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* [6, 7], *avr* [8, 9], *avr* [10, 11] and *avr* [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 mold 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

- **Monosporic isolates were obtained from typical leaf mold symptoms on *Solanum lycopersicum*, cv Elpida (Enza Zaden®), Keitor, Compak, Cherry-Colly and Colibri (Clausen®).** (Table 1)
- The mycelium originated from conidia that were transferred to 2% PDA, regular as well as mycelial fragments were performed.
- Genomic DNA of fungi was extracted using methods 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 ITS4-5 and ITS5-18 [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 dye-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.

Discussion

Among all the materials analyzed we successfully identified that, by means of morphological features as well as molecular tools, twelve isolates of *P. fulva*, the etiological 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 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.

**References**