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Original article

Physiological and molecular characterization of *Phytophthora infestans* isolates from the Central Colombian Andean Region☆

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ABSTRACT

Background: Late blight, caused by *Phytophthora infestans*, is one of the most devastating diseases found in potato and tomato crops worldwide. In Colombia it also attacks other important crops: cape gooseberry and tree tomato. The knowledge of the pathogen population is determinant to effectively design control strategies.
Aims: To determine the physiological and molecular characteristics of a set of Colombian *P. infestans* isolates.
Methods: Strains isolated from Cundinamarca and Boyacá were examined for the level of resistance to mefenoxam and cymoxanil. Virulence was tested for all strains and crosses between A1 mating type, from different hosts, and the Colombian A2 mating type were tested for the production and viability of oospores in different substrates. Additionally, the molecular diversity of the avirulence gene *Avr3a*, the β-tubulin gene, and two single copy genes showing RxLR motif, was assessed.
Results: We found all levels of mefenoxam sensitivity, with 48% of the strains resistant. A high diversity of races was detected and the population was genetically clonal. Colombian strains had the possibility of sexual reproduction.
Conclusions: These results will help in optimizing the use of fungicides and deployment of resistance as control strategies and will contribute to broader studies on diversity of this pathogen.

Caracterización fisiológica y molecular de aislamientos de *Phytophthora infestans* de la región andina central colombiana

Palabras clave:
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Ridomil gold
Avr3a
β-tubulin
Razas
Motivo RxLR

RESUMEN

Antecedentes: El tizón tardío, causado por *Phytophthora infestans*, es una enfermedad devastadora de la papa y el tomate a nivel mundial, y en Colombia también ataca otros cultivos como la uchuva y el tomate de árbol. El conocimiento de la población del patógeno es determinante para el diseño efectivo de estrategias de control.
Objetivos: Determinar las características fisiológicas y moleculares de aislamientos colombianos de *P. infestans*.
Métodos: El nivel de resistencia al mefenoxam y al cimoxamil fue evaluado en aislamientos de Cundinamarca y Boyacá. Se estimó su virulencia y se determinó la producción y viabilidad de oosporas en diferentes sustratos con cruces entre aislamientos A1 y el aislamiento colombiano A2. Además, se determinó la diversidad molecular en el gen de avirulencia *Avr3a*, el gen de la β-tubulina y otros dos genes de copia única con motivo RXLR.
Resultados: Los aislamientos colombianos tuvieron la posibilidad de reproducirse sexualmente. Encontramos todos los niveles de sensibilidad al mefenoxam, con el 48% de los aislamientos resistentes. Se detectó una diversidad de razas y a nivel genético la población fue clonal.

☆ GenBank accession numbers: *Avr3a* (JN849402–JN849407), *β-tubulin* (JN849430–JN849450), and *SC9* (JN849408–JN849429).

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Conclusiones: Estos resultados ayudarán a optimizar el uso de fungicidas y reducir la resistencia como estrategias de control, además de contribuir al conocimiento de la diversidad de este patógeno.

The knowledge of pathogen population structure and changes that these populations can experience over time is crucial for developing durable crop disease management strategies.[14] Due to variability of conditions in each country, region and even within each field, detailed local population studies must be carried out. This is especially true when disease control measures at a certain time and location are not effective or when they represent an economical or environmental burden.

Late blight is one of the most devastating plant diseases demanding a high chemical input for disease control worldwide.[11] This disease, caused by the oomycete *Phytophtora infestans*, affects a wide range of solanaceous plants. In Colombia, cultivated solanaceous plants such as *Solanum tuberosum* (potato), *Solanum phureja* (yellow potato), *Solanum lycopersicum* (tomato), *Solanum betaceum* (tree tomato), *Solanum quitoense* (lulo) and *Physalis peruviana* (cape gooseberry) are *P. infestans* hosts.[34] Optimal conditions for both pathogen and disease development, of particular importance temperature and humidity, are found everywhere potatoes are cultivated in Colombia. In addition, main potato cultivars grown in the regions Diacol Capiro, Parda Pastusa, Tuquerreña and criolla are highly susceptible to the disease.[12] Recently, severe epidemics have been reported in lulo and tree tomato crops.[37]

Chemical applications are the preferred strategy to control late blight,[5] and mixtures of systemic and protective fungicides are the most widely used.[27] Two molecules with systemic activity usually incorporated in fungicide mixtures are mefenoxam, the most active enantiomer contained in the fungicide Ridomil Gold™, which inhibits ribosomal RNA synthesis, and cymoxanil whose mode of action is still unknown.[27] Mefenoxam (also known as metalaxyl-M) is comprised almost solely of the *R* enantiomer of the molecule, while metalaxyl is a mixture composed of approximately equal proportions of the *R* and *S* enantiomers. Metalaxyl resistance has evolved rapidly ever since its introduction in 1980, with resistance being detected first in Ireland and The Netherlands.[6] Since this first report, several studies worldwide were conducted to explain the genetic resistance to metalaxyl,[8,20,21] the presence of resistant strains in the populations and the effect of numerous chemicals that may aid in reducing the spread of the disease.[15,22,29] Resistance values for this fungicide have risen up to 100 mg/l in countries where this fungicide is commonly used.[30] Although *P. infestans* has been thought to be sensitive to cymoxanil, Grünwald et al. showed that in Mexico some isolates exhibited resistance and a selection pressure directed to resistance was observed.[18] The least sensitive strain reported was one isolate from Israel, which showed an EC_{90} value of 467 mg/l. In other countries such as Mexico, The Netherlands and Ireland these values ranged between 64 and 152 mg/l for the least sensitive strains.[31]

In Colombia pathogen populations are dominated by the EC-1 clonal lineage, and a couple of isolates were determined to be variants of this clonal lineage (*e.g.* EC1.1) and the clonal lineage, CO1. Mating type A1 and mitochondrial haplotype IIa were the most commonly found in a previous study and isolates can be recovered from a wide diversity of hosts.[35] A Colombian isolate from cape gooseberry was recently characterized as US-8, mitochondrial haplotype Ia and mating type A2, indicating the possibility of sexual reproduction in Colombia.[30,35]

As a consequence, we established the potential of producing viable sexual progeny by making crosses between isolates classified as A1 mating type from different hosts and the Colombian A2 mating type from cape gooseberry. In addition, to further characterize a sample of Colombian *P. infestans* isolates gathered from the Central Andean region, we established their fungicide baseline sensitivity to two molecules (mefenoxam and cymoxanil) and we characterized their molecular diversity in the avirulence gene *Avr3a*,[1] the β-tubulin gene and two single copy potential RxLR effector genes.[36] Pathogen effectors are thought to co-evolve in an arms race with corresponding host resistance genes. Because effectors are important determinants in virulence and pathogenicity, we investigated selected gene sequences looking for polymorphisms, which may help to explain host virulence, indicate co-evolution or be used as potential markers for clonally reproducing populations of this pathogen.

Materials and methods

Isolate collection and maintenance

Twenty-five isolates were obtained from the *P. infestans* culture collection maintained in the Laboratory of Mycology and Plant Pathology at Universidad de los Andes (LAMFU), 21 from Colombia and two from Venezuela. Isolates mating type, mitochondrial haplotypes and genotypes for several microsatellites have been previously reported.[35] Colombian isolates were chosen with the aim of selecting the most diverse ones in the central states of Boyacá and Cundinamarca, based on the characteristics mentioned above (Table 1). Isolates US940480 and US940494 were provided by William E. Fry (Cornell University) and were used as reference strains. Isolates were maintained in rye A agar at 14 °C.[12]

Mefenoxam and cymoxanil sensitivity tests

The sensitivity of *P. infestans* isolates to the phenylamide fungicide mefenoxam as technical grade (90%) was evaluated using the radial growth assay method.[17] Each isolate was transferred to rye B agar medium amended with mefenoxam at 0.5 and 100 mg/l. One plug of 5 mm in diameter was placed on the center of each plate. Mycelial radial growth in each plate was recorded 7 days after inoculation. The relative growth of each isolate growing in fungicide-amended media was obtained by subtracting the plug diameter from the diameter of the colony (after 7 days), and then dividing the corrected radial growth of the isolate growing in the fungicide-amended media by the radial growth of the same isolate growing in media without fungicide. Colony diameters were determined as the average diameter between two measured diameters. One of those diameters was determined by measuring one random diameter, and then a second diameter that was perpendicular to the first. Isolates were scored as resistant when radial growth at 5 and 100 mg/l was more than 40% of the growth without mefenoxam, as intermediate when the radial growth was more than 40% at 5 mg/l but less than 40% with 100 mg/l and as susceptible when radial growth was less than 40% in both 5 and 100 mg/l.[10] These concentrations and ranges were selected because of the good resolution they provide to distinguish differences between the different levels of metalaxyl sensitivity in previous studies.[21–23] Three replicates were performed per concentration for each isolate, and the experiment was repeated twice. In addition, sensitivity to commercial products that contain mancozeb besides the systemic molecule Ridomil Gold (ai = Mefenoxam) (Syngenta, NC, USA)