

Genotypic variability of *Phytophthora capsici* isolates in Guanajuato

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Abstract

Phytophthora capsici is the causative agent of ‘chili pepper wilt’ one of the most important diseases of this crop. To establish efficient control strategies, it is necessary to know the diversity of the pathogen, for this, this research was carried out, whose objective was to determine the degree of genetic diversity of 30 monozygotic isolates obtained from pepper (*Capsicum annuum* L.) from six municipalities in the state of Guanajuato, and two reference strains. The genetic variation was determined by the AFLP technique. The similarity found between the isolates ranges from 76 to 95%. The results obtained indicate that, within the population, groups form based on the localities of isolation. No clonal organisms were found, so the 32 isolates analyzed were unique genotypes, and no correlation was found between groups defined by molecular markers and virulence, type of mating or response to metalaxyl.

Keywords: genetic variation, pepper wilt, population diversity.

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The oomycete *Phytophthora capsici* Leonian is a hemibiotrophic pathogen that causes severe epidemics in a wide range of crops worldwide (Lamour *et al.*, 2012). In the chili pepper crop (*Capsicum annuum* L.), it is part of the complex that causes the drying or wilting of the chili pepper (Erwin and Ribeiro, 1996), which, in Mexico and specifically in the state of Guanajuato, is one of the main causes of crop loss (González-Pérez *et al.*, 2004), which reaches up to 100% when environmental conditions are favorable for the development of this pathogen (Universidad Illinois, 2001).

In the field, the management of this disease is mainly based on the application of fungicides, in combination with cultural practices that limit the development of wilt, such as raised beds, plastic mulching and drip irrigation, among others (Granke *et al.*, 2012). However, the wide range of *P. capsici* hosts, its ability to recombine sexually, and the production of oospores as survival structures, has limited the effectiveness of control strategies (McDonald and Linde, 2002; Gobena *et al.*, 2012). Particularly, sexual recombination between mating types A1 and A2 of this heterothallic oomycete can generate new genotypes with greater virulence, pathogenicity, resistance to fungicides and ability to overcome host resistance (Granke *et al.*, 2012).

P. capsici sporangia are infrequently dispersed between fields by wind (Lamour and Hausbeck, 2001b). Instead, the movement of surface water sources for irrigation and the movement of infected plant material or infested soil are known to be key factors in the local spread of *P. capsici* (Granke *et al.*, 2009). These conditions limit the rapid spread over long distances of this pathogen, so it would be expected that a better understanding of the regional genetic diversity of the pathogen population would allow a more effective deployment of resistant varieties and the establishment of improvement programs that seek to generate lasting host resistance (Quesada-Ocampo *et al.*, 2011).

This assessment of genetic diversity is based on different characteristics, including molecular characteristics (Martin *et al.*, 2012). Molecular characteristics are data based on deoxyribonucleic acid (DNA) that have been used to determine genetic diversity (Mohammadi and Prasanna, 2003), among other reasons because this information is not influenced by environmental conditions. To generate this data, there are different protocols, the most used are: random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) y Ribosomal DNA e internal transcribed spacers (rDNA-ITS), among others (Gupta *et al.*, 1999). Based on the above, this study was carried out with the aim of evaluating the genetic diversity present in the DNA of *P. capsici* isolates, obtained in the main chili peppers-producing areas in Guanajuato.

Strains

30 isolates of *P. capsici* were used, obtained from plants with symptoms of 'wilt', in 12 commercial production lots of different varieties and hybrids of five types of chili peppers (poblano, güero, serrano, jalapeño and chilaca), located in the most important municipalities in the production of this crop in Guanajuato: Dolores Hidalgo, San Luis de la Paz, Juventino Rosas, Silao, Salvatierra and Cortázar (Table 1). These isolates were identified using the keys of Erwin and Ribeiro (1996). In addition, two strains PCT17 and PCC6, donated by Dr. Sylvia Patricia Fernández Pavía of the Plant Pathology Laboratory-UMSNH.

Table 1. Municipality of origin and type of chili pepper from where 30 monozygotic cultures of *Phytophthora capsici* were obtained.

Municipality	Key	Type of chili pepper	Municipality	Key	Type of chili pepper	
Dolores Hidalgo	D1	Poblano	Silao	S1	Güero	
	D2	Poblano		S2	Güero	
	D3	Poblano		S3	Güero	
	D4	Poblano		S4	Serrano	
	D5	Poblano		S5	Jalapeño	
	D6	Poblano		S6	Jalapeño	
	D7	Poblano		S7	Jalapeño	
	D8	Poblano	Juventino Rosas	J1	Jalapeño	
	D9	Poblano		J2	Poblano	
	D10	Poblano		J3	Jalapeño	
	San Luis de la Paz	D11	Poblano	Cortázar	CT1	Jalapeño
		D12	Poblano		CT3	Jalapeño
SP1		Poblano		CT4	Jalapeño	
	SP2	Poblano	Salvatierra	SA2	Chilaca	
	SP3	Poblano		SA3	Chilaca	

From all the isolates, including the reference strains, monozygotic cultures were made according to the protocol of Mitchell and Kannwischer-Mitchell (1995); Erwin and Ribeiro (1996). Cultures were kept in V8C solid medium (160 ml of V8 juice, 3 g CaCO₃ and 840 ml of deionized water), transferring them every six weeks.

Genetic diversity

The DNA of the 30 isolates and the two reference strains was obtained from mycelium of monozygotic cultures, using the method proposed by Doyle and Doyle (1987). It was quantified with the help of a spectrophotometer (NanoDrop 8000) and the dilutions were standardized at a concentration of 120 ng μl^{-1} of DNA.

Amplified fragment length polymorphisms (AFLP) were detected based on the protocol described by Vos *et al.* (1995), using a NEN Global IR2 sequencer, brand LI-COR, which generates and allows the automatic capture of the digital image. The adapter-restriction site primers Eco R1 (E+2) used in this work were: E+AT, E+AG y E+AC; the adapter- restriction site primers Tru 91 (T+2) were T+CC, T+CA, and T+CG. With these primers, the six combinations used for the analysis were formed: E+AT/T+CC, E+AT/T+CA, E+AG/T+CC, E+AG/T+CA, E+AG/T+CG, y E+AC/T+CG.

The images generated were analyzed using the program 'Cross Checker 2.91', which encodes the bands using a binary code, where each band is represented as present (1) or absent (0), it was considered that a fragment of DNA is monomorphic if 99% of the population has it and polymorphic to those that differ from this percentage (Cavalli-Sforza and Bodmer; 1981). The

binary data were analyzed with the program NTSYS-pc version 2.1 (Numerical Taxonomy Multivariate Analysis System) (Rohlf, 2005) to generate a similarity matrix based on the Dice coefficient (Núñez-Colín and Valadez-Moctezuma, 2010), which considers only the coincidences to determine the similarity between two isolates. A Bootstrap procedure was carried out with 2 000 resamples with replacement, from the average similarity matrix, the genetic relationships between the genotypes studied were determined, generating a consensus dendrogram applying the Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

Only well-defined and medium- to high-intensity bands were considered for analysis (Figure 1). A total of 807 bands were obtained, of which 78.81% were polymorphic. The percentage of band polymorphism is similar to that reported by Kersey *et al.* (2005), who, when analyzing 31 isolates of *P. capsici* from New Mexico USA, found that 80% of 1 155 bands were polymorphic. The level of polymorphism was higher than that obtained by Lamour and Hausbeck (2001a) for *P. capsici* isolates from Michigan USA, who reported an average percentage of polymorphisms of 43% for 94 bands.

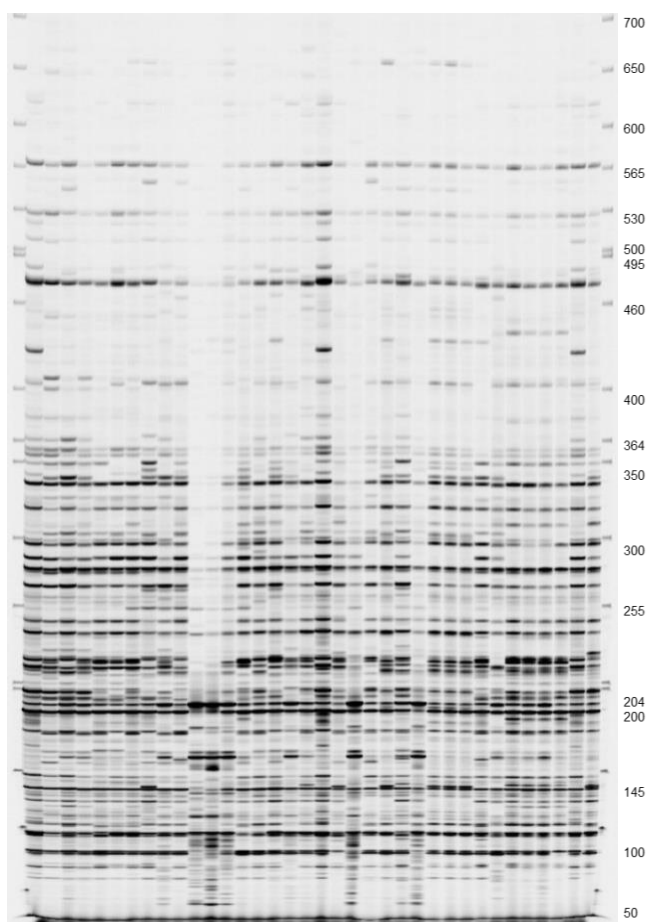


Figure 1. Amplified fragment length polymorphisms (AFLP), genetic profiles generated by the combination of E+AT/T+CA primers in 30 *P. capsici* isolates from the state of Guanajuato and 2 reference strains, showing the definition and intensity of the amplified DNA fragments (bands).

The similarity matrix based on the Dice coefficient shows values ranging from 76 to 95% similarity with an average of 86%, these genetic relationships are reflected in the consensus dendrogram obtained in this work (Figure 2). In it, one can see the formation of four well-defined groups, group I formed with two isolates from Cortázar and one from Silao, which have the least similarity with the rest; the groups with the largest number of isolates are group II, formed only by isolates from Dolores Hidalgo, and group III, formed by a mixture of isolates from Silao and Dolores Hidalgo, group IV is formed by six subgroups, highlighting that five of them are formed only with isolates from the same municipality, these are the subgroups formed only with isolates from Silao, Dolores Hidalgo, San Luis de la Paz, Juventino Rosas and the subgroup formed by the two reference strains, which, following the observed trend could, come from the same locality.

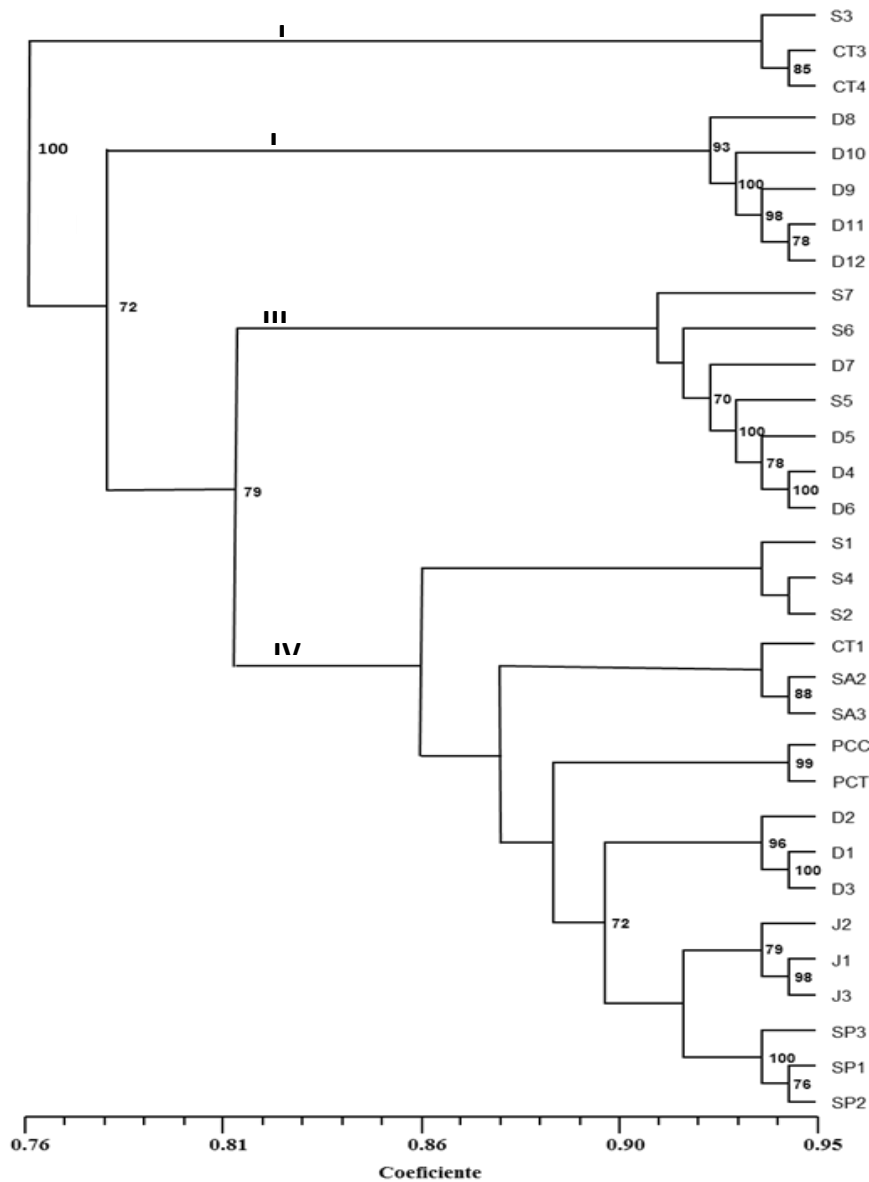


Figure 2. Consensus dendrogram of 30 *P. capsici* isolates from the state of Guanajuato and 2 reference strains. The numbers on the nodes are the percentage of dendrograms above 70% that were supported by bootstrap analysis.

In this group only a subgroup formed, which mixes two strains originating from Salvatierra with one from Cortázar. These results seem to indicate a tendency of the isolates to group according to their municipality of origin. Except for the monozoosporic cultures originating from Silao and Cortázar, which grouped with isolates from different localities, which is indicative of a high genetic diversity.

The isolation site-based association is consistent with the study of variability of *P. capsici* isolates originating from four different regions of Michigan USA reported by Lamour and Hausbeck (2001a), in which the grouping found was based on the collection site. Likewise, in what was reported by Parada-Rojas and Quesada-Ocampo (2018), who, using microsatellites to analyze the genetic relationships of 50 *P. capsici* isolates originating in several USA states, find a population structure based on geographical origin. In Mexico, Castro-Rocha *et al.* (2016), analyzing the diversity of 80 isolates originating in the north and center of this country, using SNP-type markers, point out that the isolates from Chihuahua form two groups and the rest form three closely related groups, composed mainly of isolates from Aguascalientes, Guanajuato and Michoacán, finding a separation by regions of isolation.

If one considers what was pointed out by Brasier and Hansen (1992), that the genus *Phytophthora* has a strong selection for climate, so most of its species are found in areas of cool subtropical or tropical temperatures, and that humidity, nutrient content, pH and native microorganisms are factors that influence the adaptation of this pathogen. So, it seems that the differences between the environmental conditions of the regions of Guanajuato where the collections were carried out are the main factors that influenced the formation of the groups in this study.

The municipalities of the north of the state, San Luis de la Paz and Dolores Hidalgo, have a semi-dry climate, with an annual rainfall of 387.5 mm and an average annual temperature of 16 °C; Silao, Juventino Rosas and Cortázar, which are located in the center of the state, have a subtropical-subhumid climate with an annual rainfall of 688 mm and average annual temperature of 19.4 °C, very similar to the climatic conditions of Salvatierra, which is in the south of the state, with a predominantly humid temperate climate with an annual rainfall of 730 mm and average annual temperature of 18.1 °C (INEGI, 2013). Inconsistencies in the grouping by isolation site could be explained by sampling effects, genetic drift or crossing in small populations, which can lead to changes in genetic diversity (Goodwin, 1997) and consequently changes in the groupings.

All the isolates of *P. capsici* from Guanajuato were unique genotypes, this was expected by the results obtained in the genome sequencing project, where they report that the density and diversity of the variants of a single nucleotide between genomes of *P. capsici* is notably higher than in other eukaryotic genomes, which has confirmed that the isolates of *P. capsici* have a large genetic variation in the form of SNPs, which can occur as frequently as 1 in 40 bp (Lamour *et al.*, 2012).

When including in the analysis their morphological and physiological characteristics described in a previous work (Pons *et al.*, 2020), no clear association was found between the genetic groups with morphology, type of compatibility, sensitivity to mefenoxam, the type of chili pepper or the

degree of virulence. This absence of correspondence was found in other *Phytophthora* spp. (Lebreton and Andrivon, 1998; Mahuku *et al.*, 2000; Abu-El Samen *et al.*, 2003), where no correlation was found between the groups defined by molecular markers and the virulence, type of mating or response to metalaxyl of the isolates analyzed.

Despite the absence of these correlations, the results of this work indicate that the population of *Phytophthora capsici* from Guanajuato maintain its characteristic of high levels of diversity, as has been reported in other populations of this pathogen by several authors (Lamour and Hausbeck 2001a; Hausbeck and Lamour 2004; Gevens *et al.*, 2008; Hurtado-González *et al.*, 2008; Meitz *et al.*, 2010; Lamour *et al.*, 2011; Gobena *et al.*, 2012). As the two types of mating were found in several of the collection municipalities (Pons *et al.*, 2020), it is likely that a part of this diversity results from sexual recombination and another from mutations that, according to that reported by Goodwin (1997), is the primary source of the new genetic diversity in oomycetes. Regardless of its origin, the presence of genetic diversity in the population of *P. capsici* from Guanajuato indicates that there is a genetic potential in the population of pathogens for the development of resistance to fungicides and to overcome the defenses of the host, so it is necessary to monitor it continuously and consider it for the design of strategies for its control.

Conclusions

There is genotypic variability among *P. capsici* isolates from Guanajuato. All isolates of *P. capsici* from Guanajuato are unique genotypes since no clonal individuals were found. The genetic groups that form are based on the place of origin of the isolates. No clear association was found between genetic groups and virulence, mating type or response to metalaxyl in the isolates analyzed.

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