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Differential response of pepper (*Capsicum annuum* L.) lines to *Phytophthora capsici* and root-knot nematodes

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ABSTRACT

The oomycete *P. capsici* is among the major pathogens found in pepper. A desirable, sustainable and environmentally-compatible way to manage it is through genetic resistance. Huacle and Serrano pepper lines resistant to *P. capsici* isolate 6143 have been detected; however, it is necessary to determine whether the resistance of these lines is effective against a higher number of isolates and evaluate their resistance to other important pathogens, such as root-knot nematodes. The aim of this study was to evaluate the resistance of Huacle and Serrano pepper lines to different *P. capsici* isolates and root-knot nematodes (*M. incognita* and *N. aberrans*). Ten *P. capsici* isolates from different pepper-growing regions, and two independently-inoculated nematode populations, one of *M. incognita* and the other of *N. aberrans*, were used. Serrano pepper, lines 41-1, 41-2, 42-6 and 55-2 stood out, with a resistance response to all *P. capsici* isolates followed by Huacle pepper lines 33-3, 35-3 and 34-3, which were only susceptible to one isolate. Furthermore, except for lines 34-3, 35-5 and 42-2, all the others were resistant to *M. incognita.* Serrano pepper lines 41-1, 41-2 and 42-2 and Huacle lines 35-3 and 35-5 were resistant to *N. aberrans*, while lines 41-1, 41-2 and 35-3 lines were resistant to the three pathogens evaluated. Resistance previously detected in Huacle and Serrano peppers is effective for different *P. capsici* isolates and root-knot nematodes.

1. Introduction

Pepper (*Capsicum* spp.) is one of the most important vegetable crop in the world, in terms of world production, Mexico ranks second with annual production of 2,294,400 t (FAO, 2015). It also stands out for the high genetic diversity in the genus *Capsicum* that the country has (Aguilar-Rincón et al., 2010).

Pepper cultivation is affected by biotic factors, such as wilt induced by the *oomycete P. capsici* Leonian and root-knot nematodes attack. Wilt is one of the most destructive diseases worldwide (Richins et al., 2010); in Mexico it causes losses of 25–90% (Velásquez and Amador, 2007; García-Rodríguez et al., 2010). Similarly, root-knot nematodes *Meloidogyne* spp. and *Nacobbus aberrans* Thorne & Allen are one of the main problems in pepper (Djian-Caporalino, 2012; Jones et al., 2013). The galls block the vascular system and cause a nutrient imbalance and, in some cases, the breakdown of resistance in genotypes resistant to fungi and oomycetes (Nicol et al., 2011). Chemical control of *P. capsici* and root-knot nematodes has not been effective and has generated oomycete-resistant strains, due to intensive fungicide application (Pérez-Moreno et al., 2003; Lamour, 2009). An alternative strategy for the management of both problems, which is both sustainable and environmentally compatible, is the use of resistant genotypes. Several pepper materials such as Criollo de Morelos CM334, Chilhuacle Amarillo (PI 201231) and Chile Criollo (PI 566811), among others, have been reported to have varying degrees of resistance to *P. capsici* (Guerrero-Moreno and Laborde, 1980; Foster and Hausbeck, 2010; Candole et al., 2010; Anaya et al., 2011; Mo et al., 2014); in relation to inherited resistance to this disease, studies report that it can be both specific and quantitative (Guerrero-Moreno and Laborde, 1980; Gil Ortega et al., 1991; Bonnet et al., 2007; Sy et al., 2008; Truong et al., 2012). Despite these studies, it is necessary to identify the resistant genotypes and introduce, by breeding, the resistance genes in commercial varieties (Richins et al., 2010). Within the wide genetic diversity of Mexico, pepper materials resistant to *P. capsici* have been found in the state of Morelos (Guerrero-Moreno and Laborde, 1980; Redondo et al., 1989; Anaya-López et al., 2011), of which CM-334 stands out as highly resistant. To date, it has shown resistance against all isolates of this pathogen, which is why it is considered as universally resistant (Glosier et al., 2008; Lamour et al., 2012). Using *P. capsici* resistance in commercial varieties has been limited, as is the case of

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peppers with intermediate resistance, mentioned by Foster and Hausbeck (2010). In the case of pepper CM-334, it has been difficult to incorporate its resistance into commercial varieties, because inheritance of its resistance is complex (Minamiyama et al., 2007; Lamour et al., 2012). On the other hand, CM-334 presents resistance to the three major *Meloidogyne* species [*M. incognita* (Kofoid and White, 1919) Chitwood, 1949; *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. javanica* (Treub, 1885) Chitwood, 1949] (Pegard et al., 2005), and susceptibility to *N. aberrans*.

The identification of pepper genotypes resistant to *P. capsici* and root-knot nematodes will contribute to the development of a plant health management program that favors the sustainability of this crop. At Mexico's Colegio de Postgraduados, evaluations of the resistance of pepper germplasm to *P. capsici* have been conducted, from which selfing lines 33-1, 33-3, 34-2, 34-3, 35-3, 35-5, 41-1, 41-2, 42-2, 42-6, 49-5, 55-2, 55-3, 56-2 and 56-3 have been detected as resistant to isolate 6143 (V. H. Aguilar-Rincón, and T. Corona-Torres, 2012, personal communication); however, it is necessary to determine whether the resistance of these lines is effective against a higher number of isolates. For this reason, this study aimed to evaluate the resistance of Huacle and Serrano pepper lines to *P. capsici* isolates and root-knot nematodes *M. incognita* and *N. aberrans*.

2. Materials and methods

2.1. Plant material

We used 15 pepper lines from a single selfing, six Huacle pepper lines (33-1, 33-3, 34-2, 34-3, 35-3, 35-5) and nine Serrano ones (41-1, 41-2, 42-2, 42-6, 49-5, 55-2, 55-3, 56-2 and 56-3), provided by Víctor Heber Aguilar-Rincón and Tarsicio Corona-Torres of the Colegio de Postgraduados. The Serrano CM-334 pepper was used as a control resistant to *P. capsici* and to *M. incognita,* and as a control susceptible to *N. aberrans.* The Yolo Wonder variety was used as a control susceptible to the first two pathogens.

2.2. Sowing of the plant material

The seeds of each line were disinfested with a 1% commercial sodium hypochlorite solution for 2 min. Afterwards they were placed in plastic germination boxes (28.0 × 16.5 × 4.5 cm), on paper towels moistened with sterile distilled water, and incubated at 28 ± 1 °C. Once the seeds germinated, the seedlings were transplanted into flower pots (4 cm of diameter by 4 cm of height) with 25 cm^3 of sterile fine sand (one seedling per pot) and then kept in a growth chamber at a temperature of 26 ± 1 °C, with a photoperiod of 14 h light, light intensity of 6768 lux (fluorescent light), and 10 h dark. They were fertilized weekly with nutrient solution (3.15 g of Nitrofoska®0.000378 N, 0.000378 P_2O_5, 0.000378 K_2O and 0.000063 Mg per liter of sterile water).

2.3. P. capsici inoculum

Ten isolates were used: nine from different pepper-producing regions in Mexico, labeled as Zac (Zacatecas), 3C (Chihuahua), Pue (Puebla), JTcap (Mexico), PcT17 (Michoacán), JC11 (Michoacán), J10 (Michoacán), CH11 (Michoacán) and C7P8F7 (Guanajuato), and one from the United States, identified as 6143 (New Mexico). Isolate C7P8F7 was provided by Dr. José Luis Anaya López of INIFAP's Bajío Experimental Station, and the rest by Dr. Sylvia Fernández-Pavía of the Universidad Michoacana de San Nicolás de Hidalgo.

Isolates were cultured on V8 medium for 7 days at 28 °C. After incubation, an isotonic solution of 0.9% sodium chloride (Abbott®) was added for up to 10 min to induce sporulation. The isotonic solution was decanted and, with the aid of a dissecting needle, the culture medium was divided into four parts. Each of these was placed in a petri dish and covered with sterile distilled water. The dishes were incubated under a lamp of white light at 26 °C for 48 h. To induce zoospore release, the dishes were kept at 4 °C for 30 min, to later incubate them at room temperature for 30 min. Zoospores were quantified with a cytometer (Marienfeld®), the suspension was adjusted to a concentration of 1×10^5 zoospores/mL, and 1 mL per plant was inoculated, at the base of the stem, in a development stage of 4–6 true leaves. For each isolate, five plants of each pepper material were used.

With the above-described methodology, two experiments were performed (Table 1); one nine isolates were used and in the second the isolate that was the most virulent in the first experiment (PcT17) was used. In addition, in the second experiment highly virulent isolate, C7P8F7, from the state of Guanajuato was added (García-Rodríguez et al., 2010).

2.4. Inoculum of M. incognita and N. aberrans

N. aberrans inoculum was obtained from tomato (*Solanum lycopersicum* Mill.) roots with galls, collected at the Colegio de Postgraduados, Montecillo campus, state of Mexico. *M. incognita* inoculum was obtained from bean roots with galls, collected in Los Mochis, Sinaloa, which was increased from a single mass of eggs. For both species, the eggs were extracted using the method described by Vrain (1977) and incubated at 28 °C in Petri dishes with sterile distilled water, until hatching. When the plants had two to three pairs of true leaves, they were inoculated with a suspension of 500, second stage juvenile (J2) of *M. incognita* and 1000 J2 of *N. aberrans* per plant. Two experiments were conducted: in the first one, for each species of nematode, five plants of each pepper genotype were used and 12 plants in the second one.

2.5. Resistance evaluation

2.5.1. P. capsici

Wilt induced by *P. capsici* was assessed using the severity scale proposed by Sanogo (2006) and amended for the purposes of this study: 1 = no visible symptoms, 2 = necrosis without encircling the stem, 3 = necrosis encircling the stem, 4) stem necrosis with <50% defoliation, 5 = stem necrosis with >50% defoliation, 6) wilting plant 6 and 7 = dead plant. Evaluations were performed at 3, 7, 14 and 21 days after inoculation (dai). The classification of lines, as resistant or susceptible, was made based on the average severity of each line, where if the scale was ≤3.5, the pepper line was considered as resistant (R) and if the scale was >3.5 as susceptible (S), which took place when the susceptible control had 100% of dead plants. In Experiment 1, for isolates 3C, Jtcap, CH-11, JC11, J10, Pue and Zac this occurred 21 dai, and for isolates 6143 and PcT17, 7 dai; in Experiment 2, with isolates PcT17 and C7P8F7, 7 dai.

2.5.2. M. incognita and N. aberrans

The number of eggs (extraction according to Vrian, 1977) per plant 45 dai was quantified. With these data the reproductive index (RI) was calculated, based on the ratio = number of eggs per gram of root of each pepper line/number of eggs per gram of root of the susceptible pepper × 100. The classification of lines as resistant or susceptible was based on the following categories: highly resistant (RI < 1%), very resistant (1% ≤ RI < 10%), intermediate resistance (10% ≤ RI < 25%), moderately resistant (25% ≤ RI < 50%) and susceptible (RI ≥ 50%) (Hadisoeganda and Sasser, 1982). The number of root galls was also recorded. In Experiment 2, with *N. aberrans*, the number of nematodes in the root of two plants of each material 21 dai was counted. The last two variables were complementary in determining the resistance response of the pepper lines.