Polymorphic *avr* genes in isolates of two races of *Passalora fulva* from Argentina

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Introduction

Tomatoes growing under environmental conditions such as high relative humidity and 25 C° [3] are highly affected by leaf mold [1], a disease caused by *Passalora fulva* [2] [syn. *Cladosporium fulvum*] a non-obligate biotrophic fungus.

The Tomato-Passalora interaction behaves according to a typical gene for gene relationship [4]. In the intercellular spaces of the leaf, the fungus releases avirulence proteins coded by *avr* (avirulence) and *ecp* (extracellular protein) genes, which are recognized by plant resistance genes (*Cf*), leading in this way to incompatible interactions, that is resistance [5]. Several races of the fungus have been described and also the sequences of *avr*2 [6, 7], *avr*4 [8, 9], *avr*4E [10, 11] and *avr*9 [12] are known.

The development of new races is always threatening production areas around the world since it has been demonstrated that new races of *P. fulva* developed rapidly [13, 14, 15]. Furthermore, De Wit [16] found that other fungal species closely related phylogenetically to *P. fulva* also carry *avr* genes, which might be another source of variation.

In Argentina, leaf mold is a rather new disease of tomato, that affects mostly greenhouse grown tomatoes.

Objective

The purpose of this work was to identify races of the etiological agent of diseased tomatoes with leaf mould symptoms, by means of morphological and molecular tools. Furthermore, we amplified, sequenced and analyzed the *avr* gene sequences to determine the races of the pathogens and their genetic variability.

Materials and methods

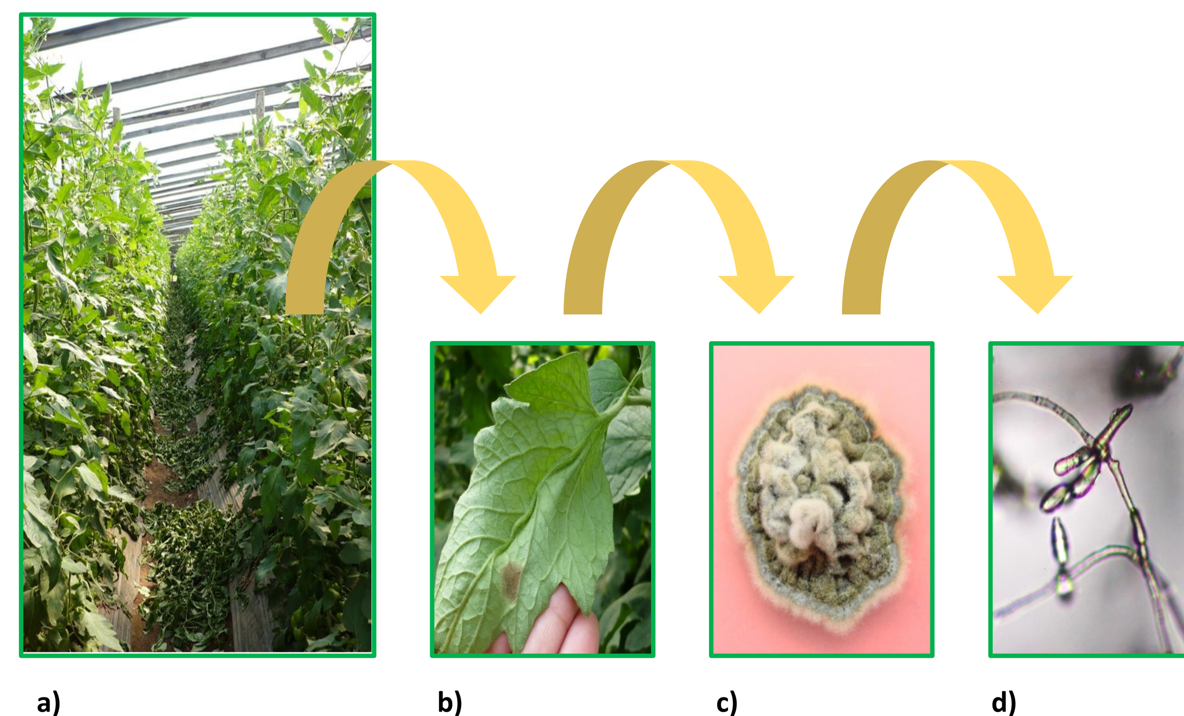

Figure 1. a) Greenhouse. B) left mold symptoms. C) Monosporic isolate. D) Microculture (1000X).

Isolate	Tomato cultivar	Site of Isolation
ALH	Elpida	Los Hornos
ELH	Elpida	Los Hornos
EMP	Elpida	Abasto
CH6	Elpida	Pcia de Corrientes
COA	Colibrí	Arana
EOP	Elpida	Olmos
ColA	Colibrí	Arana
CK813	Keitor	Pcia de Corrientes
ELS	Elpida	Etcheverry
ComA	Compak	Arana
EAV	Elpida	Arana
CHEAV	Cherry- Colly	Arana

Table 1

Primer	Sequences
AVR2f	5'-CATCAGCATATCCTCTTCCATCC-3'
AVR2r	5'-CAGTACGTTCAAAAGCAGATAAGG-3'
AVR4f	5'-ACGGTAGGTCTGTACACGCGAC-3'
AVR4r	5'-ACCGAACTGGGTCATGGAATG-3'
AVR4Ef	5'-GCCCGGTATATCGCTGTGC-3'
AVR4Er	5'-CGGAACCCCTGGCTGAGA-3'
AVR9f	5'-AATACAACCTTGAAACAGCTAGG-3'
AVR9r	5'-GGACTCTACGGGGCTTGG-3'

Table 2

- Monosporic isolates were obtained from typical leaf mold symptoms on *Solanum lycopersicum,* cv Elpida (Enza Zaden®), Keitor, Compak, Cherry-Colly and Colibrí (Clausen®). (Table 1)
- The mycelium originated from conidia that were transferred to 2% PDA , regular as well as microcultures were performed.
- Genomic DNA of fungi was extracted by means of the CTAB method [17].
- In order to amplify the 3´ end of the 18S rDNA, ITS1, 5.8S rDNA, ITS2 and the 5´ end of the 28S rDNA, PCR were run with genomic DNA mixed with primers ITS-4 and ITS-5 [18]
- In order to amplify avr genes PCR were run with the primers described by Stergiopoulos [19], which were modified, an Eco RI and HindIII sequence were included at the 5' end of the forward primer and 3' end of the reverse primer, respectively. (Table 2)
- The DNA amplicons were sequenced by the dideoxy termination method [20].
- Sequences were analyzed using Basic Local Alignment Search Tool (Blast) and other bioinformatics tools.
- The sequences of the ITS and the avr genes were deposited in the GenBank

Results

Microscopy

All the isolates described in this study belong to and are maintained in the culture collection of the CIDEFI (Table 1). Colony pigmentation was typical of *P. fulva and the* fungal structure and size type of this species were observed by × 1000 magnification (Fig 1.D)

Molecular analysis

- ITS- Amplification.

PCR products (approximately 600 pb) were resolved by electrophoresis in 1% agarose gels. (Figure 2.A). The ITS sequences of the isolates were 100 % homologous to that of *P. fulva* .

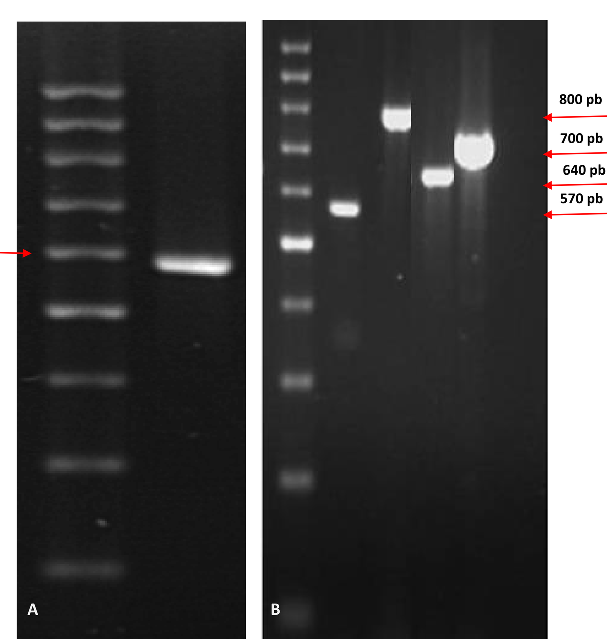

Figure 2. A) ITS amplification and B) avr amplification. avr2, avr 4, avr4E and avr9.

- *AVR* genes- Amplification.

PCR products were resolved by electrophoresis in 1,5 % agarose gels. (Figure 2.B)

The sizes of the amplified fragments (570, 806, 640 and 710 pb) were identical to those described by Stergiopoulos *et al*. (2007).

The sequences were 99% homologous to *avr 2, avr4, avr4E and avr9.*

- Alignment.

The sequences were aligned using Bioedit software and them were analyzed on search of transitions (TS), trasvertions (TV), deletions (D) and insertions (I) within the *avr* genes sequences of the isolates (Table 3).

ISOLATES	avr 2				avr 4				avr 4E				avr 9				%
	TS	TV	D	I	TS	TV	D	I	TS	TV	D	I	TS	TV	D	I	
ALH	-	-	-	-	2	0	0	0	4	2	0	0	0	0	0	0	6.89
ELH	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	4.31
EMP	-	-	-	-	2	6	0	2	3	0	0	0	0	0	0	0	11.20
CH6	-	-	-	-	3	3	1	1	3	1	0	0	0	1	0	0	11.20
COA	-	-	-	-	2	1	0	0	3	0	0	0	0	1	0	0	6.03
EOP	0	1	0	2	0	0	0	0	4	0	0	0	0	0	0	0	6.03
ColA	-	-	-	-	1	1	0	0	3	0	0	0	0	1	0	0	5.17
CK 813	-	-	-	-	nd	nd	nd	nd	4	3	0	0	0	1	0	0	≥6.89
ELS	0	0	0	0	0	0	1	0	5	7	1	0	0	1	0	0	12.93
ComA	0	0	0	0	0	1	0	0	3	0	0	0	0	1	0	0	4.31
EAV	1	1	7	0	3	7	0	0	3	1	0	0	nd	nd	nd	nd	≥19.83
CHEAV	-	-	-	-	0	0	0	0	3	0	0	0	0	1	1	0	4.31
TOTAL	1	2	7	2	13	19	2	3	41	16	1	0	0	7	1	0	116

Table 3. *avr* genes of the *Passalora fulva* isolates collected. Here we report the changes observed in the sequences of the *avr* genes analyzed. I = insertion; D =deletion; TV= transvertion; TS= transitions. (-) absent. nd : not determined.

Discussion

Among all the materials analyzed we successfully identified, by means of morphological features as well as molecular tools, twelve isolates of *P. fulva,* the ethiological agent of leaf mold disease .

The isolates belong either to race 0 or race 2.

Although *avr* genes within isolates were found to be polymorphic, we do not know the biological implications of these differences.

Though the number of races identified was of only two, the population of the fungus seems to be evolving, which might lead to the appearance either of new races or more virulent variants of the existing ones.

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