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Virulence of Spanish *Phytophthora nicotianae* isolates towards *Capsicum annuum* germplasm and pathogenicity towards *Lycopersicum esculentum*

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Abstract

The virulence of six northwestern Spanish *Phytophthora nicotianae* Breda de Haan isolates towards the pepper (*Capsicum annuum*) cultivars commonly used for the determination of *P. capsici* Leonian pathotype (SCM 334, PI201234 and Yolo Wonder), was similar to that of the latter oomycete but different to that of the German reference isolate *P. nicotianae* 411.87. When nine local *C. annuum* cultivars were inoculated with a *P. nicotianae* isolate of well known pathogenicity, the virulence recorded was significantly different to that recorded for *P. capsici*. Though the average level of resistance of the northwestern Spanish *Capsicum* germplasm to *P. nicotianae* was incomplete and weaker than that recorded for the SCM 334 and PI201234 cultivars, genotypes Co 12B and Co 3.25 showed high resistance to this oomycete. None of the six northwestern Spanish *P. nicotianae* isolates tested were pathogenic towards *Lycopersicum esculentum* cv. S. Pedro, unlike *P. capsici*, which produced clear collar and root rot in this host.

Additional key words: collar rot, pepper, resistance, root rot.

Resumen

Virulencia de aislados de *Phytophthora nicotianae* sobre germoplasma de *Capsicum annuum* y patogenicidad sobre *Lycopersicum esculentum* L.

La virulencia de seis aislados de *Phytophthora nicotianae* Breda de Haan recopilados en España sobre los cultivares de *Capsicum annuum* L. habitualmente empleados para la determinación de patotipos de *P. capsici* Leonian —SCM 334, PI201234 y Yolo Wonder— ha resultado ser muy similar a la registrada por este último oomiceto pero diferente de la registrada por el aislado de referencia 411.87 de *P. nicotianae*, recopilado en Alemania. Las virulencias de sendos aislados de estos dos oomicetos fueron diferentes cuando se inocularon sobre cultivares locales de *Capsicum*. Aunque el nivel de resistencia del germoplasma local a *P. nicotianae* no es completo e inferior al registrado por las líneas SCM 334 y PI201234, los niveles registrados por ciertos genotipos locales —como Co 12B y Co3.25— fueron elevados. Ninguno de los seis aislados de *P. nicotianae* recopilados en Galicia resultó ser patogénico sobre *Lycopersicum esculentum* cv. S. Pedro, en contraste con lo registrado por el aislado de *P. capsici* inoculado, que produjo podredumbres netas de cuello y raíz en este hospedador.

Palabras clave adicionales: pimiento, podredumbre de cuello, podredumbre de raíz, resistencia.

Introduction

Phytophthora nicotianae Breda de Haan has been described as a pathogen of a wide range of hosts worldwide, including pepper (*Capsicum annuum*) (Kendrick, 1923; Nolla, 1929; Tasugi and Ikeda, 1939; Allagui *et al.*, 1995; Allagui and Lepoivre, 1996; Erwin and Ribeiro, 1996). In Europe, this oomycete is a known pepper pathogen in Italy (Borzini, 1956) and Spain (Bartual *et al.*, 1991; Andrés *et al.*, 2003), especially northwestern Spain (Andrés *et al.*, 2005a). *P. nicotianae* has also been reported pathogenic towards tobacco (Tucker, 1931), citrus species (Klotz *et al.*, 1958; Tsao, 1969), tomato (Messiaen *et al.*, 1991), carnation (Hine and Arakagi, 1952; Tramier and Andreoli, 1969; Tello, 1990), and some 298 other plant species (Erwin and Ribeiro, 1996). Considerable evidence indicates that some isolates show host preference: isolates should not be considered pathogenic towards all potential hosts

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(Erwin and Ribeiro, 1996). Hosts susceptibility is variable, but isolates usually show the strongest pathogenicity towards the hosts from which they were isolated (Bonnet *et al.*, 1978).

Two different pathotypes of *P. nicotianae* have been identified among the isolates that affect tobacco (Apple, 1962; Van Jaarsveld *et al*., 2002), but no confirmed information is available regarding the pathotypes affecting other hosts. The existence of pathotypes within the *P. nicotianae-Lycospersicum esculentum* L. interaction has, however, been suggested (Boukema, 1982). The virulence of *P. nicotianae* towards tobacco (Nielsen, 1992; Van Jaarsveld *et al*., 2002) and tomato germplasm (Sharma *et al*., 1974; Kohli *et al*., 1996) has been examined, as well as towards that of pepper in Tunisia (Allagui, 1994) and India (Verma *et al.*, 2001). As far as we know, no previous screening for sources of resistance to *P. nicotianae* in *C. anuum* germplasm has been performed in Europe.

The aims of the present work were: (i) to study the virulence of *P. nicotianae* isolates to well known reference *C. annuum* cultivars used for determining *P. capsici* pathotype, (ii) to evaluate the resistance of northwestern Spanish pepper germplasm to local isolates of *P. nicotianae*, and (iii) to study the pathogenic behaviour of these isolates towards *L. sculentum,* a well known worldwide host of this oomycete.

Material and Methods

Pathogen material

The fungal material used comprised 12 *Phytophthora* isolates (Table 1), 10 of which belonged to *P. nicotianae* and two of which belonged to *P. capsici* (employed as reference controls). Nine *Phytopthora* spp. isolates —eight of which were *P. nicotianae* and one of which was *P. capsici*— were obtained from diseased pepper plants in four locations in northwestern Spain. The pathogen was isolated from the infected plants on potato dextrose agar (PDA) (Rapilly, 1968) and on a selective medium (Ponchet *et al*., 1972). The taxonomic criteria used for the identification of *Phytophthora* species were those described by Stamps *et al.* (1990). Isolates 370.72 and 411.87 provided by the Centraalbureau voor Schimmelcultures (The Netherlands) were used as reference controls. Isolate 00/307, collected in Burgos (northern Spain), was provided by NEIKER (the Research Centre of the Basque Regional Government).

Host plants

The pepper plants used represented 12 genotypes (Table 2), nine of which were *C. annuum* lines from northwestern Spain and conserved at the CIAM (*Centro de Investigaciones Agrarias de Mabegondo*). The remaining Yolo Wonder and SCM334 and PI201234 lines were supplied by the University of La Coruña (Spain). The commercial San Pedro tomato (*Lycopersicum esculentum* L.) cultivar was used in the virulence tests.

Host preparation

The inoculum assays were conducted under greenhouse and growth chamber conditions. Seeds of the different genotypes were first sown on plastic trays

Table 1. Isolates of *Phytophthora capsici* and *P. nicotianae* used

Isolate	Pathogen	Origin	Host	Year of isolation
370.72	*P. capsici*	USA	*Capsicum* sp.	1972
Pa-1	*P. capsici*	La Coruña-Spain	*Capsicum annuum*	2001
00/307	*P. nicotianae*	Burgos-Spain	*Capsicum annuum*	2000
411.87	*P. nicotianae*	Germany	—	1987
Ar-3	*P. nicotianae*	Orense-Spain	*Capsicum annuum*	2001
Ar-4	*P. nicotianae*	Orense-Spain	*Capsicum annuum*	2001
Hor1/03	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2003
Hor4/03	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2003
Ro-16/02	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2002
Ro-18/02	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2002
Ro-3/02	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2002
Ro-7/02	*P. nicotianae*	Pontevedra-Spain	*Capsicum annuum*	2002

Table 2. Origin of the *Capsicum annuum* genotypes used

Genotype	Origin	Pepper type[1]	Type of genotype
Co5A	La Coruña-Spain	C4	Improved line
Co10A	La Coruña-Spain	C4	Improved line
Co12B	La Coruña-Spain	C4	Improved line
Co2.20	La Coruña-Spain	C4	Landrace
Co3.25	La Coruña-Spain	C4	Landrace
Pa129	La Coruña-Spain	C4	Improved line
Pa141	La Coruña-Spain	C4	Improved line
Pa158	La Coruña-Spain	C4	Improved line
Pa172	La Coruña-Spain	C4	Improved line
Yolo Wonder	USA	A1	Commercial variety
SCM 334	Mexico	C1	Landrace
PI 201234	USA	C1	Landrace

[1] Pepper type according to Pochard's classification (1966): A1, large fruit (heavier than 100 g) with rectangular longitudinal section; C1, long fruit with triangular longitudinal section; C4, short fruit with triangular longitudinal section.

(32×30×20 cm) filled with a mixture of sterilized peat (50% in volume) and sand. For greenhouse experiments, pepper and tomato plants were grown in plastic trays at 18-22ºC. For growth chamber experiments, these trays were kept at 22-28ºC with a photoperiod of 16 h at 30,000 lux.

Inoculum assays

The *Phytophthora* isolates were grown on V8 juice agar (Erwin and Ribeiro, 1996) at room temperature for 8 days. Each inoculum was prepared by seeding isolate samples in sterile 0.01 M potassium nitrate solution in Petri dishes. Sporangia were formed by growing this culture under UV light at 24ºC for 7 days. When abundant, the potassium nitrate solution was replaced by sterile distilled water, under aseptic conditions. The Petri dishes were then kept at 5ºC for 30 min and then at room temperature (20-24ºC) for 3 h to release the zoospores. The zoospore solution was filtered using sterile Whatman paper, vibrated for 1 min and adjusted to 2×10^4 zoospores per ml (Bartual *et al.*, 1991). At the six leaf stage, the collar of each plant was inoculated with 5 ml of the zoospore solution (Gil Ortega *et al.*, 1995).

Experimental design

In the virulence tests, seven *P. nicotianae* isolates —six from Spain and one reference isolate of German origin— were inoculated into the well known reference *C. annuum* cultivars PI201234, SCM 334 and Yolo Wonder, commonly used for *P. capsici* pathotype identification. The experiments for each isolate had a complete random block design with three replications and 20 plants per replication. Assays were performed in a growth chamber inoculating with one isolate at a time. All experiments included non-inoculated controls.

The assessment of resistance to *P. nicotianae* in the local pepper germplasm was also based on a complete random block design for each isolate, with three replications and 20 plants per replication. In these tests, 10 pepper lines —nine local germplasm types and the susceptible Yolo Wonder— were inoculated with two *Phytopthora* isolates —*P. nicotianae* and *P. capsici*— from northwestern Spain. These experiments were conducted under greenhouse conditions.

The pathogenic behaviour of *P. nicotianae* towards tomato was studied in a growth chamber. Five isolates of the oomycete —four from northwestern Spain and one of German origin— plus a reference *P. capsici* strain, were inoculated into tomato cv. San Pedro plants. This experiment also had a complete randomised block design (three replications per each cultivar-isolate combination and 20 plants per replication).

Disease severity ratings

Disease severity was rated 14 days after inoculation in the virulence tests, at 30 days post-inoculation for the evaluation of the resistance of *C. annuum* to *P. nicotianae*, and 14 and 21 days post-inoculation in the study of the pathogenicity of *P. nicotianae* towards tomato. Each plant in each experiment was rated on the following scale: 0, no symptoms (0% disease); 1, small rots on the base of the stem, to up to 30% of the plant affected; 2, 31-50% of the plant affected; 3, 51-70% of the plant affected; 4, 71-90% of the plant affected; 5, dead plant, 100% of the plant affected (Kim and Hwang, 1992).

Statistical analysis

Mean disease severity ratings were compared after transforming the data for each plant using the following formula: $Y = \arcsin \sqrt{X/100}$, where X is the disease rating as a percentage. The virulence tests were performed separately for each individual isolate;

Table 3. Mean disease severity ratings of the reference *Capsicum annuum* germplasm inoculated with *Phytophthora nicotianae* and *P. capsici* isolates from northwestern Spain, 14 days after inoculation

Isolate	Pathogen	Mean disease rating[1]		
		Yolo Wonder	PI 201234	SCM 334
Ar-3	*P. nicotianae*	0.47 a[2]	0.00 b	0.00 b
Ro-3/03	*P. nicotianae*	2.62 a	0.00 b	0.00 b
Ro-7/02	*P. nicotianae*	1.32 a	0.00 b	0.00 b
Ro-16/02	*P. nicotianae*	0.38 a	0.00 b	0.00 b
Ro-18/02	*P. nicotianae*	1.65 a	0.00 b	0.00 b
00/307	*P. nicotianae*	0.63 a	0.00 b	0.00 b
411.87	*P. nicotianae*	0.00 b	0.00 b	0.00 b
Pa-1	*P. capsici*	4.97 a	0.00 b	0.00 b
370.72	*P. capsici*	5.00 a	0.00 b	0.00 b

[1] Average disease severity ratings of three replicates of 20 plants per replicate, calculated on the basis of a 0 (0% disease) - 5 (100% disease) scale at 30 days post-inoculation, under growth chamber conditions. [2] Means followed by the same letter in the same row do not significantly differ according to Duncan's multiple range test (significance set at $P < 0.05$).

means were compared using Duncan's multiple range test. The means of cultivar tolerance to *P. nicotianae* and *P. capsici* were compared separately for each oomycete using the Waller-Duncan's multiple range test. The mean pathogenicity of *P. nicotianae* isolates towards tomato San Pedro were also compared using Duncan's multiple range test. Significance was set at $P < 0.05$ for all tests. All calculations were performed using SAS System v. 8 software (SAS Institute, 1999).

Results

The *P. nicotianae* isolates from northwestern Spain produced clear disease symptoms on cv. Yolo Wonder but were not pathogenic towards cvs. PI201234 and SCM334 (Table 3). The virulence of these isolates was not different to that of the two *P. capsici* isolates — including isolate 370.72 from the USA [which is classified as pathotype 0 according to Gil Ortega *et al.* (1995)], although the *P. capsici* strains were clearly more pathogenic than *P. nicotianae* towards cv. Yolo Wonder. The reference *P. nicotianae* isolate 411.87 showed significantly different virulence to all other isolates, in fact it seemed not to be pathogenic towards any of the three inoculated cultivars.

No complete resistance to *P. nicotianae* was observed among the northwestern Spanish pepper lines, whereas the reference *Capsicum* cultivars PI201234 and SCM334 were completely resistant (Table 4). The average level of resistance to this pathogen was higher than that recorded for *P. capsici*. Although quite susceptible to *P. capsici*, lines Co 12 B and Co 3.25 showed a high level of resistance to *P. nicotianae*.

None of the *P. nicotianae* strains inoculated into tomato San Pedro produced any kind of symptoms, while the *P. capsici* isolate used as a control caused clear collar and root rots 21 days post-inoculation (although it caused no plant deaths) (Table 5). The pathogenicity of the *P. capsici* isolate was confirmed

Table 4. Mean disease severity ratings of northwestern Spanish pepper germplasm inoculated with one isolate of *P. capsici* (Pa-1) and another of *P. nicotianae* (Ar-3) collected in northwestern Spain, at 30 days post-inoculation

Pepper genotype	Mean disease rating[1]	
	Pa-1	Ar-3
Co5A	2.47 d[2]	1.50 b
Co10A	4.00 bc	0.90 bc
Co12B	3.80 c	0.10 d
Co2.20	4.70 a	0.95 bc
Co3.25	3.90 bc	0.64 cd
Pa129	4.60 ab	0.88 bc
Pa141	4.80 a	0.93 bc
Pa158	3.90 bc	1.10 bc
Pa172	4.90 a	2.40 a
Yolo Wonder	4.75 a	2.36 a
Control	0.0 e	0.0 d

[1] Average disease severity ratings of three replicates of 20 plants per replicate, calculated on the basis of a 0 (0% disease) - 5 (100% disease) scale at 30 days post-inoculation, under growth chamber conditions. [2] Means followed by the same letter in each column do not significantly differ according to the Waller Duncan's multiple range test at $P < 0.05$.

Table 5. Mean disease ratings in tomato cv. San Pedro 14 and 21 days after inoculation with *P. nicotianae* isolated from pepper plants

Isolate	Pathogen	Mean disease ratings (%)[1]		Re-isolation of the pathogen
		14 days after inoculation[2]	21 days after inoculation[2]	
411.87	*P. nicotianae*	0.00 b	0.00 b	–
Ar-4	*P. nicotianae*	0.00 b	0.00 b	–
Png04	*P. nicotianae*	0.00 b	0.00 b	–
Hor-1/03	*P. nicotianae*	0.00 b	0.00 b	–
Hor-4/03	*P. nicotianae*	0.00 b	0.00 b	–
Pa-1	*P. capsici*	1.62 a	1.81 a	+
Control	—	0.00 b	0.00 b	–

[1] Average disease severity ratings of three replicates of 20 plants per replicate, calculated on the basis of a 0 (0% disease) - 5 (100% disease) scale at 30 days post-inoculation, under growth chamber conditions. [2] Means followed by the same letter in the same column do not differ significantly according to Duncan's multiple range test (significance set at $P < 0.05$).

by positive re-isolation of the pathogen on the diseased plants at the end of the test.

Discussion

All of the northwestern Spanish *P. nicotianae* isolates were similar in their virulence towards the reference varieties proposed by Gil Ortega *et al.* (1995) for determining the *P. capsici* pathotype. Certain variation in pathogenicity towards the pepper cv. Yolo Wonder was seen among the *P. nicotianae* isolates, but these were not pathogenic towards SCM334 and PI201234; similar results were obtained with *P. capsici.* These results differ from those of Allagui and Lepoivre (1996) who reported the virulence of the *P. nicotianae* strains isolated from pepper in Tunisia when inoculated into these same varieties: the Tunisian strains were pathogenic to PI201234 but not to SCM334. Whether this means that the Tunisian and Spanish isolates belong to different pathotypes needs to be determined in future work.

These results provide information valuable in breeding programs. Most of the pepper genotypes were moderately resistant to the oomycete, and some showed strong (although incomplete) resistance. The resistant pepper germplasm from northwestern Spain (Co-12B and Co 3.25) might be useful in breeding programs after the source of its resistance is determined. The lack of complete resistance to the pathogen among the Spanish germplasm tested, in comparison with that observed for the American lines SCM334 and PI201234, implies they might have different mechanisms of resistance. This has been reported for the host-pathogen interaction of *P. nicotianae* and carnation: one mechanism appears to be related to aggressiveness and another to compatibility (Maia and Venard, 1978). A similar situation was also confirmed recently for the interaction between *P. capsici* and *C. annuum* in Spain (Andrés *et al.*, 2005b).

The lack of pathogenicity of the *P. nicotianae* isolates obtained from pepper when inoculated into tomato was also observed in Tunisia by Allagui and Lepoivre (2000): these authors proposed the possible existence of a well-defined *forma specialis* within this species. Elicitins produced by *P. nicotianae* have been reported as microbial signalling molecules involved in the host-specificity of this pathogen (Kamoun *et al*., 1993) and specifically in the *P. nicotianae*-tomato interaction (Colas *et al.*, 1998; Capasso *et al.,* 1999). This possible specialisation could be important for horticultural growers in northwestern Spain where crop rotation involving tomato and pepper (both potential hosts of *P. nicotianae*) is common.

Overall, these findings confirm the existence of *C. annuum* germplasm in northwestern Spain with relatively high levels of resistance to *P. nicotianae*. This material could be useful in breeding programs with the aim of producing more resistant plants that require the use of less fungicide.

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