Tolerance to Virulence Phenotypes of Phytophthora capsici in Pasilla Pepper Cultivars

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Abstract. Phytophthora capsici is the most important limiting factor in the production of chile pepper in Mexico. This pathogen presents virulence phenotypes capable of infecting diverse cultivars of this crop. The search and development of resistance in chile pepper is an excellent alternative for the management of P. capsici. The objective of this work was to evaluate the response of four pasilla pepper cultivars to infection with five virulence phenotypes of P. capsici. Pasilla pepper landraces PAS-1, PAS-2, PAS-3, and PAS-4 were inoculated with P. capsici isolates MX-1, MX-2, MX-7, MX-8, and MX-10. Two experiments were conducted under greenhouse conditions from April through June 2017 and April through June 2018. ‘California Wonder’ was included as a susceptible control, and uninoculated plants were included as a negative control. In each experiment, groups of six 56-day-old plants from each pepper cultivar were inoculated with each virulence phenotype. Disease severity was evaluated 20 days after inoculation using an individual rating system. All pepper cultivars were classified as resistant (R), moderately resistant (MR), tolerant (T), moderately tolerant (MT), or susceptible (S), according to the frequency of resistant plants (severity 0–1). ‘California Wonder’ and ‘PAS-4’ were susceptible to all five virulence phenotypes. The rest had different responses to the virulence phenotypes, but ‘PAS-2’ and ‘PAS-3’ were susceptible to only one of the five virulence phenotypes. Pasilla peppers with low severity exhibited a slow rate of infection, which is a mechanism we have called “slow wilting.” The pasilla pepper cultivars PAS-1, PAS-2, and PAS-3 could be used in plant breeding programs as sources of genetic tolerance and moderate resistance against P. capsici.

Chile pepper (Capsicum species) is of great economic, social, and scientific importance in Mexico, which is a country with a high level of diversification of cultivars and history of domestication of this Solanaceous crop (Aguilar-Rincón et al., 2010). During 2019, the production of pepper surpassed 3 million tons, placing Mexico among the leading pepper producers worldwide (Secretaría de Agricultura y Desarrollo Rural, 2020). The main producing states are Chihuahua, Michoacán, San Luis Potosí, Sinaloa, and Zacatecas (Secretaría de Agricultura y Desarrollo Rural, 2020). Chile pasilla (C. annuum) is one of the 64 types of Mexican chile peppers cultivated and consumed across Mexico. Different landraces (farmers or local cultivars) of pasilla peppers are cultivated in north-central Mexico (Durango, Zacatecas, Aguascalientes, San Luis Potosí, Guanajuato, Querétaro, and Michoacán). Pasilla peppers are commercialized and consumed as dried chiles to prepare moles; however, they are sometimes harvested and commercialized unripe (known as “Chilaca” peppers). The fruit is 15 to 20 cm long and 2 to 3 cm wide (Muñoz, 2000), but it can be as large as 34 cm long and 5.6 cm wide (Rincón et al., 2010). Immature fruits are deep dark green and turn to blackish brown at maturity. Once dried, fruits are moderately spicy and present a shiny and wrinkled surface similar to a “pasita” (raising), which explains its common name “pasilla” (Muñoz, 2000).

The most important factor that limits the production of this crop in most areas of the country and in other producing countries, however, is the disease Phytophthora blight caused by the oomycete P. capsici (Barchenger et al., 2018a; Leonian, 1922; Macias-Valdez et al., 2010; Silva-Rojas et al., 2009), which can cause up to 100% of losses (Barchenger et al., 2017). P. capsici can attack different parts of the plant and produce different syndromes, including rot of the root, crown, stem, and fruit (Jiang et al., 2015; Monroy-Barbosa and Bosland, 2010, 2011; Oelke et al., 2003; Reyes-Tena et al., 2019; Ribeiro and Bosland, 2012; Sy et al., 2008). Furthermore, this oomycete is a highly destructive pathogen with a wide range of economically important crops (Lamour et al., 2012b).

The high genetic diversity reported in populations of P. capsici in central Mexico (Castro-Rocha et al., 2016) increases the capacity of this pathogen for adaptation to changing environments. Furthermore, the loss of heterozygosity in P. capsici populations has been reported as an allele fixation mechanism that favors rapid adaptation to new hosts and the emergence of new genotypes (Lamour et al., 2012a). The control of P. capsici is complex because of the presence of different virulence phenotypes. Phenotyping for virulence is determined by evaluating the resistance or susceptibility of differential pepper lines against different isolates of this oomycete (Barchenger et al., 2018b). Fungicides, solarization, and crop rotation practices have been ineffective (Barchenger et al., 2018a; Bi et al., 2014). Therefore, the search and development of pepper cultivars resistant to local isolates and virulence phenotypes of P. capsici are the best alternative to manage this destructive disease (Foster and Hausbeck, 2010; Reyes-Tena et al., 2019). In addition, host resistance is an environmentally friendly strategy that can reduce the application of pesticides (Barchenger et al., 2017). Six major chromosomal regions related to resistance to P. capsici have been reported for Capsicum annuum (Castro-Rocha et al., 2012); however, understanding this interaction is complex because resistance to P. capsici is regulated by several unknown genes (Barchenger et al., 2018a).

Mexico is an important reservoir of genetic variations of chile peppers, particularly of...
**Materials and Methods**

*Phytophthora capsici* isolates. The isolates used in this study were virulence phenotypes MX-8, MX-10, MX-7, MX-2 and MX-1. They were recovered from chile pepper plants showing typical signs of wilting and root rot in commercial fields of Copándaro (MX-8), Morelia (MX-10), Queréndaro (MX-1 and MX-2), and Tarimbaro (MX-7) in the state of Michoacán. The virulence phenotypes were tested on the New Mexico Recombinant Inbred Lines (NMRILs). The level of virulence was higher on the phenotypes MX-1 and MX-2, and the phenotype MX-10 showed the lowest virulence on the lines tested. All isolates were previously characterized morphologically and molecularly (Reyes-Tena et al., 2019, 2020).

**Plant material.** The landrace cultivars of pasilla chile pepper (*Capsicum annuum*) evaluated during this study were pasilla-1 (PAS-1), pasilla-2 (PAS-2), pasilla-3 (PAS-3), and pasilla-4 (PAS-4). They were collected from pepper-producing areas in the following municipalities: Queréndaro, Michoacán (PAS-4); Ojo Caliente, Zacatecas (PAS-2); Pánfilo Natera, Zacatecas (PAS-3); and Rincón de Romos in Aguascalientes, Mexico (PAS-1). California Wonder (CW), a sweet pepper commercial cultivar was used as a susceptible control. This cultivar has been used as a standard susceptible control against *P. capsici* (Candole et al., 2012). Sufficient seedings per cultivar were produced in cell trays of 100 cm³ per cell filled with commercial substrate (Mix 3 Sunshine; Sun Gro Horticulture, Agawam, MA). All seedlings were watered at field capacity every 48 h and fertilized once per week with Miracle-Gro (24N–8P–16K).

**Inoculation.** Plants of each cultivar that were 56 d old were inoculated with the pathogen. Uninoculated plants of each cultivar were used as a negative control. The inoculation procedure was performed as described by Reyes-Tena et al. (2019), with minor modifications. When abundant sporangia formation was observed, all isolates received a low temperature shock (4 °C for 30 min) to induce the release of zoospores. A zoospore suspension of each isolate was adjusted to 1 × 10⁷ zoospores/mL. Each plant received 1 mL of inoculum at the stem base using a 5-mL dosing syringe (Ape).

**Registered variables.** All plants were evaluated according to the severity scale described by Glosier et al. (2008). This scale comprises six levels of severity as follows: 0 = no symptoms (healthy plant); 1 = chlorotic leaves without necrosis on the stem; 2 = minor stem necrosis; 3 = moderate stem necrosis and early foliar wilt; 4 = severe stem necrosis and leaf wilt; and 5 = plant death caused by necrosis and wilting. The evaluation of severity was performed 20 d after inoculation (dai), when the susceptible control reached a severity level of 4 or 5. The pathogen was re-isolated from susceptible plants (severity >3) and from tolerant plants with necrotic root tips (severity = 1) to verify its presence. The pepper cultivars were classified into five categories (Table 1) according to the percentage of resistant plants.

**Experimental design.** A completely randomized 6 × 5 factorial design with six replicates was used (Reyes-Tena et al., 2019). The design included six levels of the pathogen (five virulence phenotypes of *P. capsici* plus an uninoculated control) and five pepper cultivars (four of the pasilla-type plus the CW susceptible control). This combination yielded 30 treatments or combinations between the six phenotypes of the pathogen and the five cultivars of the host. Six replicates (plants) were used per combination and randomly distributed, yielding a total of 180 experimental units. The experiment was conducted from April through June 2017 and repeated in April through June 2018.

**Statistical analysis.** The severity data of each experiment were analyzed independently. The homoscedasticity test of variances was not significant (*P* > 0.05); therefore, a combined analysis was performed using a single data matrix of both experiments from 2017 and 2018. A two-factor analysis of variance (ANOVA) was used to analyze the interaction between the phenotype of the pathogen and the host cultivar. The independent response of each cultivar to the phenotypes of the pathogen was analyzed using a one-way ANOVA. For specific comparisons between treatments, Tukey’s test was applied. Statistical analyses were performed with the Statgraphics Centurion XVIII statistical package. In all cases, significant effects were considered when *P* ≤ 0.05.

**Results**

All CW plants (susceptible control) showed the typical symptoms of the disease (stem and root necrosis, chlorosis, and foliar wilt) when inoculated with each one of the five virulence phenotypes of *P. capsici*. Gradually advanced severity was observed starting at 3 dai. The severity of the uninoculated cultivars was 0; therefore, the positive and negative controls behaved as expected in the two experiments. Each pasilla chile cultivar evaluated had the same resistance/susceptibility reaction to the different isolates of *P. capsici* tested in the 2 years of evaluation.

The factorial ANOVA is shown in Table 2. The factorial ANOVA detected statistically significant effects for the pathogen–host interaction (Tables 2 and 3). This significant host–pathogen interaction indicates that the disease severity depends on the specific combination of the virulence phenotype of the pathogen and the cultivar of the pepper.

When the response of each cultivar of pasilla pepper to the five phenotypes of the pathogen was analyzed, significant differences (*P* < 0.05) were observed in the level of severity for all except CW (Table 3). In the case of the susceptible cultivar CW, all the isolates caused similar but high levels (>3) of severity (*P* > 0.05), thus confirming the susceptibility of CW to *P. capsici*. The PAS-1 cultivar registered a level of damage statistically similar to that of the control without inoculum against the MX-1, MX-7, and MX-10 phenotypes of *P. capsici*. During other experiments involving three virulent isolates from central Mexico, this cultivar showed tolerance with >80% of asymptomatic plants as well as higher values of dry biomass and root volume than the positive controls (unpublished data). The cultivar

<table>
<thead>
<tr>
<th>Classification</th>
<th>Resistant plants category (%)</th>
<th>severity 0–1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>R</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Moderately resistant</td>
<td>MR</td>
<td>61–90</td>
</tr>
<tr>
<td>Tolerant</td>
<td>T</td>
<td>41–60</td>
</tr>
<tr>
<td>Moderately tolerant</td>
<td>MT</td>
<td>30–40</td>
</tr>
<tr>
<td>Susceptible</td>
<td>S</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

**Table 2. Two-way analysis of variance of severity data on pasilla pepper cultivars uninoculated or inoculated with five virulence phenotypes of *Phytophthora capsici*.**

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Factorial</td>
<td>Virulence phenotype</td>
<td>5</td>
<td>23.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Pepper cultivar</td>
<td>4</td>
<td>11.90</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Virulence phenotype × chile cultivar</td>
<td>20</td>
<td>2.24</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Experimental error</td>
<td>253</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>282</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Severity of Phytophthora blight on five pasilla pepper cultivars uninoculated (control) or inoculated with five virulence phenotypes of Phytophthora capsici.

<table>
<thead>
<tr>
<th>Virulence phenotype</th>
<th>Severity of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MX-1</td>
</tr>
<tr>
<td>PAS-1</td>
<td>1.92  b</td>
</tr>
<tr>
<td>PAS-2</td>
<td>3.50  b</td>
</tr>
<tr>
<td>PAS-3</td>
<td>2.50  ab</td>
</tr>
<tr>
<td>PAS-4</td>
<td>2.92  b</td>
</tr>
<tr>
<td>CW†</td>
<td>3.83  b</td>
</tr>
</tbody>
</table>

* Different letters in rows indicate significant differences according to Tukey test (P < 0.05).
† CW = California Wonder (susceptible control).
‡ Uninoculated control.

PAS-2 registered similar results against MX-2, MX-7, MX-8, and MX-10, as did PAS-3 against MX-1, MX-7, MX-8, and MX-10. Additionally, with these treatments, higher percentages of resistant plants were found. Therefore, these cultivars were considered moderately tolerant, tolerant, and moderately resistant against the virulence phenotype evaluated (Table 4). The plants with severity levels of 1 and 2 showed slow disease progression.

According to the plant cultivar categorization criteria (R, MR, T, MT, and S), 11 combinations of cultivar × phenotype were not susceptible; the remainder had less than 33% of resistant plants (Table 4, Fig. 1). The pathogen was re-isolated from roots of infected plants with a severity level of 1.

Discussion

The present study shows the necessity of evaluating local chile pepper cultivars against virulence phenotypes of P. capsici previously characterized. The analysis of disease severity caused by the pathogen in the cultivars of pasilla chile pepper showed variations in the response. A variation in the response to isolates by cultivars of pepper was also reported by Byung-Soo et al. (2010) in South Korea, where cultivars of pepper tolerant to isolate Pe003 were susceptible to isolate Pe002. In Mexico, Morán-Baíuellos et al. (2010) also reported differences in the severity of P. capsici, which was attributable to the genetic variation of 29 native pepper populations from southern Puebla. In Mexico, different levels of resistance to P. capsici in 15 out of 32 landraces (C. annuum and C. pubescens) from 14 states were recently reported. Furthermore, the 32 landraces showed disease symptoms, but the severity was variable, with six landraces showing a high level of resistance (Retes-Manjarrez et al., 2020). However, in South Korea, Su-Jung et al. (2014) observed that cultivars of pepper tolerant to isolate C. annuum/C19 and C. pubescens/C2 from Georgia on 2301 pepper accessions and found that two accessions from Mexico, PI 201237 and PI 404532, consistently showed high levels of resistance. Recently, Retes-Manjarrez et al. (2020) identified new sources of resistance in 14 landraces of piquin.
manzano, pasilla, cola de rata, and jalapeño peppers. Similarly, genotypes of *C. annuum* with resistance to *P. capsici* have been reported in the United States (in New York and Michigan), Laos, and South Korea (Dunn et al., 2014; Foster and Hausbeck 2010; Mo et al., 2014; Su-Jung et al., 2014). A common problem among resistant pepper genotypes is that they show less resistance when assayed under different environmental conditions and *P. capsici* isolates (Dunn and Smart 2019). However, this pioneering work on the genetic tolerance and moderate resistance in different environmental conditions and *P. capsici* isolates (Dunn and Smart 2019; Messaouda et al., 2015). This is largely explained by the presence of different virulence phenotypes of the pathogen (Oelke et al., 2003; Sy et al., 2008). Therefore, the search for specific resistance to local virulence phenotypes is the best way to generate resistant pepper cultivars. The identification of local pathotypes of *P. capsici* should be part of this strategy. The pasilla pepper cultivars PAS-1, PAS-2, and PAS-3 could be used directly by producers in commercial fields, and they also have the potential to be used in plant breeding programs as sources of genetic tolerance and moderate resistance in the form of slow wilting against local pathotypes of *P. capsici*. One of the shortcomings of the present study was the necessity to evaluate a large group of virulence phenotypes against the local cultivars because it is possible to find their great diversity in the field (Reyes-Tena et al., 2019). However, this pioneering work searched for virulence phenotype-specific resistance of chile pepper cultivars.

### Literature Cited


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