III International Late Blight Conference

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Editors	G.A. Forbes, F. Govers, W.E. Fry
Publication date	25 June 2009
ISBN	978-90-66055-72-8
ISSN	0567-7572
Number of articles	24
Volumes	1
Place	Beijing, China

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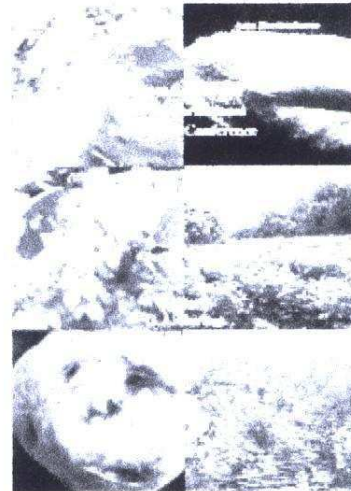
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Population Structure of *Phytophthora infestans* in the Venezuelan Andes (2004-2007)

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Abstract

During the years 2004-2007, samples of *Phytophthora infestans* were collected from 84 potato farms in the Andean states of Mérida, Táchira and Trujillo, the main potato producing region of Venezuela. At least one sample from each of 84 fields (production units) was morphologically and molecularly characterized using standard methods for mating type (PCR and mating with reference strains), mitochondrial haplotype and a few microsatellites. Some isolates were also tested for metalaxyl resistance. A subset (12) was also analyzed for sequence variability of nuclear and mitochondrial genes (β-tubulin, intron Ras, ras, *cox1*, and ITS45). Data on potato variety, use of fungicides and disease management were also collected. This assessment of the *P. infestans* population in Venezuela indicates a high degree of homogeneity. All but one (self fertile) isolate were of the A1 mating type. All but one isolate were of the Ia mitochondrial haplotype. The exception, an IIa haplotype, also showed differences in terms of the SSR loci. The *P. infestans* population from Venezuela seems to be composed of few lineages apparently not present in Colombia. Reasons that may explain the isolation of the *P. infestans* population in Venezuela are discussed.

INTRODUCTION

In a regular meeting of the Caracas Society of Physics and Natural Sciences, held in 1874, A. Ernst showed members illustrations made by Ahles of the causal agent of "the potato disease", *Peronospora infestans* (Banco Central de Venezuela, 1968). Later that year, he also informed the Society's members on the discovery made by W.G. Smith of the sexual spores produced by what we know now as *Phytophthora infestans*. While we cannot ascertain when *P. infestans* was recorded for the first time in Venezuela, it is evident that the disease "candelilla tardía" (late blight) was well known in potato producing areas of the country in the 1860s (Ernst, 1867). For instance, in a short publication aimed at promoting under-exploited crops, Miesse and Peraza (1913), when recommending the improvement of potato cultivation, state that "The cultivation of potato in Venezuela is not new but the cultivation of good class potatoes is unknown here". One of the reasons given to support that assertion was that the potatoes grown in the country suffered heavily from damage inflicted by the "potato disease", caused by *P. infestans*. Later, in 1930, Martínez Mendoza reinforces this notion, but surprisingly considers the presence of the pathogen as a consequence and not the cause of the potato disease. Chardon and Toro (1934), after a series of extensive journeys to different parts of Venezuela in 1932, reported the presence of *P. infestans* on potato and tomato in the central states but not in the Andes, although they visited the most important potato producing areas in this region. However, in a thesis published in 1929, P.J. Paredes, a student at Universidad de Los Andes (Mérida) had already described the disease, which

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Proc. IIIrd Internat. Late Blight Conference
Eds.: G.A. Forbes et al.
Acta Hort. 834, ISHS 2009

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he stated was caused by *Botrytis infestans* (Verdun de Lille), on the potatoes grown in El Valle, a locality of Mérida state (Paredes, 1929). Finally, it was a phytopathologist, Albert Müller (a disciple of Whetzel, from Cornell University), who published the first scientific report (1939) describing *P. infestans* as an important factor affecting potato production in Venezuela, and made this known to a wider and specialized audience. These references illustrate the fact that the presence of this disease in Venezuela has been recognized for over 140 years. Studies on the pathogen population over this time, however, have been limited to mating type (presence of the A1 mating type identified by Rodríguez, 2001), reports of different races (Aponte and Jiménez, 1987), or recommendations on the use of fungicides for the control of potato late blight (reviewed by García, 2002). The disease and its chemical control, more than the pathogen, have been the focus of attention of Venezuelan phytopathologists, including a few instances in which resistance was pursued, mainly by assaying the progeny of selected crosses made at the International Potato Center (CIP) (García et al., 1995; León et al., 1995; Rodríguez et al., 2008). A systematic study of the pathogen population in the country, however, has never been conducted.

Potato is one of Venezuela's 10 top commercial crops. Historically, it was produced in the Andean states and in the mountainous regions of the central states of Lara, Aragua, Monagas and Carabobo. In the past, potatoes were also cultivated on a very small scale in Miranda and Falcón. Currently, however, the Andean states of Mérida, Táchira and Trujillo account for more than 75% of the gross national production. The Andean region also reports the highest levels of productivity, but this relies on the excessive use of fungicides to control late blight, which has been recognized since the 1940s as the main problem associated with the commercial cultivation of potato. Although potato, along with maize and cucurbits, among other crops, was cultivated in the Venezuelan Andes at the arrival of Spaniards, it never had the cultural and economic importance that still characterizes other Andean countries. With time, other crops dominated the agricultural landscape of Venezuelan Andes: wheat, maize and potato in the highlands, and sugarcane, maize, tobacco and cacao in the warmer lowlands. Three factors determined that potato, now the most important crop of the Venezuelan Andes, revived during the XXth century. First, immigration from the Canary Islands increased. The migrants brought with them cultural practices associated with potato production, and possibly germplasm from the islands. In fact, it has been reported that potatoes exports from the islands to Venezuela had been common practice since the first half of the XIXth century, reflected in similarities in the vernacular names of the native potatoes in the islands and the Venezuelan Andes (e.g., "negra", "yema de huevo", etc.). Second, the increased use of imported certified potato seed, made economically possible by oil revenues and put in practice from the beginning of the XXth century, was part of a state policy aimed at diversifying agroproduction in the Andean states. Lastly, there has been a sustained increase in production and yield during the last years of the XXth century and beginning of the XXIth (FAO, 2008).

In this work we report on the structure of the *P. infestans* population in the Andean states of Venezuela as an initial step to contribute to a better understanding of an elusive pathogen that is gaining more, and well deserved, attention in Colombia and Venezuela.

MATERIALS AND METHODS

Sampling
During the years 2004-2007, *P. infestans* was isolated from potato producing areas in the Venezuelan highlands (the main potato producing zone in the country). Our survey included the Andean states of Mérida, Táchira and Trujillo, where potato is grown in mountainous areas above 2000 m asl (Fig. 1). Samples were taken from small production units (almost none bigger than one hectare). Ten infected leaflets (with abundant sporangia present on the abaxial surface) were collected per unit, one leaflet per plant, with individual plants at least 10 m apart. Thus, there was a total of 10 leaflets (= 10

lesions) per production unit. 84 production units were sampled in this way. Leaflets were kept in plastic bags and brought to the lab where, almost invariably, samples were processed no later than two days after collection. Collections were made early in the morning, mainly during the rainy season (April to October), with temperatures at time of sampling between 10 and 20°C.

Medium

M3 medium (Cedeño et al., 2008) was used routinely for all pathogen manipulations. This medium was prepared using a mixture of oat (30 g), processed chickpea meal (20 g) and processed broad bean meal (20 g) cooked in 1 L of distilled water with constant stirring for 30 min; the mixture was filtered through a four-layer cheesecloth, after which 20 g dextrose, 20 g peptone and 15 g agar previously dissolved in water was added and completed with distilled water to a 1 L final volume. The mixture was sterilized by autoclaving at 121°C and 15 psi for 15 min M3, amended with antibiotics and fungicides (see below), was the selective medium used for isolating samples from the field.

Isolation and Purification

Five discs of 4 mm were taken from each individual leaflets showing necrosis on the adaxial side and sporangia on the abaxial side, and cut to include dead and living tissue. Discs were surface sterilized by immersion in an aqueous sodium hypochlorite (0.25%) solution for 60 seconds. After washing in abundant sterile distilled water for one minute, leaflet discs were aseptically transferred to M3 medium supplemented with nistatin (19 mg/ml), Benomyl™ (5 mg/ml), Rifampicin (20 mg/ml), parachlorinenitrobenzene (50 mg/ml), ampicillin (200 mg/ml) and vancomycin (100 mg/ml). Each set of 5 discs, representing 1 lesion from a single leaflet, was incubated in an individual Petri dish in the dark at 16°C for 15 days. Agar with hyphae growing from the discs was transferred to sterile Petri dishes to secure one isolate per lesion. Every isolate was observed under the microscope following a basic lacto-fuchsine staining to check for the absence of septa and purity of every sample. For long term storage, all samples of the collection are being maintained at -80°C in 15% dimethyl sulfoxide (DMSO).

DNA Isolation, Primers, PCR Amplification and Sequencing

DNA was isolated using two different methods: in the first one, 100 mg lyophilized mycelium (grown on M3 agar in Petri dishes) was mixed with carborundum (1:1) and ground to a fine dust in the presence of liquid nitrogen. After mixing with 600 μl of extraction buffer (Tris-HCl 0.22 M pH 8.0, EDTA 22 mM, NaCl 800 mM and CTAB 0.8%), 500 μl of chloroform was added to the lysate, mixed, incubated at 58°C for 30 min and the phases separated by centrifugation (Fulton et al., 1995). The aqueous phase was collected in a new tube and mixed with 1.3 volumes of isopropanol, mixed by inversion, incubated at -20°C for one hour and centrifuged at 16,000 rcf for five min. The pellet was washed with 70% ethanol, air-dried and re-suspended in 50 μl of distilled sterile water in the presence of ribonuclease A. In a second method, a disc of 2 cm of mycelium (grown on M3 agar in Petri dishes) was aseptically cut and processed in a microcentrifuge tube with 70 μl of lysis buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.5, 1 mM EDTA and 1% SDS), and ground until a homogenate was produced. One volume of chloroform was added and mixed thoroughly, after which both phases were separated by centrifugation. The aqueous phase was mixed with one volume of isopropanol, incubated for at least five min and centrifuged, after which the DNA pellet was washed with 70% ethanol. The air-dried DNA was re-suspended in 20 μl of sterile distilled water. For both methods, integrity and concentration of DNA was checked by agar gel electrophoresis. Different sets of primers were used to amplify diverse regions of the *P. infestans* nuclear and mitochondrial genomes. Primer sequences for mating type have been described by Judelson (1996), primers for microsatellites by Knapova et al. (2001),

and primers for mitochondrial haplotype by Griffith and Shaw (1998). Amplification conditions were as indicated in the original articles cited above. In most cases, PCR products were gel-purified and sent for sequencing to commercial providers (MWG Biotech, NC) in order to check the identity of the sequence used for analysis.

Mitochondrial Haplotype and Microsatellites

An amplification product of the *P. infestans* mitochondrial genome from nucleotides 13619 to 14667 was obtained and digested according to Griffith and Shaw (1998), its size confirmed by gel electrophoresis, and gel purified and sequenced. After checking the identity of the amplified product by direct sequencing, the described amplification protocol was used on the 84 selected samples. An aliquot of the amplification products was digested with the restriction enzyme *Msp*I, run in agarose gel, and digestion products analyzed and digitally recorded. To double check the product sizes, the digestion mixture was also run in polyacrilamide gels according to standard methods (Sambrook et al., 2001). As controls of the haplotypes Ia and IIa, reference strains 280, 3839 and 3845 from CIP were used.

For microsatellite analysis, DNA obtained from lyophilized samples was used in all experiments with primers Pi4BF, Pi4GF, PiG11 and Pi2H designed by Knapova et al. (2001). Amplification products were run in 15 cm long polyacrilamide gels, and stained with silver salts according to Bassam et al. (1991). All gels were digitally recorded to estimate product sizes. Control strains were the same for haplotype analysis, plus a pair of A1 and A2 reference strains from Europe (see below).

Mating Type

Mating type was analyzed by PCR amplification (Judelson, 1996) and by controlled crosses in vitro using A1 and A2 reference strains (kindly provided by F. Groves). For the biological determination of mating type, the tested and reference isolates were grown in a pea-based medium (Cedeño et al., 2008) for eight days at 16°C. A 5 mm disk of the tested isolate was put 3 cm from one of the two reference isolates onto the surface of a Petri dish containing pea-agar. The test was performed independently for both mating type isolates. Petri dishes were incubated at 16°C for 14 days, after which sexual compatibility was assessed by observing the presence or absence of oospores under an optical microscope (Medina and Platt, 1999; Groves and Ristaino, 2000).

Sequence Analysis

Sequences obtained in this work were compared to those published in the literature or against public databases. When performing alignments, ClustalX2 version 2.0 (Larkin et al., 2007) was used with default parameters, and further analyzed with MEGA version 3.1 (Kumar et al., 2004). Genes that were used in this analysis include β-tubulin, and *cox1* (Griffith and Shaw, 1998; Kroon et al., 2004), ITS (Trout et al., 1997; Hussain et al., 2005), and intron Ras and Ras (Gomez et al., 2003). Primers for the amplification products are described in the aforementioned references and were bought from commercial providers (MWGBiotech, NC). Amplification products were directly sequenced (Operon Technologies) and outputs edited using BioEdit (Hall, 2001).

RESULTS

Sampling and Phenotypic Characterization of the Isolates

All production units that were sampled for this work contributed with at least one single lesion isolate, all collected during the years 2004-2007. Potato production in the sampled units was dominated by very few varieties, most of which are susceptible to *P. infestans* (e.g., "Granola", Table 1). Not surprisingly, heavy applications of chemicals were recorded for potato production in the Venezuelan Andes (García, 2002), a fact that was corroborated throughout the sampling period, where we found that no less than 7 applications of a mixture of fungicides per season seems to be the rule. It was exceptional

to find a disease-free field, but, on the other hand, very few production units were severely affected to the point of crop loss. In general, it can be said that the potato grower of the Venezuelan Andes deals with late blight by relying almost exclusively on the (excessive) frequent use of fungicides, instead of using resistant varieties. Recovery of pathogen isolates from the field samples was good, as more than 500 isolates were recovered from the approximately 840 leaflets collected (10 per 84 production units). All isolates were observed under the microscope, and 84 (one per production unit) were selected for additional characterization.

All 84 selected samples were subjected to mating with reference strains of *P. infestans*. All except one were of the A1 mating type. The exception was a self-fertile isolate that produced oospores spontaneously under almost any circumstance and medium (isolate "Santa Rosa"). No A2 types were found after our extensive travels (more than 4000 km) sampling the main producing areas of potato in the Venezuelan Andes. However, no other host than potato was considered for this analysis. The "Santa Rosa" isolate produced oospores with the reference A1 more profusely than with the referential strain A2. Interestingly, when crossed with the A2 reference isolate, this particular isolate produced fewer oospores than in single culture.

Several isolates were tested for sensitivity to metalaxyl, and none were able to grow in the presence of high concentrations of the fungicide. We did not proceed further with this analysis since we had to rely on the use of commercial formulations of metalaxyl, instead of the pure formula, which is not available in the country and extremely difficult to import.

Molecular Assays

To fully characterize the collection of 84 isolates representing the sampled units, diverse molecular markers were used, with different purposes. In all cases, molecular markers that were isolated and sent for sequencing, confirmed the identity of the pathogen under scrutiny. Primers for mating type, however, proved to be unreliable and tended to give false positive results for the mating type A2. All possible candidates for the A2 type identified by the amplification assay were also tested biologically using reference strains and shown to be phenotypically A1.

The analysis of mitochondrial haplotype revealed that all 84 tested isolates were of the lineage Ia, with the exception of one from Táchira state ("Las Porqueras") which was IIa (Fig. 2). Isolates were screened for variability at four microsatellite loci. Three of these proved to be monomorphic; and one (Pi4GF) polymorphic, but with only one isolate differing from the others. This variant isolate was also the only IIa isolate of the collection (i.e., "Las Porqueras"). Interestingly, when all ten isolates of "Las Porqueras" collection site were assayed for mitochondrial haplotype and SSRs, the other nine isolates were found to conform to the majority of the 84-collection; the first isolate described from "Las Porqueras" was unique.

Gene Sequence Analysis

Since, contrary to our expectations, the population of *P. infestans* analyzed with the markers already described yielded no evidence of variability (except for the single IIa isolate), diverse genes were amplified and sequenced in order to find differences at the nucleotide level. To attain this goal, genes with different cellular locations, rate of mutation and usability to detect differences at diverse hierarchical levels were used. Regarding ITS, no single nucleotide difference was found among the analyzed sequences, which were found to be identical to numerous of the *P. infestans* isolates sequences reported for Colombia by Vargas et al. (2009), although information on the specific host is not provided in that study. The ITS sequences from our samples are also identical to the corresponding sequences reported for *P. andina*, from a non identified solanaceous host (AY770741), and *P. mirabilis* from *Mirabilis jalapa* (EU257523). The ITS sequences are generally the same for isolates of *P. infestans* and the closely related species, *P. andina* and *P. mirabilis*. Therefore, our results corroborate the species identity of our isolates.

No variability was seen among isolates for the β-tubulin gene sequence, which was identical to that of the reference isolate PD_00102 (P10650), EU079626, of Blair et al. (2008), from potato in Mexico, and 98% similar to *P. andina* (from *Solanum brevifolium* of Ecuador).

Likewise, the Intron Ras sequence was invariable among the samples and identical or 99% similar to sequences from other *P. infestans* isolates. This sequence was also 94% similar to the *P. andina* (DQ864574) corresponding sequence. Regarding Ras, all sequences analyzed were identical among themselves and to sequences from many different isolates of *P. infestans*. No equivalent sequences are reported for *P. andina*.

A single, corroborated nucleotide difference was found between the *cox1* gene sequence of isolate "Las Porqueras" and the rest of the Venezuelan isolates analyzed. The former showed identity to the sequence from isolates of the haplotype IIa (EF366793 and others) and IIb, and a 99% similarity to isolates of haplotypes Ia (AF348600) and Ib. In contrast, the rest of the isolates (all Ia) were identical to another set of *P. infestans* isolates of haplotype Ia, and Ib (EF366781 and others), and 99% similar to IIa (EF366793 and others) isolates. Both showed a 98% similarity to the sequences of *P. andina* (AY564160) and other *Phytophthora* species.

DISCUSSION

Contrary to our expectations of finding a high degree of variability of *P. infestans* in Venezuela, we have found a homogeneous, and more importantly, apparently isolated population of the pathogen when compared to neighboring countries. Our expectations arose from the fact that we assumed that legal and illegal introduction of potato for seed and fresh consumption from neighboring countries might introduce inoculum of the oomycete. Traditionally, commercial potato production in Venezuela has relied on the use of imported seed. Even at the end of the XIXth century we found reports of potato growing in Táchira State reportedly of the "American potato" type, which was further corroborated by Pittier when he confirmed the US origin of most of the seeds used for the commercial production of potato in Venezuela (Pittier, 1920). Later, although the notion persisted that imported seeds were the better option for increasing potato production, some interest arose in finding areas where seed could be produced. This did not happen until the 1960s, however, probably because of the high incidence of late blight in the areas more appropriate for potato seed production, as reported by many phytopathologists in the country during the XXth century. Even today, seed production is negligible in Venezuela. None of the local Andean varieties, on the other hand, have been successful in competing with "Granola" and "Única", which dominate the market and which are distributed as certified seed. The single fact that only certified seed is used might explain why we were unable to find *P. infestans* isolates resembling those of the countries where the seed originates. Moreover, the fact that growers can keep the pathogen under control economically using chemicals means that there is little pressure to test the regional blight-resistant potato varieties. Producers in the Andean states prefer to play safe and stick to known varieties that are accepted by the market and can be easily transported without sprouting or changing color.

It has been argued (Adler et al., 2002) that the predominant (or at least, known) *P. infestans* in Venezuela belongs to the IIa mitochondrial lineage. This assumption is based on a reference to an article by Perez et al. (2001) where a personal communication supports the claim that the EC-1 lineage is present in Venezuela. Our results are clear in demonstrating the overwhelming dominance of the Ia mitochondrial lineage, combined with the A1 mating type. Only one isolate out of the 84 analyzed had the IIa haplotype (typical of EC-1), and this was unique among the ten samples from the same site. Sequence analysis of various genes, particularly the *cox1* gene, further corroborates that there is a single predominant clone in the Venezuelan Andes, which has the haplotype Ia. It is possible that the isolates from Venezuela analyzed by Perez (2001) came from the only place where the haplotype IIa is found in Venezuela; that is, Táchira state, close to the Colombian border. Another possibility is that the IIa population has been recently

displaced by the Ia, but the historical data needed to examine this hypothesis is lacking. More isolates from Táchira are now being analyzed, and a more extensive area sampled.

The haplotype IIa is the predominant lineage present in Colombia, where the A1 mating type also dominates, although A2 has also been reported (Vargas et al., 2008). In another neighboring country, Brazil, on the other hand, the population of *P. infestans* is characterized by an equal presence of A1 and A2 isolates, and mostly of the IIa lineage (Suassuna et al., 2004). Brazil and Venezuela are connected by only one paved road that runs through a tropical rain forest, very far from the Andes, and we are unaware of any significant quantity of seed being imported from Brazil. It is thus likely that these two populations have very little contact with each other. The situation regarding Colombia requires further examination (Vargas et al., 2007), however, and three aspects are worth noting when trying to explain why the populations of *P. infestans* in both countries are different. First, despite the weighty presence of Colombians in Venezuela (ca. 4 million in a country of 28 million), each country has its preferred potato varieties. This shows that Colombians have not influenced the production and taste for particular varieties of potatoes grown and consumed in Venezuela. In other words, a cultural barrier exists between these countries regarding patterns of potato production and consumption. Second, a huge area between Táchira State and Central Colombia has not been sampled to see if populations of the pathogen start to mingle. Interestingly, however, the only IIa isolate of the Venezuelan *P. infestans* population was sampled less than 60 km from the border between the two countries, and IIa is the haplotype dominating the characterized isolates of *P. infestans* in Colombia. Third, our collection only includes isolates from potato, since in the Andean region of Venezuela this is the only solanaceous species grown commercially. Tomato, for example, is mostly cultivated, in the Andean states of Venezuela, in lower, warmer and drier regions, far from potato growing areas. In Colombia, it is easy to find tomato, tree tomato and uchuva (*Physalis peruviana*) close to potato fields. The larger diversity of potential hosts in Colombia may therefore influence the diversity of the pathogen population in that country, even that part of the population associated with potato.

In summary, the Venezuelan population of *P. infestans* is composed almost solely of a single lineage, of mitochondrial haplotype (Ia), and only one mating type (A1) with a very low variability, as demonstrated by microsatellites and sequence analysis of diverse genes. One isolate of this lineage varied in the sense that it was self-fertile ("Santa Rosa" isolate), and only one isolate (the IIa isolate) differed from the bulk of the (Ia) isolates. In order to shed light on the reasons why this population is different from our neighboring country Colombia, a thorough sampling of the bordering regions of both countries is necessary, including a larger number of genes and more hosts of the pathogen. A new study with these sampling criteria is already under way.

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Table 1. Summary of results for the sampling of *Phytophthora infestans* in the Andean states of Venezuela (2004-2007).

| | State | | | | | | Totals (%) | |
| | Mérida | | Táchira | | Trujillo | | | |
Variety	Samples	Units	Samples	Units	Samples	Units	Samples	Units
Granola	170	24	24	6	16	2	210 (37.2)	32 (38.1)
Única	82	14	15	2	12	2	109 (19.3)	18 (21.4)
R12	48	5	8	1			56 (9.9)	6 (7.1)
Roja	26	3					26 (4.6)	3 (3.6)
Caribay	22	3					22 (3.9)	3 (3.6)
Andina	8	1					8 (1.4)	1 (1.2)
Idrafvin	8	1					8 (1.4)	1 (1.2)
Andinita	7	1					7 (1.2)	1 (1.2)
Sin nombre	7	1					7 (1.2)	1 (1.2)
Tibisay	5	1					5 (0.9)	1 (1.2)
Merengue	9	1					9 (1.6)	1 (1.2)
Revoltijo			5	1			5 (0.9)	1 (1.2)
Reinosa	4	1					4 (0.7)	1 (1.2)
Unknown	35	5	53	9			88 (15.6)	14 (16.7)
Totals (%)	431 (76.4)	61 (72.6)	105 (18.6)	19 (22.6)	28 (5.0)	4 (4.8)	564	84

Table 2. Summary of consolidated results for the characterization of 12 different geographical isolates of *Phytophthora infestans* from the Venezuelan Andes.

Isolate	Geographical origin	Haplotype	Mating type A1/A2?[1]	Microsatellites			Variety	Genes				
				SSR1	SSR2	SSR3		1	2	3	4	5
V1	Santa Rosa, Mérida	Ia	A1/A2	=	=	=	Granola	=	=	=	=	=
V2	Las Porqueras, Táchira	IIa	A1	=	=	≠	Sin nombre	=	=	=	=	=
V3	El Valle, Mérida	Ia	A1	=	=	=	R12	=	≠	≠	=	=
V4	Pueblo Llano, Mérida	Ia	A1	=	=	=	Granola	=	=	=	=	=
V5	Pueblo Llano, Mérida	Ia	A1	=	=	=	Única	=	=	=	=	=
V6	Pueblo Llano, Mérida	Ia	A1	=	=	=	Granola	=	=	=	=	=
V7	El Cobre, Táchira	Ia	A1	=	=	=	Granola	=	=	=	=	=
V8	Valle del Chama, Mérida	Ia	A1	=	=	=	Granola	=	=	=	=	=
V9	Valle del Mocoties, Mérida	Ia	A1	=	=	=	Granola	=	=	=	=	=
V10	Valle del Tuñame, Trujillo	Ia	A1	=	=	=	Unknown	=	=	=	=	=
V11	El Trompillo, Mérida	Ia	A1	=	=	=	Única	=	=	=	=	=
V12	Santo Domingo, Mérida	Ia	A1	=	=	=	Única	=	=	=	=	=

* Genes referred in the table are: 1, β-tubulin; 2, ITS; 3, *cox1*; 4, Ras; 5, Intron Ras.
[1] This isolate is self fertile but produces more oogonia when mated to an A1 tester
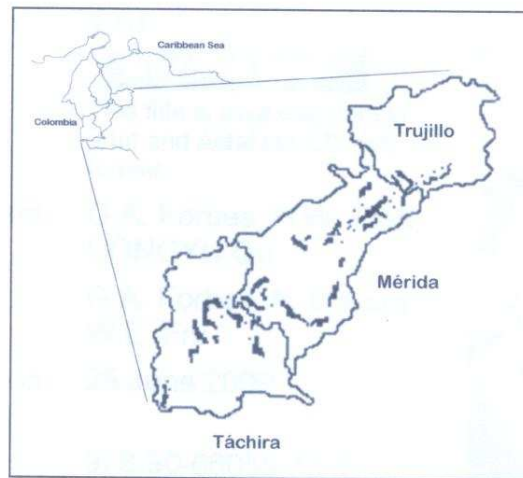
Figures

Fig. 1. Schematic representation of the main sampling localities in the potato producing areas of the Andean states of Táchira, Mérida and Trujillo in Western Venezuela.
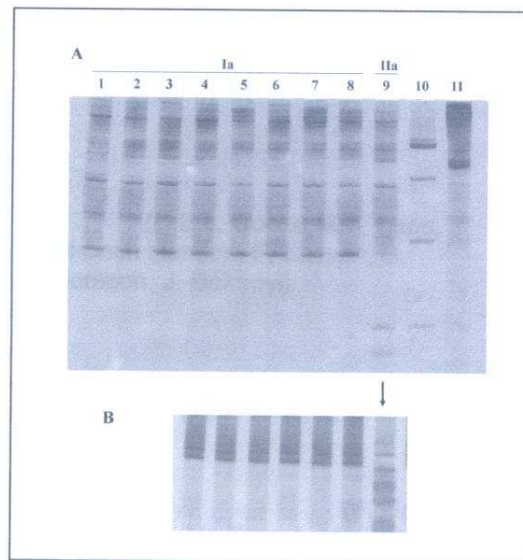

Fig. 2. A. Polyacrylamide gel electrophoresis (PAGE) of the *Msp*I-digested products after amplification of mitochondrial DNA of all geographical isolates of *Phytophthora infestans* from collection site "Las Porqueras". Lanes 1 to 8, isolates Ia from same collection unit; lane 9, isolate IIa; lane 10 molecular ladder (M. E-Gel® Low Range Quantitative DNA Ladder), and lane 11, a non digested amplification product. B. PAGE of amplification products for microsatellite Pi4G from isolates from different geographical origins; an arrow indicates the IIa isolate in lane 9, Fig. 2A.

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