Polymorphisms at DNA level of *avr* genes of P.*Fulva* in Argentina

The allelic variation in four avirulence *(Avr)* and four extracellular protein *(Ecp)*–encoding genes of the tomato pathogen *Cladosporium fulvum* was analyzed for a worldwide collection of strains. The majority of polymorphisms observed in the *Avr* genes are deletions, point mutations, or insertions of transposon-like elements that are associated with transitions from avirulence to virulence, indicating adaptive evolution of the *Avr* genes to the cognate *C. fulvum* resistance genes that are deployed in commercial tomato lines. Large differences in types of polymorphisms between the *Avr* genes were observed, especially between *Avr2* (indels) and *Avr4* (amino-acid substitutions), indicating that selection pressure favors different types of adaptation (Stergiopoulos *et al* 2007)

Four *Avr* genes (*Avr2*, *Avr4*, *Avr4E*, and *Avr9*) have been sequenced from *C. fulvum*, and their encoded proteins trigger a hypersensitive response (HR) in tomato plants that carry the cognate *Cf-2*, *Cf-4*, *Hcr9-4E* (also known as *Cf-4E*), and *Cf-9* genes, respectively (Joosten *et al*. 1994; Luderer *et al.* 2002; Van Kan *et al*. 1991; Westerink *et al*. 2004b). However, several strains of *C. fulvum* exist in natural populations of the fungus that are able to overcome as many as five different *C. fulvum* genes that are present in commercial tomato lines (Lindhout *et al.* 1989). Such strains with complex virulence spectra are thought to have emerged as a result of strong selection pressure imposed on the fungus after the introduction of the different *C. fulvum* genes into tomato cultivars since the 1930s (Lindhout *et al*. 1989; Rivas and Thomas 2005). The transition from avirulence to virulence is associated with DNA modifications in the *Avr* genes that include deletions, point mutations, or insertions of transposon-like elements, resulting either in complete loss of the Avr protein or in the production of altered forms that no longer trigger HR in tomato plants carrying the cognate *C. fulvum* genes (Westerink *et al.* 2004a). In most cases, loss or mutation of Avr proteins does not significantly affect the fitness of the fungus on cultivated tomatoes, suggesting that Avrs mostly represent virulence factors with minor effects (De Wit 2002; Westerink et al. 2004a).

The purpose of this work was to isolate the organisms causing typical symptoms of leaf mold disease in tomato and identify the races of *P. fulva* from genomic DNA either of the organisms or leaf lesions. Five isolates were identified as *Passalora fulva* based on the ITS sequence. One isolate was identified as race "0", which carried *avr2*, *avr4*, *avr4E* and *avr 9* while the rest of the isolates were identified as race 2 that lacks *avr2*. These isolates provoked disease on tomato cv Money Maker, race 0 (MMCf-0) and the remaining isolates on both cultivars MMCF-0 and MM Cf-2.

MATERIALS AND METHODS

Genomic DNA of fungi was isolated by means of the CTAB method and quantified by comparison with a Lambda *Hind*III marker (Invitrogen), after electrophoresis in a 0.7% agarose gel stained with ethidium bromide.
Genomic DNA was mixed with primers ITS-4 (5`-AAGCTTTCCTCCGCTTATTGATATGC-3`) and ITS-5 (5`-GAATTCGGAAGTAAAAGTCGTAACAAGG-3`) to amplify the 3` end of the 18S rDNA, ITS1, 5.8S rDNA, ITS2 and the 5` end of the 28S rDNA
The primers used in the amplification of genes avr were essentially those described by Stergiopoulos (Stergiopoulos et al 2007) which were modified since they lack the universal MP13 sequence and had an *Eco* RI and *Hind*III sequence at the 5' end of the forward primer and 3'end of the reverse primer, respectively.
The amplified DNA fragments were precipitated by adding 10% of a 3M NaAc solution and 1 volume of isopropilic alcohol. The DNA was sequenced by the dideoxy termination method using the BigDye Terminator Cycle Sequencing Ready Reaction kit and the automated ABI Prism 3730 DNA sequencer (Applied Biosystems). The sequences of the ITS and the *avr* genes were deposited in GenBank.

Isolates	**Site of Isolation**	**Tomato cv**	**Reference**

ALH	Los Hornos	Elpida	This work
ELH	Los Hornos	Elpida	This work
EMP	Los Hornos	Elpida	This work
CoA	Arana	Colibri	This work
CH6	Corrientes	Elpida	This work

Results

P. fulva isolates only two races were found; one that carried *avr2*, *avr4*, *avr4E* and *avr9*, and the other one that carried *avr*4, *avr*4E and *avr*9 (Fig 1). Interestingly, we found that the avr gene sequences were polymorphic between them and also with those sequences available at the ncbi database that corresponded to *P. fulva* representatives isolated in Europe.

1) En el caso de avr 2 no se observa polimorfismo.

2) En el caso de avr4 se encontraron todo tipo de mutaciones en la secuencia analizada.

Isolate	transition	transvertion	deletion	insertion
ALH	2	1	ND	G
ELH	1	6	ND	ND
EMP	1	4	ND	A
CH6	3	1	ND	2x G
CoA	2	1	-A	ND

3) En el caso de avr4E se observan transiciones y transversiones, pero no deleciones ni inserciones. En el cuadro se indica el número de eventos encontrados en la secuencia obtenida.

Isolate	AVR4E	
	transition	transvertion
ALH	3	3
ELH	3	2
EMP	3	-
CH6	4	2
CoA	4	-

4) En el caso de avr9 solo se encontró una transversión en el aislamiento CoA en el extremo 3´ (G x T)

Conclusión

Stergiopoulos, De Kock, Lindhout,and De Wit 2007. Allelic Variation in the Effector Genes of the Tomato Pathogen Cladosporium fulvum RevealsDifferent Modes of Adaptive Evolution. The American Phytopathological Society. MPMI Vol. 20, No. 10, 2007, pp. 1271–1283. doi:10.1094 /MPMI -20-10-1271.

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