDA GRIN
PI 183668	Turkey	Europe	USDA GRIN
PI 183922	India	Asia	USDA GRIN
PI 184039	Serbia	Europe	USDA GRIN
PI 194259	Ethiopia	Africa	USDA GRIN
PI 194261	Ethiopia	Africa	USDA GRIN
PI 194910	Ethiopia	Africa	USDA GRIN
PI 197408	Ethiopia	Africa	USDA GRIN
PI 201232	Mexico	N. America	USDA GRIN
PI 201234	Mexico	N. America	USDA GRIN
PI 201237	Mexico	N. America	USDA GRIN
PI 201239	Mexico	N. America	USDA GRIN
PI 203524	Cuba	S. America	USDA GRIN
PI 206950	Turkey	Europe	USDA GRIN
PI 207727	India	Asia	USDA GRIN
PI 213915	Bolivia	S. America	USDA GRIN
PI 224438	Mexico	N. America	USDA GRIN
PI 224442	Nicaragua	Africa	USDA GRIN
PI 226633	Iran	Asia	USDA GRIN
PI 241641	Colombia	S. America	USDA GRIN
PI 241644	Colombia	S. America	USDA GRIN
PI 243936	Turkey	Europe	USDA GRIN
PI 244669	India	Asia	USDA GRIN
PI 249635	India	Asia	USDA GRIN
PI 249908	Portugal	Europe	USDA GRIN
PI 250141	Pakistan	Asia	USDA GRIN

Table 3.1 (cont'd)

PI 257044	Colombia	S. America	USDA GRIN
PI 257047	Colombia	S. America	USDA GRIN
PI 257048	Colombia	S. America	USDA GRIN
PI 257283	Spain	Europe	USDA GRIN
PI 260452	Argentina	S. America	USDA GRIN
PI 262172	Germany	Europe	USDA GRIN
PI 262902	Spain	Europe	USDA GRIN
PI 263075	Former Soviet	Asia	USDA GRIN
PI 263076	Former Soviet	Asia	USDA GRIN
PI 263077	Former Soviet	Asia	USDA GRIN
PI 263113	Former Soviet	Asia	USDA GRIN
PI 263114	Former Soviet	Asia	USDA GRIN
PI 264281	USA	N. America	USDA GRIN
PI 264662	Germany	Europe	USDA GRIN
PI 267730	Cuba	S. America	USDA GRIN
PI 269455	Pakistan	Asia	USDA GRIN
PI 269458	Pakistan	Asia	USDA GRIN
PI 273415	Italy	Europe	USDA GRIN
PI 281318	USA	N. America	USDA GRIN
PI 281341	El Salvador	S. America	USDA GRIN
PI 281433	USA	N. America	USDA GRIN
PI 298646	Spain	Europe	USDA GRIN
PI 298647	Spain	Europe	USDA GRIN
PI 302987	Canada	N. America	USDA GRIN
PI 322720	India	Asia	USDA GRIN
PI 339005	Turkey	Europe	USDA GRIN
PI 339006	Turkey	Europe	USDA GRIN
PI 339007	Turkey	Europe	USDA GRIN
PI 339009	Turkey	Europe	USDA GRIN
PI 339010	Turkey	Europe	USDA GRIN
PI 339019	Turkey	Europe	USDA GRIN
PI 339048	Turkey	Europe	USDA GRIN
PI 339075	Turkey	Europe	USDA GRIN
PI 339079	Turkey	Europe	USDA GRIN
PI 339083	Turkey	Europe	USDA GRIN
PI 339132	Turkey	Europe	USDA GRIN
PI 342949	USA	N. America	USDA GRIN
PI 357503	Serbia	Europe	USDA GRIN
PI 357531	Serbia	Europe	USDA GRIN
PI 368396	Serbia	Europe	USDA GRIN
PI 369996	India	Asia	USDA GRIN
PI 371867	USA	N. America	USDA GRIN
PI 379182	Serbia	Europe	USDA GRIN
PI 385960	Kenya	Africa	USDA GRIN
PI 390612	Peru	S. America	USDA GRIN

Table 3.1 (cont'd)

PI 409141	South Africa	Africa	USDA GRIN
PI 410407	Brazil	S. America	USDA GRIN
PI 427290	USA	N. America	USDA GRIN
PI 432802	China	Asia	USDA GRIN
PI 432818	China	Asia	USDA GRIN
PI 438565	Guatemala	N. America	USDA GRIN
PI 438624	Mexico	N. America	USDA GRIN
PI 438633	Mexico	N. America	USDA GRIN
PI 441628	Brazil	S. America	USDA GRIN
PI 511879	Mexico	N. America	USDA GRIN
PI 511882	Mexico	N. America	USDA GRIN
PI 511884	Mexico	N. America	USDA GRIN
PI 511886	Mexico	N. America	USDA GRIN
PI 550700	USA	N. America	USDA GRIN
PI 555649	Sudan	Africa	USDA GRIN
PI 566808	Mexico	N. America	USDA GRIN
PI 566811	Mexico	N. America	USDA GRIN
PI 585246	Ecuador	S. America	USDA GRIN
PI 593493	Mexico	N. America	USDA GRIN
PI 593495	Mexico	N. America	USDA GRIN
PI 593511	Mexico	N. America	USDA GRIN
PI 593561	USA	N. America	USDA GRIN
PI 593564	Mexico	N. America	USDA GRIN
PI 593572	Brazil	S. America	USDA GRIN
PI 593573	Brazil	S. America	USDA GRIN
PI 593920	Ecuador	S. America	USDA GRIN
PI 593929	Venezuela	S. America	USDA GRIN
PI 593933	Ecuador	S. America	USDA GRIN
PI 595906	Venezuela	S. America	USDA GRIN
PI 600934	USA	N. America	USDA GRIN
PI 601110	USA	N. America	USDA GRIN
PI 631126	China	Asia	USDA GRIN
PI 631131	Yemen	Asia	USDA GRIN
PI 631140	Guatemala	N. America	USDA GRIN
PI 631143	Guatemala	N. America	USDA GRIN
PI 631147	India	Asia	USDA GRIN
PI 639641	Poland	Europe	USDA GRIN
PI 640448	Taiwan	Asia	USDA GRIN
PI 640460	China	Asia	USDA GRIN
PI 640461	China	Asia	USDA GRIN
PI 640480	France	Europe	USDA GRIN
PI 640516	Taiwan	Asia	USDA GRIN
PI 640532	Mexico	N. America	USDA GRIN
PI 640560	Netherlands	Europe	USDA GRIN
PI 640579	Egypt	Africa	USDA GRIN

Table 3.1 (cont'd)

PI 640581	Nigeria	Africa	USDA GRIN
PI 640582	Nigeria	Africa	USDA GRIN
PI 640588	USA	N. America	USDA GRIN
PI 640641	Indonesia	Asia	USDA GRIN
PI 640659	Thailand	Asia	USDA GRIN
PI 640663	Taiwan	Asia	USDA GRIN
PI 640670	India	Asia	USDA GRIN
PI 640671	Sri Lanka	Asia	USDA GRIN
PI 640676	Kenya	Africa	USDA GRIN
PI 640682	Tanzania	Africa	USDA GRIN
PI 640744	Japan	Asia	USDA GRIN
PI 640791	Egypt	Africa	USDA GRIN
PI 640803	Philippines	Asia	USDA GRIN
PI 640809	Denmark	Europe	USDA GRIN
PI 640815	Zambia	Africa	USDA GRIN
PI 640833	USA	N. America	USDA GRIN
PI 645520	Italy	Europe	USDA GRIN
PI 653650	Bangladesh	Asia	USDA GRIN

peat mix (Suremix Michigan Grower Products, Inc Galesburg, MI) in a polyethylene greenhouse at the Michigan State University Horticulture Research Farm (East Lansing, MI.) Germinated seedlings were transferred to 4" black plastic pots containing the same soilless peat mix and grown to maturity. Immature fruit were detached, and bulked for each line. Fruit were taken to the laboratory for inoculation and subsequent evaluation.

Two isolates were selected from the long-term collection of Dr. Mary K. Hausbeck (Michigan State University). Isolates were characterized by host, mefenoxam sensitivity (insensitive=I, sensitive=S) and mating type (A1 or A2). Isolate 12889 (pepper, I, A1) and isolate OP97 (cucumber, S, A1) were maintained on unclarified V8 agar during the experiment. Prior to inoculations, isolates were activated by inoculating and recovering each isolate from a single pepper fruit.

Peppers were surface disinfested in 10% bleach for 5 minutes, rinsed with distilled water, and dried prior to inoculation. For inoculations, a single 6mm plug of V8

agar from the actively growing isolate was placed, mycelium side down, onto the surface of the fruit. Plugs were covered with a sterile microcentrifuge tube cap and affixed into place with petroleum jelly. Peppers were placed into a humidity chamber consisting of a clear, covered plastic box with a ring of moistened paper towel around the outside edge, and placed on a light bench under constant light at room temperature (25 °C). Peppers were blocked by isolate and completely randomized within the boxes. Two control peppers were inoculated with a sterile plug of V8 agar for each line. Five pepper fruit from each accession per isolate were evaluated and the experiment was performed three times for a total of 15 peppers per isolate, per line. Pepper landrace CM334 was used as the resistant control and Johnny's Breeding line JN571 was evaluated as the susceptible control. Due to poor fruit set only two experimental replicates were performed on lines Grif 972, JN570, 262902, 339083, 511886, 593572, 631131, 631140, 640803, 182646, 194261, and197408 when inoculated with isolate OP97 and lines 194261, 182646, 164560, 640641 631131, 631126, 593572, 593511, 511882, 438633, 566811, 123469, 273415, JN570, and 224442 with isolate 12889.

Peppers were evaluated at 3 and 5 days post inoculation (dpi). Lesion area in cm^2 (maximum length by maximum width) was measured using a hand caliper. For some accessions the visible lesion area was not solidly filled (e.g. multiple spots of diseased tissue) and a coverage score was determined. If symptomatic tissue covered less than 25% of the lesion area measured, the coverage score=0.25, 25% to <50% the coverage score was 0.5, if symptoms covered 50% to <75% the score =0.75, if the visible lesion coverage was >75% the coverage score =1. For recovery of the isolates, isolations were performed on approximately 10% of the symptomatic fruit. Three pieces of fruit were

plated onto V8 agar amended with ampicillin, rifampicin and benomyl (2mL, 2mL, and 0.05g, respectively per liter). Cultures of *P. capsici* were identified by morphological characteristics according to Waterhouse et al (120). Mefenoxam sensitivity was confirmed by transferring isolates to V8 plates amended with 100ppm mefenoxam and determining growth.

Control fruits (inoculated with sterile V8 plug) were removed prior to analysis to avoid violating variance assumptions. Experimental replicates were combined for statistical analysis. Final lesion area was calculated by multiplying the lesion area by the coverage score. Final lesion score for 3dpi and 5dpi relative to line, country of origin and host of origin, was analyzed by ANOVA using the PROC MIXED function of SAS v9.3. Line, isolate and interaction means were separated using LSD at P=0.05, when significant. When analyzing unequal sample sizes, degrees of freedom were accounted using the Kenward Rogers option implemented in SAS. Countries represented by less than four lines were removed from analyses. Data was log transformed for 3dpi values when analyzing by line and transformed $1/x^2$ where x indicates the line value at 3 and 5dpi when analyzed by countries and continents to normalize the data. The percentage of diseased fruit was calculated by combining all replicates for a single line and isolate (total diseased out of 15 total peppers). A diseased fruit was considered to be fruit with a visible lesion, regardless of lesion size.

Genomic DNA was extracted from young green leaves of peppers using the Nucleo Spin II DNA extraction kit (Machery-Nagel Germany, CAT#740770) according to the manufacturer's instructions. DNA was normalized to 10 ng/ul using the NanDrop

ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE).

One hundred ninety-two primers from previously published SSR markers (76), solgenomics.org or designed (Primer 3 http://primer3.sourceforge.net/) from putative Solanaceae defense-related genes (NCBI ncbi.nlm.nih.gov) were tested against a subset of the pepper collection to identify polymorphic markers. Reactions were performed in 15 ul total volume and contained 1 ul DNA, and 0.15 ul GoTaq (Promega Corporation Madison, Wisconsin), 0.9 ul 25uM $MgCl_2$, 0.3 ul dNTPs, and 0.6 ul each of forward and reverse primers (Integrated DNA Technologies, Inc.), with 8.45 ul ddH_2O. PCR reactions were performed in a programmable thermal cycler (Eppendorf, Westbury, NY) using the program: initial denaturation, 94 C (3 min) followed by 35 cycles at 94 C (30s), 60 C (30s) and 72 C (1 min), with a final extension step of 10 min at 72 C. PCR products wereanalyzed by electrophoresis in 4% (wt/vol) agarose gel in 1x Tris-borate-EDTA buffer, stained with ethidium bromide (5 ug/ml) for visualization and compared to a 100-bp ladder (Invitrogen Life Technologies Burlington, ON Canada) to determine amplicon sizes. SSR markers identified as polymorphic in the population were used for genetic diversity, and population structure analyses (Table 3.2).

Table 3.2. Simple sequence repeat (SSR) markers tested against the pepper genotypes and their respective genetic diversity and polymorphism information content (PIC) within the population.

SSR	Primer sequence		Source	G_D	PIC[a]
E492334	GCTGGTTGTGGTTGTACGAG	TGCTCACATATCAATAGATTCAGC	SOLCAP	0.51	0.44
U221402	AAGCCTCCTTGACAAATGCATAT AG	AGATATAGCTACAGTGGCAGCTTCA TC	SOLCAP	0.57	0.52
T0633	GATGGGCTATGCTTGCTGTT	ACATCCCCAATGTTGTTGTG	SOLCAP	0.26	0.24
C2_At2g30100	CAAACTATTTCAGATTTACACTT AAATG	ACCGTTCAAGTTGGCTCTTCACAAC AG	SOLCAP	0.24	0.22
CA516044	ATCTTCTTCTCATTTCTCCCTTC	TGCTCAGCATTAACGACGTC	SOLCAP	0.63	0.58
asu5	GGAAGATCCCTTGAATGAGTATG TCTC	GGCTGAAAATGTCTGATGGAACTG G	SOLCAP	0.33	0.31
GPMS159	AAGAACATGAGGAACTTTAACC ATG	TTCACCCTTCTCCGACTCC	SOLCAP	0.38	0.32
GP20117	TGACAGCTACCGAAAATGA	CCTCTAATGCTGACGTGAA	SOLCAP	0.49	0.45
CP10023	CACCATGTAGCATCTGGG	GATGGATGGATCGACAGA	SOLCAP	0.48	0.44
GP1127	CACCACCAGTCACAAAGTTAC	CCCTTCAAATACATCCCATGC	SOLCAP	0.54	0.46
CA515275	CTCTGCCCTCCTCAACCC	AAAATATGGTCGGAGATCCG	SOLCAP	0.21	0.20
CP10023	CACCATGTAGCATCTGGG	GATGGATGGATCGACAGA	SOLCAP	0.55	0.52
GP20087	CCCTCTCCTCAATTCACA	CCTTTACCCCTAAATTTGAT	SOLCAP	0.28	0.26
GQ386945	GCTGCTATGCCCCCAAGGAT	TTTCTAGACAAGGCAGCTCACCAAT	NCBI	0.26	0.25
AF348141	CCTTACGGGGAAAACCTAGC	CCATACGGACGTTGTCCTCT	NCBI	0.64	0.57
EF645679	GCGCGAGAGACTACAAATCC	CACTCCTTCGTATCCCTCCA	NCBI	0.62	0.54
EF100893	GTTTGGTCTTGTGGGGTCAC	GGCTTTTCTCCACCATTCAC	NCBI	0.29	0.27
GU295217	TTTCGGATTGCCCTATGCTTGTT	AAATTTGTGAGGGCTGTTAGGT	NCBI	0.14	0.13
GU116570	ATTCGGGGTGTGATGAGGTGGA G	AAAAACAAACATAGGGCAAGACGA A	NCBI	0.13	0.12
HPMSHSMAD	TGCTTTCAAAACAATTTGCATGG	GCGTCTAATGCAAAACACACATTAC	Minamiyama	0.70	0.65

Table 3.2 (cont'd)

CAMS319	TCACCTTCCACAGCATCAAG	CAAACGCAAACACCAATCAG	Minamiyama	0.50	0.42
CAMS839	GCAAGCACATCATGCTGAAT	CGAGCGCATTATTGAAGTGA	Minamiyama	0.61	0.57
C2_At5g13200	TATGGGTCCGCCTGCAGTTCCAAC	AAGTTTTCCCCATGCCGCTTCTGT	SOLCAP	0.75	0.71

[a] Genetic diversity for each marker

[b] Polymorphism information content for each marker

Genetic diversity was estimated using Powermarker v3.25 (71) and significance at each locus was determined with 1000 permutations using the Exact test; overall genetic diversity was estimated using the Mantel test as implemented in Powermarker.

Population structure of the germplasm was analyzed using STRUCTURE v2.3.4 (92). Following preliminary analyses, burnin length, MCMC chain replication and lambda were selected to be 200,000, 500,000 and 0.49, respectively. Population number (k) was determined empirically by comparing posterior distribution likelihoods independently among 3 independent runs of K=1 to 20 as described by Evanno et al. (27). Data included 23 polymorphic SSRs and were analyzed using the admixture model and correlated allele frequencies without previous population information (28,92). Fst significance between populations was determined using 95% confidence intervals based on 1000 bootstrap replicates as implemented in Powermarker.

Visualization of the resulting Q (proportion of membership) of each individual into predefined categories (country, continent, and disease susceptibility) was generated using the Population Sorting Tool (PST) in R (99), J.J. Morrice unpublished.) Individuals with $Q \geq 0.6$ membership in a single subpopulation were labeled as such. Individuals with $Q < 0.6$ membership in a single subpopulation were considered admixed. Significant population structure by continent, country and Phytophthora fruit rot resistance was estimated using the population differentiation test implemented in Powermarker. Significance at each locus and overall was determined using 1000 permutations. Countries represented by less than four individuals were excluded from analyses. Significance of pairwise Fst differentiation was based on 2.5 and 97.5% confidence intervals calculated from 1000 bootstrap replications. At 3dpi, fruit were grouped into

resistant (lesion area 0<2 cm^2), moderately resistant 2<5cm^2, moderately susceptible (5<10cm^2) and susceptible (10 cm^2 and greater) categories. At 5dpi, fruit categories were resistant (0<5 cm^2), moderately resistant (5<10 cm^2), moderately susceptible (10 <20 cm^2) and susceptible (20 cm^2 and greater).

RESULTS

Significant differences were identified among pepper genotypes at both 3 and 5dpi. At 3dpi, isolate was not significant (P=0.319) and line was highly significant (P<0.001). Line-by-isolate interactions were also significant (P=0.0001) at 3dpi. Breeding line JN571 was the most susceptible with an average lesion area of 27 and 30 cm^2 for isolates 12889 and OP97, respectively. When inoculated with isolate OP97 line PI 640516 had the smallest average lesion area of 0 cm^2. When inoculated with 12889, line 640803 had the lowest lesion area (0 cm^2). Most lines, 111 and 121 for isolates 12889 and OP97, respectively, were moderately resistant or resistant to *Phytophthora capsici* at 3dpi.

Significant line-by-isolate interactions (P=0.0008) were identified at 5dpi. Line was highly significant (P<0.0001) and isolate was not significant (P=0.302). Most genotypes evaluated were susceptible to both isolates: 74 lines for isolate OP97 and 103 for 12889 (Table 3). No accession was completely resistant to both isolates of *P. capsici*. JN571 had an average lesion area of 50 cm^2, indicating the fruit were completely covered

Table 3.3. Pepper fruit disease susceptible at 3 days post inoculation (dpi) and 5dpi when inoculated with isolates OP97 and 12889.

	OP97			12889		
PI	**Day 3[a]**	**Day 5[a]**	**% Incidence**	**Day 3[a]**	**Day 5[a]**	**% Incidence**
CM334	1.06	17.32	0.53	1.37	14.42	0.93
Early Jalapeño	2.69	43.33	0.87	2.80	28.63	1.00
Grif 9094	9.71	48.50	1.00	9.98	46.23	1.00
Grif 9105	8.47	46.97	1.00	6.35	44.12	1.00
Grif 9109	1.56	14.60	0.47	1.37	16.68	0.47
Grif 972	5.10	27.80	0.70	-	-	-
Jn566	3.72	36.71	0.80	4.80	35.07	0.87
Jn570	17.44	49.91	1.00	12.65	50.81	1.00
Jn571	28.24	50.00	1.00	30.49	53.33	1.00
Jn574	6.77	35.51	0.87	9.76	46.92	1.00
PI 102883	3.06	25.26	0.87	-	-	-
PI 123469	1.75	30.00	0.60	1.58	34.26	0.80
PI 123474	10.98	34.87	0.87	6.74	37.39	0.80
PI 124078	1.40	27.71	0.60	1.03	20.77	0.47
PI 127445	2.38	26.67	0.53	4.20	38.09	0.80
PI 135822	2.89	27.39	0.60	2.08	23.50	0.60
PI 135826	3.15	32.02	0.80	2.63	27.45	0.67
PI 135874	1.87	21.09	0.53	1.15	20.32	0.47
PI 138557	4.58	34.34	0.73	2.51	25.32	0.67
PI 138558	2.49	31.89	0.73	4.43	34.38	0.80
PI 138560	6.59	34.87	0.80	2.35	29.05	0.73
PI 138565	1.58	17.31	0.40	1.76	27.43	0.79
PI 142832	3.26	36.52	0.86	3.26	34.25	0.85
PI 148628	2.13	36.68	0.80	5.18	30.00	0.60
PI 159256	4.49	32.56	0.73	4.25	36.55	0.87
PI 164311	4.79	37.30	0.87	10.38	43.33	0.87
PI 164560	1.26	26.38	0.73	2.95	30.03	0.70
PI 167063	8.61	48.90	1.00	6.01	46.76	1.00
PI 169129	7.94	39.32	0.93	9.07	45.10	1.00
PI 169140	1.57	18.28	0.53	3.36	33.70	0.87
PI 174114	1.25	22.00	0.47	2.19	33.34	0.80
PI 176888	5.38	30.43	0.73	8.89	37.33	0.80
PI 177294	5.78	33.16	0.67	3.42	30.80	0.80
PI 177301	11.94	46.21	1.00	5.87	35.12	0.73
PI 181733	3.00	33.55	0.73	4.62	38.46	0.87
PI 181734	2.41	27.54	0.80	9.23	46.67	0.93
PI 181934	5.46	41.48	0.93	-	-	-
PI 182646	1.33	31.02	0.70	2.47	35.81	0.70
PI 183668	2.06	21.53	0.53	2.54	33.67	0.73
PI 183922	3.22	37.40	0.80	3.40	36.67	0.80
PI 184039	5.76	38.75	0.93	5.90	44.19	0.93

Table 3.3 (cont'd)

PI 194259	1.41	13.33	0.27	3.71	33.33	0.67
PI 194261	1.66	16.82	0.50	6.18	35.81	0.70
PI 194910	1.52	19.76	0.53	5.96	36.81	0.80
PI 197408	1.21	23.22	0.70	-	-	-
PI 201232	1.48	15.54	1.00	1.51	13.02	0.80
PI 201234	1.33	30.62	0.73	2.23	30.90	0.87
PI 201237	1.28	9.59	0.60	1.06	4.07	0.60
PI 201239	4.39	27.31	0.67	1.75	24.14	0.80
PI 203524	1.75	22.46	0.60	1.02	14.71	0.47
PI 206950	4.40	39.17	0.87	4.60	40.06	0.93
PI 207727	-	-	-	3.05	33.33	0.67
PI 213915	1.36	10.06	0.33	4.23	26.67	0.60
PI 224438	1.06	13.72	0.60	1.54	28.64	0.64
PI 224442	-	-	-	6.32	40.81	0.80
PI 226633	4.64	37.49	0.80	7.39	46.93	1.00
PI 241641	1.27	23.84	0.53	1.11	14.08	0.73
PI 241644	7.60	36.67	0.73	6.22	32.20	0.53
PI 243936	6.48	32.21	0.87	2.21	28.89	0.80
PI 244669	1.61	26.67	0.53	2.55	35.62	0.87
PI 249635	1.75	27.83	0.60	8.68	42.92	0.86
PI 249908	6.66	46.67	0.93	4.08	40.96	1.00
PI 250141	2.20	13.33	0.27	1.32	14.06	0.33
PI 257044	1.96	25.78	0.67	1.83	30.95	0.67
PI 257047	1.52	19.03	0.53	2.29	24.85	0.80
PI 257048	4.21	31.70	0.67	3.14	35.44	0.87
PI 257283	1.77	25.52	0.73	3.26	36.91	0.93
PI 260452	2.24	30.64	0.67	1.81	30.73	0.67
PI 262172	5.51	43.10	1.00	5.63	37.10	0.93
PI 262902	3.62	25.54	0.60	1.63	24.58	0.73
PI 263075	4.21	40.12	0.93	2.54	32.02	0.60
PI 263076	1.18	39.27	0.93	1.73	28.01	0.80
PI 263077	6.17	46.67	0.93	4.51	36.65	1.00
PI 263113	1.74	46.67	0.93	7.23	50.00	0.73
PI 263114	2.07	35.70	0.87	1.50	17.26	0.60
PI 264281	1.29	11.81	0.40	1.31	16.13	1.00
PI 264662	6.06	42.70	0.93	6.25	44.75	0.53
PI 267730	3.71	20.00	0.40	2.20	26.67	0.67
PI 269455	1.09	10.06	0.27	-	-	-
PI 269458	1.75	28.39	0.60	4.12	28.83	0.50
PI 273415	2.00	21.86	0.80	1.65	16.00	0.73
PI 281318	1.53	23.97	0.67	1.64	28.10	1.00
PI 281341	-	-	-	6.41	45.92	0.60
PI 281433	1.52	30.50	0.67	2.48	30.00	0.67
PI 298646	1.58	18.33	0.53	1.77	23.60	0.93
PI 298647	4.34	47.48	1.00	1.91	30.52	0.87

Table 3.3 (cont'd)

PI 302987	4.62	40.72	0.87	4.36	42.52	0.60
PI 322720	1.39	7.09	0.20	1.41	24.92	0.93
PI 339005	5.01	26.67	0.53	17.70	45.51	0.93
PI 339006	10.88	48.50	1.00	17.68	46.67	1.00
PI 339007	2.46	30.49	0.73	10.17	50.00	0.93
PI 339009	4.33	36.19	0.80	10.13	41.48	0.93
PI 339010	2.05	27.42	0.60	10.33	45.57	1.00
PI 339019	6.60	30.00	0.60	10.50	47.06	0.67
PI 339048	2.16	25.70	0.60	3.65	31.53	1.00
PI 339075	11.44	46.87	1.00	14.67	50.31	0.80
PI 339079	10.59	44.90	0.93	9.01	40.13	0.73
PI 339083	6.98	40.20	0.80	-	-	-
PI 339132	3.86	31.13	0.67	3.87	35.50	0.93
PI 342949	6.14	34.77	0.87	4.26	38.66	0.93
PI 357503	24.80	45.61	1.00	26.41	46.94	1.00
PI 357531	3.73	42.85	1.00	8.99	44.91	1.00
PI 368396	16.78	45.59	0.93	16.76	50.10	0.93
PI 369996	3.43	46.14	0.93	2.29	29.63	1.00
PI 371867	2.02	25.80	0.80	3.05	28.40	1.00
PI 379182	3.47	30.23	0.67	8.89	48.05	1.00
PI 385960	7.38	40.80	0.93	10.31	47.84	0.67
PI 390612	8.83	40.00	0.80	5.63	31.91	0.73
PI 409141	1.04	20.00	0.40	1.42	30.05	0.93
PI 410407	4.89	45.21	1.00	19.38	47.26	0.73
PI 427290	1.13	7.57	0.27	1.64	26.80	0.93
PI 432802	6.58	47.68	1.00	8.08	42.32	0.93
PI 438565	1.08	26.67	0.47	-	-	-
PI 438624	17.87	40.00	0.80	13.92	46.67	1.00
PI 438633	12.58	45.57	0.93	20.72	48.86	0.73
PI 441628	1.91	18.65	0.73	2.05	30.53	0.47
PI 511879	1.75	23.65	0.67	1.41	13.62	1.00
PI 511882	1.86	14.05	0.87	5.21	36.05	0.60
PI 511884	1.58	30.75	0.87	1.07	11.28	0.44
PI 511886	2.33	27.66	0.56	3.56	20.81	1.00
PI 550700	3.34	39.76	1.00	7.51	45.95	0.87
PI 555649	1.96	24.34	0.80	3.12	37.27	1.00
PI 566808	7.21	35.86	0.73	17.12	47.21	0.60
PI 566811	1.08	0.66	0.69	1.06	3.81	0.87
PI 585246	2.41	37.17	0.87	3.54	36.68	0.47
PI 593493	1.00	16.67	0.67	1.69	23.33	1.00
PI 593495	1.91	30.02	0.80	1.10	23.53	0.50
PI 593511	1.74	20.01	0.53	1.31	20.82	0.93
PI 593561	2.59	39.85	0.87	3.75	40.19	0.60
PI 593564	6.68	40.00	0.80	2.85	30.00	0.67
PI 593572	2.17	20.99	0.50	5.40	34.33	0.53

Table 3.3 (cont'd)

PI 593573	1.78	20.92	0.47	2.34	18.08	0.40
PI 593920	1.77	25.03	0.50	1.37	20.00	0.93
PI 593929	3.26	39.10	0.87	1.97	32.38	0.73
PI 593933	6.15	33.33	0.67	2.79	35.77	0.33
PI 595906	4.82	30.00	0.60	2.21	13.40	0.87
PI 600934	3.18	31.67	0.93	2.18	23.45	1.00
PI 601110	9.04	43.34	0.93	24.41	47.26	1.00
PI 631126	2.80	39.32	0.93	2.37	20.23	0.70
PI 631131	5.27	34.55	0.80	3.24	28.87	0.73
PI 631140	1.10	32.63	0.67	1.31	36.67	0.67
PI 631143	5.38	35.70	0.71	1.71	33.33	0.47
PI 631147	2.78	33.35	0.73	1.43	17.68	1.00
PI 639641	7.68	37.80	0.93	18.75	50.82	0.87
PI 640448	1.59	29.76	1.00	2.43	20.67	0.93
PI 640460	4.65	36.00	0.87	3.44	35.65	0.80
PI 640461	2.34	28.56	0.57	5.30	35.82	0.93
PI 640480	3.59	37.21	0.87	2.92	41.53	0.50
PI 640516	1.00	30.76	0.69	2.35	25.12	0.86
PI 640532	1.75	18.21	0.73	2.09	19.35	1.00
PI 640560	3.39	36.03	0.80	3.08	35.16	1.00
PI 640579	6.97	34.64	0.93	3.93	40.60	0.57
PI 640581	1.36	21.49	0.50	1.07	3.78	0.60
PI 640582	1.01	6.67	0.13	1.53	26.72	0.73
PI 640588	1.66	34.03	0.87	1.93	33.93	0.60
PI 640641	1.04	6.86	0.33	2.03	26.40	0.80
PI 640659	1.12	20.05	0.60	1.47	18.27	0.93
PI 640663	1.93	20.73	0.93	1.63	26.23	0.57
PI 640670	1.69	26.67	0.53	2.54	28.69	0.93
PI 640671	6.58	37.32	0.80	9.41	37.13	0.79
PI 640676	1.00	24.63	0.79	1.16	24.83	0.73
PI 640682	1.72	20.00	0.40	1.94	33.44	0.93
PI 640744	1.93	37.86	0.93	1.56	26.31	1.00
PI 640791	9.43	41.60	0.87	13.68	44.10	0.30
PI 640803	1.18	12.00	0.70	0.97	5.68	0.67
PI 640809	3.90	37.19	1.00	2.90	30.00	0.67
PI 640815	2.02	16.67	0.33	2.35	30.00	0.93
PI 640833	1.23	8.26	0.85	1.25	0.42	1.00
PI 645520	7.44	45.24	1.00	12.12	48.79	0.40
PI 653650	1.10	14.27	0.29	1.30	16.68	0.93

a Mean lesion area (cm^2)

by the lesion. CM334, the resistant control, had an average lesion area of 14 cm^2 and 17 cm^2 when inoculated with isolates 12889 and OP97, respectively. One line, PI 566811

had an average lesion area of 1 cm^2 and was significantly more resistant than CM334 when inoculated with OP97. This same line had a lesion area of 3.8 cm^2 when inoculated with isolate 12889.

When analyzed by country and continent of origin significant differences were evident at 3 and 5dpi (Table 3.4, 3.5). Country was highly significant (P<0.0001), isolate was

Table 3.4. *Capsicum spp*. Phytophthora fruit rot resistance among countries of origin

	Lesion area[b]			
Category[a]	**3dpi**		**5dpi**	
Pakistan	5.66	A	19.44	A
Ethiopia	9.21	AB	25.56	BC
Mexico	7.58	B	23.64	B
Colombia	9.71	B	27.41	BCD
Guatemala	5.89	A	33.01	BCDE
India	9.47	BC	31.28	CDE
Brazil	10.13	CD	29.55	BCDE
Iran	10.78	D	32.60	DEF
Spain	4.88	BC	29.44	EFG
USA	8.81	E	33.16	FGH
Turkey	13.69	E	36.78	FGH
Soviet	7.61	E	38.62	HI
China	11.23	E	34.55	GHI
Serbia	18.82	F	43.72	I

[a]Categories with less than four individuals representing a country were not included in analyses

[b] Mean lesion area (cm^2) for both isolates combined at 3 days post inoculation (3dpi) and 5 days post inoculation (5dpi); different letters within a time period indicate significant differences among transformed values using LSD at P=0.05 for both isolates

Table 3.5. *Capsicum spp.* Phytophthora fruit rot resistance among continents of origin

	Lesion area[b]							
	3dpi				5dpi			
Category[a]	12889		OP97		12889		OP97	
Africa	10.29	BCD	5.89	A	33.10	BC	23.31	A
Asia	9.28	C	7.77	BC	30.50	BC	30.71	BC
Europe	14.28	E	11.50	D	39.88	D	35.67	C
N. America	8.26	C	7.84	BC	29.34	B	28.39	B
S. America	9.79	BC	9.49	B	28.84	B	28.17	B

[a] Categories with less than four individuals representing a country were not included in analyses

[b] Mean lesion area (cm^2) for both isolates combined at 3 days post inoculation (3dpi) and 5 days post inoculation (5dpi); different letters within a time period indicate significant differences among transformed values using LSD at P=0.05 for both isolates

also significant (P<0.0001 and P=0.007), and the interaction between country and isolate was not significant (P=0.3448 and P=0.0581) at 3 and 5dpi. Fruit from Pakistan were the least susceptible, while fruit from Serbia were the most susceptible at both 3 and 5dpi. When analyzed by continent, significant interactions (P=0.0369 and P=0.0269) were detected between continent and isolate at 3dpi and 5dpi. Isolate OP97 was significantly more virulent than isolate 12889 at both 3 and 5dpi. At 3dpi, fruit from Africa and S. America were the least susceptible when inoculated with OP97 and 12889, respectively. Fruit from Europe were the most susceptible when inoculated with 12889 or OP97 3dpi. At 5dpi, fruit from Africa and S. America were the least susceptible and fruit from Europe were the most susceptible when inoculated with OP97 and 12889, respectively (Table 3.5).

The percentage of diseased fruit varied greatly (13-100% incidence) between lines and isolates at 5dpi (Table 3.3). Most lines evaluated had incidence greater than 50%,

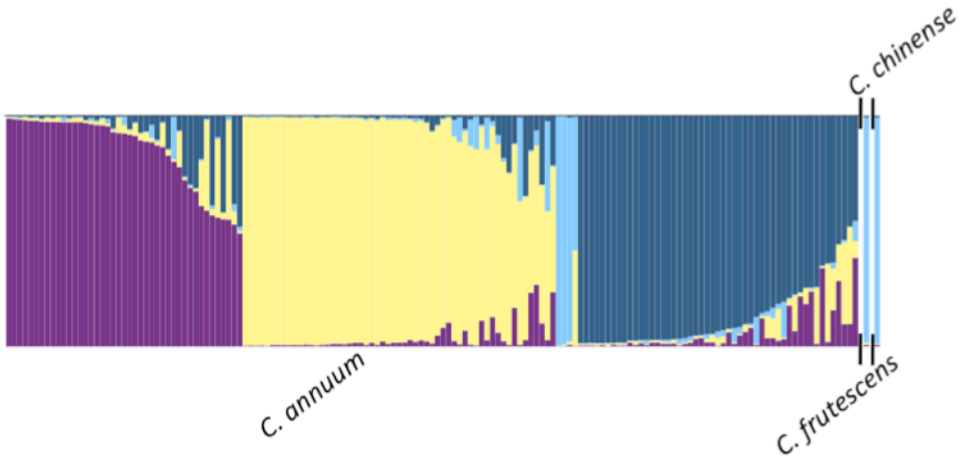
150 and 148 for isolates 12889 and OP97, respectively. When inoculated with isolate 12889, line PI 640803 had the lowest incidence with 30% diseased fruits. CM334 had a disease incidence of 93%. Line JN571 had 100% disease incidence as did 32 other lines. When inoculated with isolate OP97, line PI 640582 had the lowest disease incidence with 13% of fruits infected. CM334 had a disease incidence of 53%. JN571 had 100% disease incidence along with 18 other lines.

The 192 SSRs evaluated yielded 23 polymorphic markers that were used for characterizing and evaluating population structure of the pepper collection. Population structure for 155 of the 170 genotypes was estimated using the Bayesian analysis software, STRUCTURE (92). Pepper lines were grouped into 4 genetic clusters (Ln=-5058). The two *C. frutescens* and *C. chinense* accessions were placed into genetic cluster 3, while *C. annuum* individuals were distributed through each of clusters (Figure 3.1). One hundred forty-two individuals could be assigned to a single cluster based on membership. The remaining fifteen individuals could not be assigned to a single cluster and were classified as admixed. For the STRUCTURE inferred clusters, mean Fsts varied from 0.12 to 0.45. Cluster 1 had moderate, very great, and little differentiation from clusters 2, 3, and 4, respectively. Cluster 2 had moderate and very great differentiation from cluster 3 and cluster 4, respectively. Cluster 3 had very great differentiation from clusters 1, 2 and 4 as according to Hartl and Clark (45).

When individual genotypes were grouped by continent and country of origin, species or diseases resistance, population structure was detected. Significant differences were detected among individual markers and the predefined categories. No clusters were perfectly correlated with predefined categories, but some clusters were more frequently

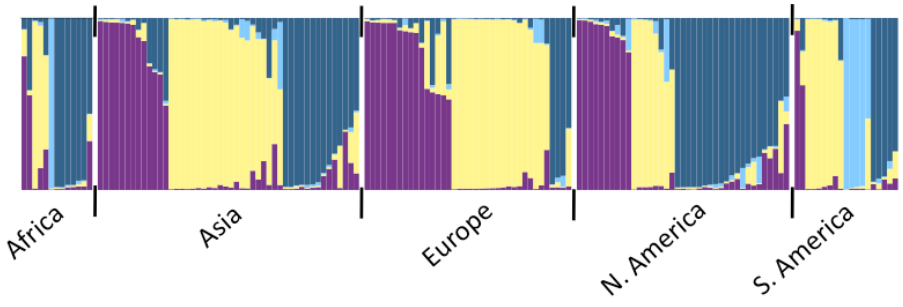
found in some categories. *Capsicum frutescens* and *C. chinense* individuals were only represented by cluster 3 (Figure 3.1).

Figure 3.1. Population structure grouped by species. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue), Cluster 4 (steel blue).



Cluster 3 individuals were only represented in S. America and Africa, and cluster 1 individuals less prevalent in Africa and S. America (Figure 2).

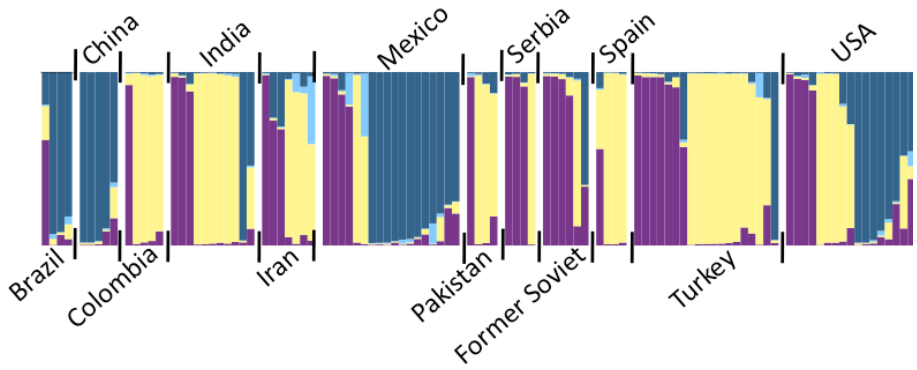
Figure 3.2. Population structure grouped by continent of origin for pepper germplasm. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue), Cluster 4 (steel blue). A white space and black tick marks separate subgroups of individuals.



Cluster 2 individuals less prevalent in Africa, but constituted a higher proportion of individuals from Europe, S. America and Asia. Cluster 4 individuals were a low

proportion of individuals from Europe and S. America. Among countries, variation in cluster representation was more evident than among continents (Figure 3.3).

Figure 3.3. Population structure grouped by country of origin for the *C. annuum* germplasm. Only countries represented by four or more individuals were included. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue), Cluster 4 (steel blue). A white space and black tick marks separate subgroups of individuals.



Individuals in cluster 1 were not found in China, Colombia, or Spain. Individuals from cluster 2 were not found in Brazil or China. Cluster 3 individuals were not found in any of the countries represented by at least four lines. Cluster 4 was only represented in individuals from Brazil, China, India, Mexico Turkey and the USA. At 3dpi, each cluster was represented in the resistant category for isolates OP97 and 12889. When grouped by disease resistance for both isolates at 5dpi, individuals from clusters 3 and 4 were not represented in the moderately resistant/resistant categories and cluster 2 had a low prevalence in the resistant/moderately resistant categories for isolate 12889 (Figures 3.4, 3.5).

Figure 3.4. Population structure grouped by Phytophthora fruit rot resistance to isolate OP97 at A) 3 days post inoculation and B) 5 days post inoculation. Individuals were grouped into a resistant and moderately resistant category (R/MR), a moderately susceptible category (MS), and a susceptible category (S) based on their mean disease ratings. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue), Cluster 4 (steel blue). A white space and black tick marks separate subgroups of individuals.

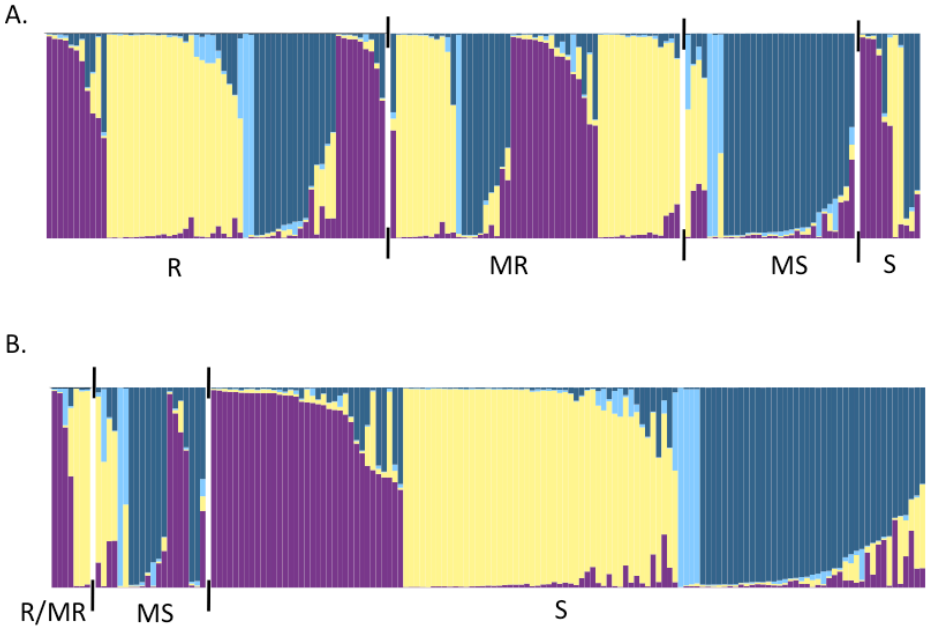
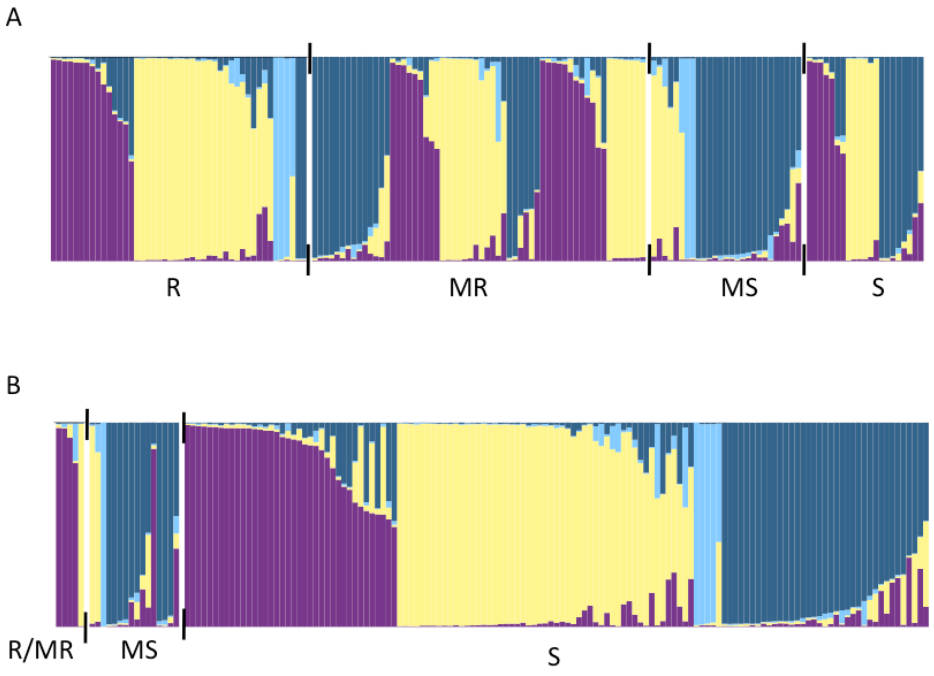


Figure 3.5. Population structure grouped by Phytophthora fruit rot resistance to isolate 12889 at A) 3 days post inoculation and B) 5 days post inoculation. Individuals were grouped into a resistant and moderately resistant category (R/MR), a moderately susceptible category (MS), and a susceptible category (S) based on their mean disease ratings. Cluster 1 (purple), Cluster 2 (light yellow), Cluster 3 (sky blue), Cluster 4 (steel blue). A white space and black tick marks separate subgroups of individuals



A total of 102 alleles were detected among the 23 SSRs, evaluated in the collection, ranging from 2 to 7 alleles per locus with an average allele diversity of 4.9 alleles per locus. The mean genetic diversity index of the collection was 0.44. The mean polymorphism information content (PIC) value was 0.40 for the collection and individual markers ranged from 0.12 to 0.71 for the population (Table 3.2). When individual genotypes were evaluated, the highest PIC value was 0.54 in PI 640809 and the lowest PIC value was 0.03 in lines JN566, PI 148628, and PI 640803. Genetic diversity was similarly distributed within continents (0.38-0.47), and pairwise Fsts indicated little to moderate differentiation between continents (0.00 – 0.07) (Table 3.6, Table 3.7).

Table 3.6. Genetic diversity of pepper genotypes among countries and continents

Category[a]	Diversity Estimates[b]	
	G_D	PIC
Africa	0.40	0.36
Asia	0.44	0.39
Europe	0.38	0.34
N. America	0.47	0.43
S. America	0.41	0.37

Category[a]	Diversity Estimates[b]	
	G_D	PIC
Afghanistan	0.28	0.23
Brazil	0.35	0.27
China	0.35	0.29
Colombia	0.27	0.22
Former Soviet	0.35	0.29
India	0.34	0.29
Iran	0.43	0.38
Mexico	0.45	0.41
Pakistan	0.36	0.30
Serbia	0.26	0.22
Spain	0.27	0.22
Turkey	0.36	0.31

[a] Categories represented by less than four lines were excluded from analyses and are not shown

[b] Average values for SSRs are presented; Mean values are presented for the genetic diversity (G_D) and the polymorphism information content (PIC)

Table 3.7. Genetic differentiation (F_{ST} pairwise differentiation) of pepper genotypes among countries and continents

	Fst[b]										
Category[a]	**Afghanistan**	**Brazil**	**China**	**Colombia**	**Former Soviet**	**India**	**Iran**	**Mexico**	**Pakistan**	**Serbia**	**Spain**
Brazil	0.18										
China	0.26*	0.19*									
Colombia	0.24	0.20	0.19								
Former Soviet	0.20	0.14	0.17*	0.09							
India	0.25*	0.17	0.17*	0.09	0.06						
Iran	0.24*	0.19*	012*	0.15	0.06	0.10*					
Mexico	0.37*	0.23*	0.18*	0.28*	0.14*	0.13*	0.06*				
Pakistan	0.19*	0.17*	0.16*	0.13	0.13*	0.14	0.11*	0.22*			
Serbia	0.23	0.20	0.17	0.16	0.09	0.21	0.11	0.27*	0.16		
Spain	0.11	0.20	0.19	0.09*	0.11	0.13	0.18*	0.33*	0.19	0.19	
Turkey	0.32*	0.21*	0.18*	0.16	0.06	0.07	0.00	0.07*	0.11	0.22	0.25
USA	0.35*	0.24*	0.16*	0.21*	0.13*	0.08	0.08*	0.05*	0.21*	0.25*	0.29*

	Fst[b]			
Category[a]	**Africa**	**Asia**	**Europe**	**N. America**
Asia	0.06			
Europe	0.02	0.02		
N. America	0.07*	0.00*	0.03	
S. America	0.01	0.05*	0.01	0.06*

[a] Categories with less than four lines were excluded from analyses and are not shown

[b] Average values for SSRs are presented; * indicates value was outside the 2.5% and 97.5% confidence intervals at 1000 bootstraps

Individuals from N. America, Asia and S. America had the highest genetic diversity and genetic differentiation was highest in individuals from N. America. Genetic diversity within countries varied greatly (0.26 – 0.45) and pairwise Fst values detected little to very great genetic differentiation among countries (Table 3.6, Table 3.7). Genetic diversity was highest in individuals from Iran, Mexico and the USA, the greatest differentiation was seen with individuals from Mexico and the USA. Pairwise Fsts for disease resistance to 12889 and OP97 showed little to great (0 to 0.22) genetic differentiation between categories (Table 3.8).

Table 3.8. Genetic differentiation (F_{ST} pairwise differentiation) among pepper disease resistance categories 3 (shaded values) and 5 days post inoculation (dpi) when inoculated with OP97 and 12889

Isolate OP97				
	Pairwise Fst[b]			
Category[a]	**MR**	**MS**	**R**	**S**
MR		0.18	-	0.50*
MS	0.01		-	0.00*
R	0.01	0.01		-
S	0.05	0.04	0.04	

Isolate 12889				
Category[a]	**MR**	**MS**	**R**	**S**
MR		0.01*	0.01	0.02*
MS	0.12*		0.03	0.01*
R	-	-		0.03
S	0.49	0.00*	-	

[a] 3 dpi are shaded above the diagonal and 5dpi values are below the diagonal for OP97 and 12889; MS = moderately susceptible, R = resistant MR = moderately resistant, S=susceptible

[b] Average values for SSRs are presented; * indicates value was outside the 95% confidence interval at 1000 bootstraps

When inoculated with isolate OP97, no significant genetic differentiation was evident between any of the categories at 3dpi. At 5dpi, individuals in the resistant/moderately resistant (R/MR) category and the susceptible (S) category were significantly differentiated, as were individuals in the moderately susceptible (MS) and R/MR categories. When inoculated with 12889, differentiation between categories was significant at 3dpi for MS and MR categories, and MS and S categories. Significant differentiation was detected at 5dpi between the MR and S, MS and S and the MR and MS categories at 3dpi when inoculated with OP97.

DISCUSSION

Pepper is an important crop grown and cultivated around the world and *P. capsici* is a devastating pathogen that causes economic losses annually. In this study, we evaluated Phytophthora fruit rot resistance, population structure and genetic diversity of a diverse collection of peppers from around the globe. Significant differences among lines and countries were detected for disease resistance. Significant population structure was detected when grouped by predefined categories of disease resistance, country and continent of origin.

Most accessions evaluated in this study were highly susceptible to both isolates of *P. capsici* at 5dpi, while many displayed partial resistance at 3dpi. At 5dpi, disease incidence varied greatly among lines, and lines with partial resistance, in general, had lower incidence than the most susceptible lines. The resistant control, CM334, had a high incidence of disease, and the mean lesion area was moderately susceptible, 14 and 17 cm^2, depending on the isolate. The susceptible control, JN571, also had a high incidence

of disease, and lesion area was high (~50 cm^2). Several individuals were identified with higher resistance than CM334 and are potential sources of resistance for breeding programs. Inoculations were made under ideal disease conditions, and those lines with lower incidence may prove to be resistant under field conditions. No lines evaluated were completely resistant to Phytophthora fruit rot at 5dpi, this included lines with known resistance to Phytophthora root rot (18). Markers known to segregate for *P. capsici* root rot were also not informative when searching for population structure related to fruit disease resistance (76). These results are consistent with previous reports that found no associations between fruit and root rot (31,111).

Certain geographic regions were significantly less susceptible than others (countries and continents). In particular, fruit from Serbia were the most susceptible and fruit from Pakistan were the least susceptible at 3 and 5dpi. Country and continent were not predictive of resistance however, with all countries and continents contributing very susceptible, 20 cm^2 or greater lesion area at 5dpi, individuals. Mexico and S. America, the center of origin for *C. annuum* and some *Phytophthora spp*. (14,39) were among the more resistant countries/continents. These results were not unexpected, since most pepper lines with resistance to *P. capsici*-induced diseases are landraces from Mexico and Mexico is known to be the center of origin for *C. annuum*.

Genetic diversity within this collection of *C. annuum* was moderate (0.44), and most individuals were grouped into 1 of 4 genetic clusters based on the Bayesian analysis. Clusters did not completely correspond with predefined categories of continent, country, or disease susceptibility though some clusters were better represented in categories than others. Few individuals (15 of 157) were admixed, consistent with

previous results (1,51,65). *C. frutescens* and *C. chinense* individuals were grouped within cluster 3 as were several *C. annuum* lines. These *C. annuum* lines in cluster 3 were predominantly from S. America and are likely the result of introgression or misclassification. Many of these individuals had growth habits or flower color reminiscent of *C. frutescens*, *C. baccatum* or *C. chinense* (data not shown). These data suggest limited introgression from related species into individuals of *C. annuum* within available germplasm, and minimal cross pollination among genetic clusters.

When based on geographic regions, populations in Asia and North America were represented by individuals from each *C. annuum* cluster. In Asia, the small-fruited lines and landraces commonly grown may be more akin to ancestral populations and may be the result of selection for different local markets. In N. America the high diversity and broad population profile is likely due to the inclusion of different market classes (sweet fruited bells and pungent chiles) and breeding lines in the population (51,87). Individual countries in Asia did not display the diversity seen across the continent. The USA (the only country represented by four individuals in N. America) was represented by multiple market classes and maintained this diverse population profile seen for N. America.

Phytophthora fruit rot resistance, population structure, and genetic diversity demonstrated significant differences between countries, and continents in this diverse collection of peppers. Disease susceptibility was significantly associated with population structure, continents and countries of origin at both 3 and 5dpi. These results provide the groundwork for Phytophthora fruit rot resistance, genetic diversity and population structure of *Capsicum annuum* on a global scale. Further work is needed to identify pepper lines with full resistance to Phytophthora fruit rot.

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