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CHARACTERIZATION OF MOROCCO ISOLATES OF *PHYTOPHTHORA INFESTANS* FOR MATING, TYPE GENOTYPE AND METALAXYL SENSITIVITY

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Abstract: In Morocco Tomatoes (*Lycopersicum esculentum* Mill) and potatoes (*Solanum tuberosum*) has become an important segment of the domestic food supplies and provide significant export income (O.C.E.). These crops are grown year-around in northern and southern Morocco along the Atlantic coast and inland in the Atlas Mountain region. In spite of ubiquity of susceptible hosts, late blight, caused by *Phytophthora infestans* (Mont) de Bary, has not been an important disease until recent years (Plant Protection Service, 1996). On the continent of Africa, it was first reported in 1941, on Irish potatoes being grown for allied troops in South Africa (Coxet al.,1960). Late blight has been observed for decades as small-delimited foci in Moroccan potato and tomato fields but has not limited the production. High daytime temperatures were though to inhibit epiphytotic development by the pathogen. In the past several years, due to population shrifts of *P. infestans*, late blight has become epidemic regardless of the control measures imposed. Significant losses have occurred in potatoes planted in the spring. This study was undertaken to characterize the parasitic fitness of Moroccan isolates of *P. infestans* on the most widely grown potato and tomato cultivars.

MATERIALS AND METHODS

Source of isolates. Samples were collected from diseased plants in commercial fields in Larache, Leaves were placed in plastic bags which were transported in an ice chest to the laboratory. Individual leaflets were cut into 2-5 mm sections, soaked for 3 min in 2% hypochlorite and rinsed with sterile distilled water and dried on sterile filter paper. Pieces of tissue were then plated on pea agar amended with antibiotic and incubated at 20°C in the dark for two wk (Ribeiro O.K. 1978). Pure cultures maintained on V8 agar and Rye agar (Deahl,et al, 1993; 1995). Plugs, containing mycelia and sporangia, were sporangia, were transferred to fresh media at two-three wk intervals.

Potato Nicola and superior and tomato bonny best pixie and better boy's plants were inoculated with each isolates to establish pathogenicity.

Quarantine. Isolates prepared in Morocco were imported into the U.S.A. under permit of the U.S. Department of Agriculture. All work was performed into a laboratory under a biosafety hood. All materials were autoclaved before disposal and greenhouse studies were conducted in the winter months.

Mating types. Mating types were determined by placing 5 mm plugs of mycelia from test isolates, two cm from known types on clarified V8 agar plates (Kadish, d. and Cohen, y 1988). Moroccan isolates were tested against Al type and A2 type. Plates were incubated in the dark at 20°C for 5-10 days. The presence of oospores was determined by microscopic study.

Matelaxyl resistance. In vitro test, the method used by Deahl et al. (1993; 1995) was performed in this experiment. Isolates from Moroccan US-1 and US-6 (from Dr Kim S.H, PA. Dept. of

agr.) and the Delaware isolates collected in 1995 (US-8), all references herein to *P. infestans* genotypes designated "US" are sensu Goodwin et al(1994), were assessed to portion of molten rye seed agar prior to autoclaving, to yield final concentration from 1 to 250 ug/ml. For each test, inocula agar plugs (5 mm) cut from edge of two week culture were placed in the center of the plate and incubated at 20°C in the darkness, each test was replicated five times. Growth was measured after 5, 10 and 15 days of incubation. In vitro growth was calculated according to Deahl, et al equation (Deahl,et al, 1993; 1995)

$$\% \text{ growth} = \frac{\text{Avg diam (-5mm) on metalaxyl containing media}}{\text{Avg diam (-5mm) on metalaxyl-free media}} \times 100$$

In vitro tests, potato leaflets from 8 weeks old plants, European cultivar 'Nicola' and American cultivar 'Superior' were floated upper surface down on water in 9cm petri dishes containing from 1.0 to 250 µg metalaxyl/ml. Each leaflet was inoculated with 1 droplet (12µl) of a zoospore and sporangia suspension containing 12000 sporangia/ml. Each inoculum was prepared by incubating 2 weeks culture at 4°C for 2 hours to induce zoospore release. Inoculated leaflets were then incubated in growth chamber at 14°C with 16-hr illumination. Three leaflets were used for each treatment or concentration exposure. Disease severity was rated after 10 days according to Deahl et al. (1993; 1995). Scale 0, 1, 2, 3, 4, and 5 representing 0, 0-20, 20-40, 40-60, 60-80, 80-100% respectively.

Potato tuber disk biossay was performed to assess for metalaxyl resistance according to the method developed by Kadish and Cohen (Kadish, d. and Cohen, y 1988) was performed to confirm the metalaxyl response of the same isolates on the same cultivars used in floating leaflet test. Three disks of 5-10 mm x 3 mm were placed in 9 cm petri dishes on filter paper wetted with 3 ml deionized water or with concentration from 1,0 to 250 ug metalaxyl/ml. the inoculum was prepared similar to the one prepared for leaflet biossay. Droplet (12 µl) of inoculum was deposed on each tuber disk and the same growth chamber and condition described above in leaflet experiment. Metalaxyl response was assessed 10 days after inoculation using the same scale.

Allozyme analysis.

The method used in this study was the Cellulose acetate electrophoresis (CAE) developed in 1995 by Goodwin et al (8). A small quantities of mycelia growth in rye broth (filtrate from 200 g autoclaved rye seed/liter) and piece of mycelia from pure culture were used in this analysis. Tissue samples (mycelia and sporangial washtes) were placed into 1.5ml microcentrifuge tubes with 1 ml of water and centrifuged at 13,000x g for 2 minute to pellet. Excess supernatant was eliminated and the pellet was ground by hand using a tapered pestle Samples were then centrifuged as above to pellet cell debris. Small aliquot of each sample were pipetted from the supernatant into wells on sample well plate Blue dye spotted along to along to monitor the progress of electrophoresis and to provide an orientation. After 15-20 minutes of electrophoresis, gels were stained using modification of agar overlays. The gels were then incubated until bands appeared.

The CAE was used in this study because it provide excellent resolution of allozyme genotypes of *P. infestans* at the two loci Glucose-6 phosphate isomerase (Gpi) and Peptidase (Pep) .

Statistical analysis

The experiment design was factorial with four factorial: isolate, dose, time and cultivar, using a randomized complete block (RCB) where the blocks were replicated in time; Computations were done using the GLM procedure of SAS (1).

RESULTS

Allozyme genotype and mating type. All Moroccan isolates collected in 1995 from North Morocco (Larache) isolated from a single lesion. Isolates from Morocco were A1 mating type and produced oospores with A2, the Moroccan isolates, allozyme analysis revealed the presence of an individual homozygous for 100 allele with a single band at Gpi locus and two different alleles (92/100) at Peptidase locus (i.e.US-6). Based on the mating type and the allozyme analysis, the isolates from Morocco belong to the genotype group of US6.

Pathogencity. Pathogencity tests revealed that the Moroccan isolates (genotype group US-6) infected both tomato and potato with equal severity (Table 1).

Table 1. Pathogencity of *P. infestans* isolates on tomato potato cultivars by artificial inoculation

	Isolates	Tomato		Potato	
	Bonny best	"Pixie"	" Better Boy"	"Superior"	"Nicola"
M-1[y]	++	++	++	++	++
D-2[z]	+	+	+	++	++
US-6	++	++	++	+	+
US-1	-	-	-	++	++

[x] Observation of foliar infection after 10 days.
\+ = less than 50% of whole plants showing lesions
++ = More than 50% " " " "
\- = No lesions
[y] M1 = Moroccan isolate
[z] D2 = Delaware isolate.

Reponses to metalaxyl: The Moroccan isolates exhibited a large variation in fitness with regard to radial growth of mycelium on metalaxyl amended media. The difference between means was significant based on statistical analysis (table 2).

Table 2. Assay of sensitivity of *P. infestants* isolates to metalaxyl in rye agar at different time intervals.

Isolates	Percentage of Radial Growth in Days [x]		
	5	10	15
Moroccan isolates (M-1)	81.166ay	85.750za	92.333a
Delaware isolate (D-2)	72.833b	74.916b	73.916b
US-6	73.583c	79.000c	82.250c
US-1	3.000d	3.250d	4.833d
LSD(0.05)	0.85	0.88	1.12

[x] Average of 12 replications.
[y] Means with the same letter are not significantly different at P=0,05 according to Fisher's LSD tests.

The % of mycelial radial growth the following equation (3,4):

$$\% \text{ growth} = \frac{\text{Avg diam (-5 mm) on metalaxyl-containing media}}{\text{Avg diam (-5mm) on metalaxyl-free media}} \times 100$$

The EC50 values for the Moroccan and references isolates were 250 and 144 respectively (table3). The cultivar Superior was more susceptible to all *P.infestans* isolates tested than the cultivar "Nicola". The Moroccan isolate was highly pathogenic on both cultivars. The responses to metalaxyl in floating leaflets and tuber disks were similar. Based on statistical analyses, there was no significant difference between. This supports the results of the metalaxyl agar plate tests

Table 3. Concentration of metalaxyl (a.i) to reduce mycelial radical growth by 50% (EC 50) based on logistic analysis

Isolates	EC50	Response
Moroccan isolates (M-1)	>250	Highly resistant
Delaware isolate (D-2)	144	Resistant
US-6	190	Resistant
US-1	0.005	Susceptible

DISCUSSION

This study was the first done on the genotype identification of *P. infestans* in Morocco. Results show the appearance of a new A1 genotype in Morocco that is highly resistant to metalaxyl (group US-6).

This genotype (M-1) is equally aggressive in causing late on tomato and potato. Isolate M-1 infected all tomato and potato cultivars used in this study with no significant difference. In both situation the pathogen was probably imported with potato tubers used as seed. In Morocco, thousands of tons of potato tuber seeds are imported from Europe, mainly from countries where the new genotype (US-6) has already been reported. The Moroccan isolate (M-1) exhibited a level of metalaxyl resistance compared to the genotypes US-6 and US-8 (also resistant). Since the M-1 and US-6 isolates are in the same group, it is interesting that the Moroccan isolates (M-1) is more resistant. Two possible explanations are:

1) it could be due the presence of this new genotype in Morocco for many years (since it was reported in Europe in 1984, compared to the US-6 isolate, which was reported just recently (1992) in the United states;
2) it could be related to the effect of environmental factors, mainly the early use of metalaxyl (since 1984) and the frequency of metalaxyl application, (up to 12 applications per season) in Morocco. Either of or a combination of or a combination of these factors could have favored the creation of more resistant isolates in Morocco.

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