SHORT COMMUNICATION

PATHOGENIC VARIABILITY OF *PHYTOPHTHORA NICOTIANAE* ON PEPPER IN TUNISIA

M. Saadoun and M.B. Allagui

Institut National de la Recherche Agronomique de Tunisie, Laboratoire de Protection des Végétaux, rue Hédi Karray, 2080 Ariana, Tunisia

SUMMARY

Pathogenic variability of 15 different isolates of *Phytophthora nicotianae* was determined following inoculation of six pepper (*Capsicum annuum* L.) varieties at the seedling stage. Cvs Beldi, Baker and D'hirat were susceptible, whilst Baklouti and Nabeul II showed a significant interaction variety x isolate, and the Mexican landrace Criollo de Morales 334 was strongly resistant to all isolates. Results clustered the 15 *P. nicotianae* isolates into four pathogenic groups. The first included the weakly virulent isolate *Pnt*317, which was able to attack only cvs Beldi and Baker. Isolate *Pnt*341 constituted the second group which was more pathogenic than the first, being able to attack also cv. D'hirat. Isolates *Pnt*314, *Pnt*323 and *Pnt*326 formed the third group, characterized by a high pathogenic power on cvs Beldi, Baker, D'hirat and Nabeul II. The fourth group, which comprised the widest isolate range was the most pathogenic since it attacked all the local varieties but not CM 334. To improve varietal resistance to *P. nicotianae*, pepper breeders in the Mediterranean region should take into account the pathogenic variability determined in this study.

Key words: Pepper, differential reactions, pathogenic groups, *Phytophthora nicotianae*.

Pepper (*Capsicum annuum* L.) production in Tunisia is challenged by various abiotic and biotic stresses, such as virus and fungus diseases, adverse environmental factors, and irrigation water quality. Among these, the dry root necrosis syndrome associated with wilting differs from that caused by *Phytophthora capsici* Leonian, described in other parts of the world (Allagui, 1999). In fact, no *P. capsici* infections to pepper have been reported so far from Tunisia. According to analyses based on morphological, biological and molecular criteria, the isolates of *Phytophthora* attacking pepper in Tunisia were identified as belonging to *Phytophthora nicotianae* Breda de Haan (Allagui *et al.*, 1995; Allagui and Tello-Marquina, 1996; Allagui and Lepoivre, 2000).

P. nicotianae is a significant agricultural pathogen worldwide, infecting many economically important species including citrus, tomato, tobacco and sesame (Erwin and Ribeiro, 1996). Studies addressing the pepper-*P. nicotianae* pathosystem are few and restricted to pathogen isolation and symptom expression in the northern Mediterranean countries (Borzini, 1957; Bartual *et al.*, 1991; Andrés *et al.*, 2003). This paper, however, evaluates the pathogenicity of some *P. nicotianae* isolates, thus may aid future breeding programmes.

Six pepper varieties were used in this study, including five susceptible local cultivars and the landrace CM334, that bears small fruits with a very hot flavor (Table 1). This landrace was selected to take advantage of its resistance to *P. nicotianae,* previously ascertained in tests containing also the pepper lines PI 201232 and PI 201234 (Allagui and Lepoivre, 1996).

Fifteen isolates of *P. nicotianae* recovered from infected peppers in different Tunisian localities (Table 2) were used as inoculum. The isolates were maintained at room temperature (20-25°C) on potato dextrose agar (PDA). To obtain zoospores for inoculation, mycelium discs excised from the edge of 7-day-old colonies were transferred onto pea agar (Allagui and Lepoivre, 2000) and maintained on this medium for 9 to 12 days at 20-25°C under a 16 h photoperiod. When the mycelium had completely colonised the plates, it was cut into rectangular portions *ca.* 4x2 cm in size. These were covered with sterile distilled water and incubated for 3 days at 25°C under a 16 h photoperiod, then cooled to 4°C for 30 min and left at room temperature for 10 to 20 min to stimulate zoospore discharge from sporangia. Zoospores recovered were immediately used to inoculate pepper seedlings.

Seedlings were grown from seeds that were surface-sterilized with 4% sodium hypochlorite (40 g active chlorine/liter) for 10 min, rinsed twice with distilled water and dried on filter paper at 20°C. The substrate used for sowing and planting was a mixture of claysoil, sand and peat (2:1:1, v/v/v), pasteurized at 100°C for 90 min. Fifteen days after transplanting in alveolated plates, seedlings (2-leaf stage) were inoculated by dripping a sus-

Corresponding author: M. Saadoun
Fax: +216. 71 235 317
E-mail: saadoun2003@yahoo.fr

Table 1. Pepper varieties used in inoculation tests.

Varieties	Donor	Characteristic[a]
Beldi	Local	Susceptible in field to *Pnt*
Baker	Local	Susceptible in field to *Pnt*
D'hirat	Local	Susceptible in field to *Pnt*
Nabeul II	Local	Susceptible in field to *Pnt*
Baklouti	Local	Susceptible in field to *Pnt*
CM334	Inia (Zaragoza, Spain)	Resistant to *Pc* (Guerrero and Laborde, 1980)

[a] *Pnt* = *Phytophthora nicotianae* from Tunisia – *Pc* = *Phytophthora capsici*

pension of *ca.* 280,000 zoospores (3.5 to 7 ml according to the isolate) at the collar of each of them. Inoculated and uninoculated seedlings were maintained in a greenhouse at 18-30°C. Irrigation was with tap water each 3 to 4 days depending on substrate humidity. After 20 days incubation, the root system of each seedling was delicately removed from the substratum by washing in a water bowl and root necrosis intensity was evaluated according to an arbitrary scale from 0 (healthy plant) to 5 (dead plant) (Allagui and Lepoivre, 2000). Each isolate was tested for pathogenicity on nine seedlings of each variety distributed in three random replicates. Mean root rot scores ≤ 1 classified the variety as resistant (R) and mean scores > 1 as susceptible (S).

Table 2. *Phytophthora nicotianae* isolates used in the study.

Isolate	Type of culture, plant stage	Visual estimation of plant attack (%)	Locality	Year of isolation
Pnt301	greenhouse	-	Monastir	1999
Pnt304	open field; adult plant in production	40	Korba-beach (Daroufa)	1999
Pnt310	open field; seedling stage	10	Korba-beach	2000
Pnt314	open field; seedling stage	10	Korba-beach	2000
Pnt317	greenhouse	whole line infected	Oued Ellil	2000
Pnt318	open field; start of production	5	Korba-Tazarka	2002
Pnt323	open field; start of production	5	Korba-Tazarka	2002
Pnt325	open field; start of production	5	Korba-Tazarka	2002
Pnt326	open field; start of production	10	Korba Km 9 to Nabeul	2002
Pnt329	open field; start of production	10	Korba Km 9 to Nabeul	2002
Pnt332	open field; start of production	10	Korba Km 9 to Nabeul	2002
Pnt334	open field; start of production	10	Tazarka	2002
Pnt336	open field; start of production	10	Tazarka	2002
Pnt341	open field; start of production	30	Korba Km 9 to Nabeul	2002
Pnt343	open field; start of production	30	Korba Km 9 to Nabeul	2002

Table 3. Mean intensity of root necrosis produced by 15 isolates of *Phytophthora nicotianae* on different pepper varieties.

Isolates	Varieties						Pathogenic groups	Means by isolate
	Beldi	Baker	D'hirat	Nabeul II	Baklouti	CM334		
Pnt317	1.3 (0.5-3.0) S	1.3 (0.5-5.0) S	1.0 (0.5-2.0) R	0.9 (0.5-2.0) R	1.0 (0.0-5.0) R	0.5 (0.0-1.0) R	1	1.0 f
Pnt341	3.3 (1.0-5.0) S	3.1 (0.5-5.0) S	2.3 (0.5-5.0) S	1.0 (0.5-2.0) R	0.5 (0.5-1.0) R	0.4 (0.0-0.5) R	2	1.8 cde
Pnt314	2.2 (0.5-5.0) S	2.1 (0.0-5.0) S	2.4 (0.5-5.0) S	1.5 (0.5-4.0) S	0.8 (0.0-4.0) R	0.3 (0.0-0.5) R		1.5 e
Pnt326	2.7 (0.5-5.0) S	2.3 (0.5-5.0) S	1.8 (0.5-5.0) S	2.0 (0.5-5.0) S	1.0 (0.5-5.0) R	0.4 (0.0-0.5) R	3	1.7 de
Pnt323	3.4 (0.5-5.0) S	3.3 (0.5-5.0) S	3.2 (0.5-5.0) S	1.9 (1.0-5.0) S	0.4 (0.0-0.5) R	0.4 (0.0-0.5) R		2.1 bcde
Pnt334	2.9 (2.0-5.0) S	2.0 (0.5-4.0) S	2.9 (1.0-5.0) S	2.3 (1.0-5.0) S	1.5 (0.5-5.0) S	0.4 (0.0-0.5) R		2.0 cde
Pnt343	3.3 (1.0-5.0) S	3.1 (1.0-5.0) S	3.9 (3.0-5.0) S	1.2 (0.5-2.0) S	1.5 (0.5-3.0) S	0.4 (0.0-0.5) R		2.2 bcd
Pnt310	1.9 (0.5-3.0) S	3.0 (2.0-5.0) S	1.7 (0.5-3.0) S	2.0 (0.5-3.0) S	2.1 (0.5-5.0) S	0.4 (0.0-0.5) R		1.9 cde
Pnt332	3.2 (2.0-5.0) S	2.1 (0.5-5.0) S	1.6 (0.5-3.0) S	2.3 (2.0-3.0) S	2.0 (0.5-5.0) S	0.5 (0.0-1.0) R		1.9 cde
Pnt301	2.5 (2.0-5.0) S	1.9 (0.5-3.0) S	3.0 (2.0-5.0) S	1.9 (0.5-5.0) S	2.1 (1.0-5.0) S	0.4 (0.0-1.0) R	4	2.0 cde
Pnt325	4.1 (3.0-5.0) S	4.4 (3.0-5-0) S	3.5 (2.0-5.0) S	3.3 (2.0-5.0) S	1.5 (0.5-3.0) S	0.5 (0.0-1.0) R		2.9 a
Pnt336	4.5 (3.0-5.0) S	3.6 (0.5-5.0) S	3.1 (2.0-4.0) S	4.0 (3.0-5.0) S	2.4 (0.5-5.0) S	0.3 (0.0-0.5) R		3.0 a
Pnt329	3.7 (2.0-5.0) S	3.5 (2.0-5.0) S	3.0 (1.0-5.0) S	3.1 (1.0-5.0) S	1.8 (0.5-5.0) S	0.4 (0.0-1.0) R		2.6 ab
Pnt318	3.4 (2.0-5.0) S	3.2 (0.5-5.0) S	3.4 (1.0-5.0) S	1.5 (0.5-2.0) S	1.8 (1.0-4.0) S	0.4 (0.0-0.5) R		2.3 bc
Pnt304	2.8 (1.0-5.0) S	2.0 (0.5-3.0) S	3.0 (2.0-5.0) S	1.7 (0.5-4.0) S	1.9 (0.5-5.0) S	0.4 (0.0-0.5) R		1.9 cde
Means by variety	3.0 a	2.7 ab	2.6 b	2.1 c	1.5 d	0.4 e		

Data are averages of three replicates; minimum and maximum values of the scale (0-5) of the necrosis intensity are in brackets.
R = resistant, with mean intensity of root necrosis ≤ 1.
S = susceptible, with mean intensity of root necrosis > 1.
Means by isolate and means by variety followed by the same letter did not differ significantly at P = 0.05.

For the analysis of variance (ANOVA) the following formula was used:

$$Yij = \mu + Vi + Ij + (V \times I)ij + eij$$

where Yij is the value of root necrosis intensity in variety i inoculated with isolate j; μ is the overall mean; Vi is the effect of the ith variety, Ij is the effect of the jth isolate, (V x I)ij is the interaction variety x isolate, and eij is the residual error. Mean comparisons were made using Duncan's multiple range test at *P=0.05*. Statistical analyses were made using the SAS System *v. 9.1* software (SAS Institute Inc, Cary, NC, USA).

Pathogenicity of the 15 *P. nicotianae* isolates on the six pepper varieties was evaluated 20 days post inoculation. Variance analysis of the root necrosis measures showed a highly significant (P<0.001) 'isolate' effect. Isolate *Pnt*336 was highly pathogenic with a mean necrosis intensity of 3, whereas *Pnt*317 was the least pathogenic with a mean necrosis intensity that did not exceed 1. *Pnt*325 and *Pnt*329 were not significantly different from *Pnt*336, with means of 2.9 and 2.6, respectively. The remaining isolates, with a mean intensity of root necrosis between 2.3 and 1.5, were significantly different from *Pnt*336 and *Pnt*317 (Table 3).

Analysis of variance showed that the varietal effect was highly significant (P<0.001), indicating that there was a differential reaction of the cultivars to the isolates tested. Beldi was the most susceptible with a mean intensity of root necrosis of 3; cvs Baker, D'hirat, Nabeul II and Naklouti showed decreasing susceptibility with a mean necrosis intensity from 2.7 to 1.5. CM334 landrace was strongly resistant with a mean necrosis intensity not higher that 0.5 (Table 3).

The interaction varieties x isolates was also highly significant (P<0.001). Beldi and Baker were susceptible to all isolates with mean intensity of necrosis always higher than 1.3; cvs D'hirat, Nabeul II, and Baklouti were susceptible to 14, 13 and 10 isolates, respectively. The resistant CM334 landrace showed very little root necrosis, which ranged from 0.3 to 0.5 (Table 3). Control plants, exposed to tap water, had healthy roots.

Inoculation tests under greenhouse conditions on a set of six pepper varieties showed *P. nicotianae* isolates from different Tunisian sites to be differentially pathogenic. Evaluation of root necrosis intensity, revealed the occurrence of four distinct pathogenic groups in the 15 isolates, based on the interactions with pepper varieties, as shown in Table 3 where cultivars with mean intensity of necrosis higher than 1 are considered susceptible. In other pathosystems, such as pepper-*P. capsici* (Palloix *et al.*, 1990; Gil-Ortega *et al.*, 1991) and strawberry-*P. fragariae* (Nickerson and Murray, 1993) a root necrosis intensity of 1 was used as threshold between resistance and susceptibility. Accordingly, isolate *Pnt*317 was pathogenic only to cvs Beldi and Baker and was classified in a separate group. Isolate *Pnt*341 constituted the second group, characterized by a high pathogenic power on cvs Beldi, Baker and D'hirat. Isolates *Pnt*314, *Pnt*326 and *Pnt*323 can be placed in a third group of pathogenicity being able to attack four varieties (Beldi, Baker, D'hirat and Nabeul II), and being ineffective on Baklouti and CM334. The difference between the third and the second group lies in the behaviour of cv. Nabeul II which is resistant to *Pnt*341 and susceptible to *Pnt*314, *Pnt*326 and *Pnt*323. The fourth group, the most pathogenic one, comprises the isolates capable of attacking all cultivars, except for CM334.

The response of different host genotypes to any given isolate of a plant pathogen was used by Van Der Plank (1968) to determine physiological races. The same approach was used in trials with *P. capsici* and pepper to identify compatible/incompatible interactions (Clerjeau *et al.*, 1976; Pochard *et al.*, 1983; Kim and Hwang, 1992). Physiological races in this pathosystem were reported from the USA (Polach and Webster, 1972) and Taiwan (Anonymous, 1999). The latter described three races of *P. capsici* denoted 1, 2 and 3, using differential pepper hosts. Gil-Ortega *et al.* (1995) reported from Spain two vertical pathotypes of *P. capsici* named P0 and P1. However, *P. nicotianae* races attacking pepper have not been reported. As pepper lines having different resistance genes required for race identification are not available, a way to approach this subject could be based on intra-varietal variability shown by inoculation tests. Such variability needs to be tested in seed lines to confirm whether genetic or environmental factors are implied. Intra-varietal selection programmes could be undertaken if variation is supported by genetic background. Selection of individual plants within a variety and testing the stability of plant resistance might offer a support for inheritance studies of a multigenic system.

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