AP AGRO PRODUCTIVIDAD

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Reaction of inbred lines of habanero hot pepper (*Capsicum chinense* Jacq.) to inoculation of *Fusarium oxysporum*

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ABSTRACT

Objective: To evaluate the reaction of five inbred lines of habanero pepper (*Capsicum chinense* Jacq.) to the inoculation of *Fusarium oxisporum* and to identify genotypes with possible levels of resistance to this pathogen.

Design/methodology/approach: Samples of hot pepper plant stems with typical symptoms of vascular wilt disease were obtained. Then, due to microscopic morphological characteristics of mycelium and conidia in the samples, patogen was identified as *Fusarium oxisporum*. By immersion of roots, at a concentration of 1×10^6 conidia per mL, five inbred lines of habanero pepper were inoculated. In addition to the serrano type cultivar Criollo de Morelos 334 (CM-334). The percentage disease index and area under the disease progress curve (ABCPE) were estimated in a randomized complete block design, with three replicates.

Results: Significant differences ($p\leq0.05$) were observed for disease index and ABCPE in the genotypes evaluated; evidencing the difference in the genetic basis of the genotypes and the resistance or susceptibility to *Fusarium oxisporum*. The CM-334 cultivar, the Habanero 5 and Habanero 8 lines showed the lowest percentage of incidence of the disease (10, 20 and 30%, respectively) and ABCPE of 300, 560 and 880. In turn, lines Habanero 9 and Habanero 6 reached the highest ABCPE values with 2220 and 2190, respectively.

Study limitations/implications: The disease resistance response in plants is complex and contains multiple interactions between genes, proteins, and metabolites.

Findings/conclusions: The grouping of genotypes according to their reaction to *Fusarium* wilt disease facilitated the identification of resistant and susceptible lines, which can be considered in subsequent genetic improvement studies for hot pepper cultivation.

Keywords: genetic resistance, inoculation, inbred lines, plant breeding.

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INTRODUCTION

The genus *Capsicum* is made up of around 35 species, among which the most important for their domestication and cultivated area are *Capsicum annuum* L., *Capsicum frutescens* L.,

Capsicum pubescens Ruíz & Pavón, *Capsicum baccatum* L. and *Capsicum chinense* Jacq. (Carrizo *et al.*, 2013). Mexico is the center of origin and diversity of *C. annuum* and the species *C. frutescens*, *C. pubescens* and *C. chinense* are also present. Aguilar-Rincón *et al.* (2010) reported for Mexico 56 types of chilli pepper among those cultivated, semi-cultivated and wild.

The habanero pepper (*Capsicum chinense* Jacq.) is a crop of importance in Mexico, its demand is increasing in national and international markets (Medina-Lara *et al.*, 2008). In 2019, 1135 ha of habanero pepper were planted, with an average yield of 18.4 Mg ha^{-1} and a total production volume of 20 829.61 Mg (SIAP, 2019). This crop is distinctive of the Yucatan Peninsula, Mexico; it was introduced by the Caribbean, adapted to the agroecological conditions and traditional management of Yucatecan farmers. In addition, it was integrated into the culture and gastronomy of the region, from where it was dispersed throughout the country and is currently planted at commercial scale in at least 17 states (Latournerie *et al.*, 2002).

This type of hot pepper is used as an important ingredient in the cuisine of countries such as Mexico, China, Thailand and South Korea (Nass *et al.*, 2015). It is known for its high pungency, ranging from 250 000 to 700 000 Scoville Heat Units (SHU), in addition to the unique aroma of the fruits. The size of the fruit normally varies from 2.9 to 6.0 cm long, 2.5 to 4.6 cm wide and from 7 to 12 g in weight. Habanero pepper is also an important source of phytochemical compounds for the human diet, such as vitamin C (Teodoro *et al.*, 2013), capsaicinoids (Jeeatid *et al.*, 2018), phenolic compounds (Campos *et al.*, 2013), carotenoids, flavonoids (Butcher *et al.*, 2012) and other secondary metabolites with antioxidant properties (Castro-Concha *et al.*, 2014).

Fusarium wilt is one of the most important diseases in chilli pepper cultivation; the causative agent *Fusarium oxysporum* causes significant yield losses (Mushtaq & Hashmi, 1997). The pathogen invades the vascular system causing wilt, chlorosis and leaf necrosis. The first symptoms are observed in the basal leaves as a unilateral yellowing, subsequently the leaves wither, dry but remain attached to the plant. The main roots and the base of the stem present vascular necrosis; when cutting transversely or longitudinally the diseased stems or the base of the petioles, necrotic tissue in the vessels of the xylem is observed (Villa *et al.*, 2014). The pathogen causes vascular wilt in a wide variety of economically important crops worldwide (Ortoneda *et al.*, 2004). It is a necrotrophic, soil-borne fungus with worldwide distribution in tropical to subtropical areas (Booth, 1971).

Chemical control is not effective in treating this disease, in addition to increasing production costs, it causes damage to the health of consumers and represents a negative environmental impact (Moran-Bañuelos *et al.*, 2010). In this regard, the use of resistant cultivars is a cheap, safe, non-polluting and reliable control method to treat diseases in agricultural production. Hence, one of the important options is to find varieties of cultivated plants capable of resisting the damage caused by these diseases (Cheema, 2018). A key aspect for the incorporation of resistance to a variety of commercial hot pepper is to use parents with genetic resistance.

Therefore, the objective of this study was to evaluate the reaction of five lines of habanero pepper (*Capsicum chinense* Jacq.) to the inoculation of *Fusarium oxisporum* and identify genotypes with possible levels of resistance to this pathogen.

MATERIALS AND METHODS

Genetic material

Five lines of habanero pepper (*Capsicum chinense* Jacq.) were evaluated, the seed was provided by the National Institute of Forestry, Agricultural and Livestock Research (in Mexico, INIFAP), obtained from the Genetic Improvement Program of chilli pepper cultivation. The serrano type cultivar Criollo de Morelos 334 (CM334) was also evaluated.

Isolation and identification of the pathogen

Samples of stems were obtained in chilli pepper plants (*Capsicum annuum* L.) with typical symptoms of vascular wilt disease, in which longitudinal cuts were made to the stem for the purpose of observing symptoms of internal brown necrosis in the conductive vessels. Then, sections of the stem of approximately 3 mm were cut, disinfected, and sown in a potato dextrose agar (PDA) culture medium in Petri dishes, incubated at 25 °C for eight days. After which hyphae tip transfer was performed to obtain monosporic cultures (Amini, 2009). The identification of *Fusarium oxysporum* (Fo) was made considering the symptomatology present in diseased plants and by the microscopic morphological characteristics of mycelium and conidia in culture medium (Gerlanch and Nirenberg, 1982, Leslie and Summerell, 2006).

Inoculum preparation and inoculation

The *Fusarium* cultures (10-day-old) grown in PDA medium were washed with sterile distilled water to obtain an inoculum suspension of the pathogen. For each isolate, the concentration of spores was measured by performing macro conidia counts using a Neubauer hematocimeter in a 40X magnification microscope. For inoculation, Habanero pepper seedlings with 68 d of germination were used, using the technique of immersion of roots in a suspension of 1×10^6 conidia per mL. After inoculation, the plants were placed in polyethylene vessels of 240 mL capacity with a mixture of soil and peat moss, kept in a greenhouse for 20 d at a temperature of 25 ± 2 °C.

The response of plants to inoculation was performed using a severity scale proposed by Apodaca-Sánchez *et al.* (2004) and Clavijo-Castro (2014) to estimate the severity of the disease, where 0: corresponds to plants without visible symptoms of the disease, 1: necrotic points in hypocotyl, 2: withered leaves, darkening at the base of the hypocotyl or decrease in plant growth, 3: wilting, necrotic lesions and decrease in plant growth, 4: necrotic injury, defoliation and decrease in growth, 5: death of the plant. Using the scale values assigned to the plants, a percentage disease index was estimated for each of the genotypes using the following formula:

$$IE=\left[\left(\frac{\sum_{i=1}^{n}X_i}{n}\right)0.2\right]100$$

where: X_i=severity of the disease in the *i-th* seedling; n=number of seedlings evaluated; 0.2=correction factor for disease percentages.

The disease indices obtained were used to determine the progression of the disease and the response of these materials to inoculation of the pathogen, by calculating the area under the disease progress curve (ABCPE) according to the following equation:

$$ABCPE = \sum_{i=1}^{n-1} \left\{ \left(\frac{y_{i+1} + y_i}{2} \right) \left(t_{i+1} - t_i \right) \right\}$$

where "t" is the time of each reading, "y" is the percentage of plants affected in each reading and "n" is the number of readings (Shaner and Finney, 1977).

Statistical analysis

All pathogenicity tests were performed in a random complete block design, with three replicates. The experimental unit consisted of 5 pots per genotype. With the values obtained for disease index and ABCPE, an analysis of variance was performed using the GLM procedure (SAS Institute, 1999) and comparison of means with the Tukey test ($p \leq 0.05$). The analysis of variance for the disease index was performed with values obtained at 6, 12 and 18 d after inoculation.

RESULTS AND DISCUSSION

The results obtained in this study show significant differences ($p \leq 0.05$) for disease index and ABCPE in the genotypes evaluated. The above mentioned highlights the difference in the genetic basis of the evaluated lines and the resistance or susceptibility they presented to *Fusarium oxisporum*. One way to assess the damage caused by pathogens in chilli pepper plantations is through the quantification of the incidence, which measures the number of affected plants expressed as a percentage. It is also used to determine resistance to this pathogen.

In this study, the first symptoms of the disease (chlorosis) were observed 8 d after inoculation (DDI), with increased severity over time. The CM-334 cultivar, the Habanero 5 and Habanero 8 lines, were the genotypes that showed the lowest percentage of incidence of the disease (10, 20 and 30%, respectively) and ABCPE of 300, 560 and 880. On the other hand, lines Habanero 9 and Habanero 6 presented the highest ABCPE values, 2220 and 2190, respectively (Table 1). In this regard, the area under the disease progress curve is a quantitative summary of the inoculation of the pathogen; the lowest values correspond to the materials with the lowest incidence of disease, that is, with a higher level of resistance (Bautista *et al.*, 2009).

The disease resistance response in plants is complex and contains multiple interactions between genes, proteins and metabolites. Horizontal and vertical resistance are a continuum of different mechanisms developed by the plant to interact with the external environment, including pathogens (Burbano-Figueroa, 2020). In this regard, plants and pathogens develop complex mechanisms of attack and defense. The defense system of plants is the ability to perceive pathogens and activate effective defense responses (Grube *et*

Table 1. Percentage of disease development over time and area under the disease progress curve (AUDPC) in chili pepper plants after artificial inoculation by root immersion of *Fusarium oxisporum*.

Genotypes	Disease index			AUDPC
	12 DAI[†]	16 DAI	20 DAI	
Habanero 10	75.0 a	85.0 a	97.0 a	2120 c
Habanero 9	75.0 a	75.0 b	80.0 b	2220 a
Habanero 6	70.0 a	75.0 b	80.0 b	2190 b
Habanero 8	30.0 b	30.0 c	30.0 c	880 d
Habanero 5	20.0 c	20.0 d	20.0 d	560 e
CM-334	10.0 d	10.0 e	10.0 e	300 f

[†]Days after inoculation.

al., 2000). Resistance in plants involves resistance proteins (R) that detect specific effector proteins (Avr) produced by the pathogen.

The use of chilli pepper varieties with genetic resistance represents a good alternative of sustainable production replacing frequent applications of pesticides. Therefore, the management of plant diseases is one of the main objectives in genetic improvement programs. However, host-pathogen interactions involving resistance are far from simple. Plants develop resistance mechanisms, pathogens develop strategies to overcome plant resistance; plants, in turn, develop new defensive measures that select for additional changes in the pathogen (Stahl and Bishop, 2000; Gururani *et al*., 2012).

Figure 1 shows the progress of vascular wilt disease along the development of plants inoculated with *Fusarium oxysporum*. During the evaluation time, it can be observed that genotypes CM-334, Habanero 5 and Habanero 8 presented the lowest incidence values of the disease in the former days of evaluation, delaying the onset of the disease probably as a defense mechanism of the plants to the attack of the pathogen.

This does not prevent plants from being infected but reduces the rate of increase of the disease in each of the points of infection. Therefore, this delays the spread of the disease

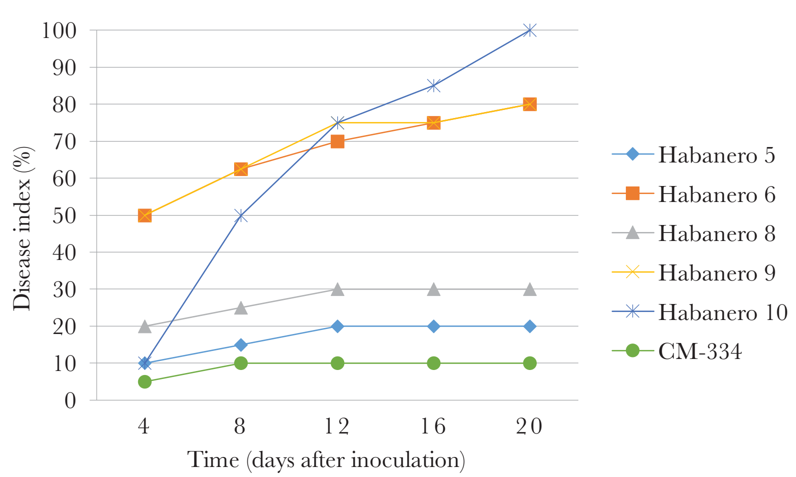

Figure 1. Response to *Fusarium oxysporum* inoculation in six chili pepper genotypes.

and the development of outbreak events in the field (Van der Plank, 1984). In this study, disease incidence values greater than 80% were observed; in contrast to other studies, the incidence and severity was variable, because the environmental conditions, variety and virulence of the pathogen were different.

The grouping of genotypes according to their reaction to *Fusarium* wilt disease facilitated the identification of resistant and susceptible lines, which can be considered in subsequent genetic improvement studies for hot pepper cultivation. Vallejo-Gutierrez *et al*. (2020) assessed variation in *Fusarium* wilt damage response in 16 genotypes (M1-M16) of Manzano pepper (*Capsicum pubescens* R. and P.), identifying the M8 genotype with resistance to *F. oxysporum* and *R. solani*. In this regard, Anaya-López *et al*. (2011) collected chilli pepper plants with symptoms of wilt and identified 26 collections resistant to *Fusarium* spp. Thus, they represent sources of resistance genes potentially useful in programs of genetic improvement, oriented to the control of the wilt on chilli pepper in the producing regions of central and northern Mexico.

CONCLUSIONS

The Habanero 5 and Habanero 8 lines were identified as the genotypes with the lowest incidence of this pathogen under greenhouse conditions. Therefore, they represent a potentially useful alternative in genetic improvement programs, aimed at controlling chilli pepper wilt.

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