Aggressiveness and Genotyping of 
Phytophthora Infestans Isolates from Nicaragua

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Abstract

The late blight pathogen, P. infestans, is a hemi-biotrophic, heterothallic, diploid oomycete that remains a major constraint for potato and tomato production worldwide and causes economic losses yearly calculated to be over four billion euros [1]. In compatible interactions with potato, the biotrophic phase of P. infestans can last from three to five days, after which macroscopic symptoms are evident (necrotrophic phase). In tomato leaves, an extended period of biotrophy has been observed due to a compatible interaction with tomato-specialized isolates [2-4]. As a heterothallic oomycete, P. infestans possesses two compatibility groups, referred to as mating types A1 and A2, which enables the production of oospores in locations where both A1 and A2 mating types are present contributing in that way to its overwintering survival and shaping the nature of its population structure [5]. In locations where only the asexual cycle is found, P. infestans survives as mycelium in infected potato tubers and debris [6] and also probably in alternate wild hosts.

Prior to the 1980s, worldwide populations of P. infestans were dominated by a single clonal lineage known as the US-1 “old” genotype, having the A1 mating type [7,8]. On the contrary, in the Toluca Valley in central Mexico the A1 and A2 mating types were present in approximately equal frequencies and the populations of P. infestans were entirely different from populations in other locations [5,9]. Since the mid-1980s changes in the population structure of P. infestans outside Mexico have been reported [10]. These changes brought about the displacement of the ‘old’ genotypes by ‘new’ ones, which are characterized by increased fitness and aggressiveness in addition to metalaxyl resistance [11]. Migration has played an important role in the observed changes in global population structure of P. infestans in the latter part of the last century [5]. In some areas where both mating types are present, it has been shown that late blight epidemics start earlier than before due to the presence of oospores as initial inoculum. There are also reports of more aggressive isolates, which has resulted in an increase in the use of fungicides for the disease control [12,13].

Introduction

The late blight pathogen, P. infestans, is a hemi-biotrophic, heterothallic, diploid oomycete that remains a major constraint for potato and tomato production worldwide and causes economic losses yearly calculated to be over four billion euros [1]. In compatible interactions with potato, the biotrophic phase of P. infestans can last from three to five days, after which macroscopic symptoms are evident (necrotrophic phase). In tomato leaves, an extended period of biotrophy has been observed due to a compatible interaction with tomato-specialized isolates [2-4]. As a heterothallic oomycete, P. infestans possesses two compatibility groups, referred to as mating types A1 and A2, which enables the production of oospores in locations where both A1 and A2 mating types are present contributing in that way to its overwintering survival and shaping the nature of its population structure [5]. In locations where only the asexual cycle is found, P. infestans survives as mycelium in infected potato tubers and debris [6] and also probably in alternate wild hosts.

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Suggested management strategies for this disease include use of clean seed, elimination of real and potential sources of inoculum (infected cull piles, volunteer potato plants, and wild alternate hosts), fungicides, Decision Support Systems (DSS), intercropping, cultivar mixtures and extended crop rotations (3-4 years) to avoid early infections developed from oospores. Along with these strategies, the use of resistant cultivars against late blight is of utmost importance, especially in locations where environmental conditions are conducive for disease development and potato growers cannot afford the numerous fungicides required to control the disease [5,12,14-16]. However, the development and use of resistant cultivars may depend on the variability of the pathogen, including isolate aggressiveness. In plant-pathogen interactions, “aggressiveness” or quantitative pathogenicity [17], refers to the ability of a given pathogen to induce severe epidemics on compatible hosts. The quantitative traits frequently used to measure aggressiveness are infection efficiency, latency period, sporulation rate, infectious period and lesion size [18]. Differential interactions for aggressiveness components between current population of \( \text{P. infestans} \) and cultivars have been studied [15,19-21].

The host range of \( \text{P. infestans} \), generally, has been considered to be restricted to two important crops, potato and tomato, and various solanaceous weeds [22], but the factors determining this host range remain unknown [23]. \( \text{P. infestans} \) has been reported affecting various species in the genus \( \text{Solanum} \) and also non solanaceous host plants [24-26]. Some degree of pathogenic specialization of \( \text{P. infestans} \) to potato or tomato has been shown in the early 1900’s and later [2,4,5,27-29]. In USA, for example, the US-8 genotype has been detected to occur on potatoes and the US-7 and US-17 genotypes have been recovered from tomatoes [30]. A study in Kenya and Uganda clearly showed that late blight epidemics in potato and tomato were caused by two separate, host-adapted populations of \( \text{P. infestans} \) [4]. On the contrary, in a recent study carried out in Taiwan, no host specificity on potato or tomato among \( \text{P. infestans} \) isolates from tomato was found [31]. Negative relationships (genetic trade-offs) between qualitative and quantitative traits required to infect one or another host can drive the appearance of pathogenic specialization mediated by antagonistic pleiotropy, in which one or more genes favour pathogen’s performance in one host, but impair its performance in another [18,32,33].

In a previous study conducted with \( \text{P. infestans} \) isolates from Nicaragua, no evidence of population differentiation among isolates collected from potato and tomato plants was found, based on phenotypic (mating type, virulence and fungicide sensitivity) and genotypic analysis [Simple sequence repeats markers (SSRs, also known as microsatellites) and mitochondrial DNA (mtDNA) haplotyping]. Consequently, it was concluded that Nicaraguan population of \( \text{P. infestans} \) belonged to a single clonal lineage which was dominated by the NI-1 genotype. However, in the previous work aggressiveness tests were not conducted, hence, it is not known if there is any difference in aggressiveness between potato and tomato isolates in Nicaragua. Therefore, this study was undertaken to test the following hypotheses: i) \( \text{P. infestans} \) isolates from Nicaragua do not differ in their aggressiveness toward potato or tomato; and ii) \( \text{P. infestans} \) population from Nicaragua is formed exclusively by a single clonal lineage.

**Material and Methods**

**Phytophthora infestans cultures**

Potato and tomato leaflets with a single lesion of \( \text{P. infestans} \) were collected in Nicaragua from July 2009 to January 2010. Tomato fruits with initial symptoms of late blight were collected as well. Samples were taken from commercial fields in the main potato and tomato growing areas of Nicaragua. Infected leaflets with a single lesion were washed with distilled water and dried with filter paper. Thereafter, they were individually placed upper surface down in inverted Petri dishes lined with 1.5% water agar and incubated at 18 °C to promote sporulation. Mycelia and sporangia were then transferred to a pea agar medium [34], amended with antibiotics (0.2g ampicillin and 10mg pimaricin L\(^{-1}\)) and incubated at 18 °C in darkness. One week later, the isolates were transferred to a pea agar medium without antibiotics and incubated at 18 °C for growth and sporulation. Seventy-two isolates (53 from potato and 19 from tomato leaflets and fruits) of \( \text{P. infestans} \) were obtained. These isolates were kept at 12 °C and transferred to rye pea agar [(30g organic rye, 60g frozen green peas, 10g sugar or sucrose, 15g agar and 1L deionised water); [35]] prior to their use in the different tests. All of the 72 isolates were used for SSR analysis, mtDNA haplotyping and mating type determination. A subset of 31 isolates (16 potato isolates and 15 tomato isolates) was used for aggressiveness testing.

**Mating type determination**

Mating type was determined by pairing each unknown isolate individually with known tester isolates of A1 and A2 mating type on rye pea agar. Hyphal interaction zones were microscopically observed after 7-14 days incubation at 18 °C in darkness. Oospores were produced in the margins of opposite mating types. Isolates that produced oospores with the known A1 tester isolates were designated as the A2 mating type and isolates that produced oospores with the known A2 tester isolates were designated as the A1 mating type.

**Plant material and inoculum preparation**

Potato plants (cv. Bintje) and tomato plants (cv. Homestead 24) were grown in 3L plastic pots filled with a soil mixture (pea-sand-clay-lime-compost), in a greenhouse at 18-20 °C (night/day temperature) and 16 h photoperiod. Plants were watered weekly with a fertilizer solution [N(8%)-P(3%)-K(5%)] supplemented with micronutrients. These plants were used to test the pathogenicity of the potato and tomato isolates on their host of origin as well as to produce inocula for the subsequent tests. Detached leaflets of 6-8 weeks old potato plants were individually placed, abaxial surface up, on the lids of inverted Petri dishes lined with 1.5% water agar acting as humid chambers. Sporangia from 2-week-old potato isolates in Petri dishes were collected in distilled water with a paintbrush. Prior to inoculation, sporangial suspensions were kept...
at 4 °C for 2h to release zoospores. Thereafter, each potato leaflet was inoculated with the mixture of sporangia and zoospores. Each leaflet was covered as evenly as possible with 20µl droplets of the sporangia/zoospore suspension to ensure infection. Inoculated potato leaflets were incubated at 16 °C (day/night temperature) and 90% relative humidity. After a week of incubation, sporangia were collected from the infected leaflets with a paintbrush in distilled water and sporangial concentration was adjusted to 2x10^4 sporangia mL^-1 using a hemocytometer. The resulting sporangial suspensions were further used to inoculate potato leaflets and determine the aggressiveness parameters. The same procedure was followed to test the pathogenicity of tomato isolates in tomato leaflets and to obtain the appropriate (pathogenic) inoculum with the abovementioned sporangial concentration.

**Aggressiveness determination**

Sixteen isolates from potato and fifteen isolates from tomato were used in these cross-inoculation assays, i.e., potato leaflets were individually inoculated with potato and tomato isolates and the same was done with tomato leaflets. The inoculum was prepared directly from infected potato and tomato leaflets as described above. Each isolate-host combination was repeated five times (one leaflet of potato or tomato per Petri dish). Potato or tomato leaflets were placed abaxial side up on the lids of inverted Petri dishes lined with 1.5% water agar in the base and inoculated with a 20µl droplet of sporangial suspension adjusted to 2x10^4 sporangia mL^-1 of the appropriate test isolate (potato or tomato). Thereafter, the inoculated leaflets were incubated at 16 °C and 16h day length. The incubation period (IP), latency period (LP), lesion area (LA), lesion growth rate (LGR), spore production (SP), sporulation area (SA) and sporulation rate (SR) were determined as described elsewhere [28,36,37]. An aggressiveness index (A_i) for each isolate-host combination was calculated using the following equation: 

\[ A_i = \ln \left( \frac{LA \times SP \times 1}{LP} \right) \]

\[ \text{(21,38)} \]

**SSR fingerprinting and mtDNA haplotyping**

Microsatellite analysis was carried out using seven loci: Pi4B, PiG11 [39] and Pi16, Pi70, PiD13, Pi63 and Pi04 [40]. Forward primers 4B, Pi16, D13 and Pi04 were labelled with 6-FAM (TAG Copenhagen), whereas G11, Pi70 and Pi63 were labelled with NED (Applied Biosystems). PCR amplifications were performed in 15µL containing approximately 10ng of genomic DNA, 0.2mM dNTPs, 0.4µM of each forward and reverse primers, 0.04U µL^-1 DNA polymerase (Saveen & Werner AB) and 1X reaction buffer Y (containing 2mM MgCl\(_2\)) supplied by the manufacturer. For the primers for locus 4B and Pi70, 4mM MgCl\(_2\) was used. The PCR conditions were as follows: an initial denaturation at 94 °C for 3min, followed by 30 or 33 cycles (dependent on the primers) at 94 °C for 30s, 30s annealing temperature ranging from 50 °C to 62 °C dependent on the primers, elongation at 72 °C for 1min and final extension at 72 °C for 25min (GeneAmp® PCR System 2700, Applied Biosystems). The annealing temperature and the number of cycles for each primer were as follows: 50 °C and 33 cycles for primer D13, 58 °C and 33 cycles for primers Pi4B and Pi70, 60 °C and 33 cycles for Pi63, 60 °C and 30 cycles for Pi63, 60 °C and 33 cycles for Pi16 and 62 °C and 30 cycles for primers PiG11 and Pi04. For dried leaflet samples, the annealing temperature and the number of cycles for primer Pi63 were 58 °C and 33 cycles respectively. Separation of the amplified fragments was done using an ABI 3730xl DNA analyzer at Uppsala Genome Center, Rudbeck Laboratory, Uppsala University in Sweden. The fragment length of the fluorescently labelled fragments was visualized and scored using the software GeneMarker® version 1.6 (Softgenetics). The allele sizes were adjusted to the sizes obtained at SCRI [40]. Mitochondrial DNA (mtDNA) haplotypes of 72 P. infestans isolates were identified by using the method previously described by Griffith [41].

**Data analysis**

In this study the hypothesis that potato and tomato isolates are equally aggressive in both hosts, potato and tomato detached leaflets (H_iµPotato isolates=µTomato isolates) was tested with a significance level of (α=0.05). The effects of the isolate origin-host (potato or tomato) combination on aggressiveness components were tested through ANOVA using the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The mean separation was done using the statement LSMEANS and the differences between the two groups of isolates (potato or tomato origin) were tested by a t-test procedure (Table 1).

**Table 1:** Simple sequence repeat (SSRs) multilocus genotypes detected in Phytophthora infestans isolates from Nicaragua collected from July 2009 to January 2010.

| Gr^[g] | No^[f] | Host | Allele Sizes^[f] Detected with Seven SSR Loci |
|-------|-------|------|---------------------------------------------|
|       |       |      | 4B   | G11 | Pi16 | Pi70 | D13 | Pi63 | Pi04 |
| NI-1  | 63    | P/T  | 205  | 132 | 176  | 192  | 98  | 148  | 166  |
|       |       |      | 213  | 156 | 176  | 192  | 108 | 157  | 170  |
| NI-2  | 2     | P/T  | 205  | 132 | 176  | 192  | 98  | 148  | 166  |
|       |       |      | 213  | 156 | 180  | 192  | 108 | 157  | 170  |
| NI-3  | 2     | P    | 205  | 156 | 176  | 192  | 98  | 148  | 166  |
|       |       |      | 213  | 156 | 176  | 192  | 108 | 157  | 170  |
| NI-4  | 2     | P/T  | 205  | 132 | 176  | 192  | 98  | 148  | 166  |
|       |       |      | 213  | 156 | 176  | 192  | 108 | 157  | 170  |
| NI-5  | 3     | P    | 205  | 132 | 176  | 192  | 98  | 148  | 166  |
|       |       |      | 213  | 156 | 176  | 192  | 108 | 157  | 170  |
(a) Gt: Genotypes found using seven SSR markers. The genotype NI-1 was the most predominant one, as it was determined in an earlier study addressing genotypic characterization of *P. infestans* isolates from Nicaragua. The frequency of the other genotypes was very low.

(b) NoI: Number of isolates in a given genotype. The NI-1 genotype was common to 46 potato isolates and 17 tomato isolates, whereas 1 potato and 1 tomato isolate shared the same allele sizes and were grouped in the NI-2 and NI-4 genotypes.

(c) Allele sizes in bold are indicating where the variation was found. Allele sizes were adjusted to the sizes obtained by Lees et al. [40].

(d) P/T: Potato or tomato host.

**Result**

**Aggressiveness testing**

The potato isolate NIC-58 did not grow on potato leaflets, but it grew, although weakly, on tomato leaflets. The tomato isolates 6T, 8T, 9T and NIC-96 did not grow on potato leaflets, but they grew on tomato leaflets (Table 2). A significant effect among potato isolates with regard to IP (P=0.04), LA (P=0.05) and LGR (P=0.01) was found. Potato isolates induced necrotic spots in tomato leaflets earlier than on potato leaflets, produced larger LA in potato leaflets and the LGR was greater in potato leaflets than in tomato ones. Highly significant differences among potato isolates for LP (P<0.001), SP (P<0.001), SR (P<0.001) and AI (P<0.003) were found. Potato isolates had a shorter LP, produced more sporangia (SP), had a greater SR and were more aggressive (AI) on tomato leaflets than in potato ones. Potato isolates were not statistically different regarding SA (P=0.73). No significant differences for IP (P=0.75), LA (P=0.95) and LGR (P=0.33) among tomato isolates were found. Highly significant effects among tomato isolates for LP (P<0.0001), SP (P<0.0001), SA (P<0.006) and AI (P<0.0001) were detected. The mean values for SR among tomato isolates were statistically significant (P=0.05). Tomato isolates had a shorter LP, produced more sporangia (SP), had a greater SA and were more aggressive on tomato leaflets than on potato ones. Potato and tomato isolates both had a shorter LP and higher SP on tomato leaflets compared to potato leaflets. Tomato isolates had the longest LP among tested isolates and that was observed on potato leaflets, whereas potato isolates had the highest SP and that was detected on tomato leaflets. The smallest SA was produced in potato leaflets by tomato isolates. It is noteworthy to point out that, both potato and tomato isolates were more aggressive (AI) in tomato leaflets and produced offspring in greater abundance (SP) on tomato leaflets than in potato ones, i.e., they were prolific in tomato leaflets (Table 3) (Figure 1 & 2).

**Table 2:** Potato and tomato isolates of *Phytophthora infestans* from Nicaragua used for determining their aggressiveness on potato and tomato leaflets in cross-inoculation experiments.

| Potato Isolates | Mating Type | SSR Genotype | mtDNA Haplotype | Metalaxyl Sensitivity | Race |
|----------------|-------------|--------------|-----------------|-----------------------|------|
| 1P             | A2          | NI-1         | la              | nd                    | nd   |
| 2P             | A2          | NI-1         | la              | nd                    | nd   |
| 3P             | A2          | NI-1         | la              | nd                    | nd   |
| NIC-3          | A2          | NI-1         | la              | R                     | R1.2.3.4.5.6.7.10.11 |
| NIC-6          | A2          | NI-1         | la              | R                     | R1.2.3.4.7.11 |
| NIC-17         | A2          | NI-1         | la              | nd                    | nd   |
| NIC-40         | A2          | NI-1         | la              | R                     | R1.2.3.4.5.6.7.9.11 |
| NIC-42         | A2          | NI-1         | la              | R                     | R1.3.4.5.10.11 |
| NIC-44         | A2          | NI-1         | la              | R                     | R1.3.4.11 |
| NIC-46         | A2          | NI-1         | la              | R                     | R1.3.4.5.7 |
| NIC-49         | A2          | NI-1         | la              | R                     | R1.2.3.4.6.7.10.11 |
| NIC-58(*)      | A2          | NI-1         | la              | R                     | R1.3.4.7.11 |
| NIC-59         | A2          | NI-1         | la              | R                     | R1.3.4.7.11 |
| NIC-60         | A2          | NI-1         | la              | R                     | R1.3.4.5.6.7.10.11 |
| NIC-75         | A2          | NI-1         | la              | *I*                   | R3.4.7.11 |
| NIC-98         | A2          | NI-1         | la              | R                     | R1.2.3.4.5.6.7.11 |

| Tomato Isolates | Mating Type | SSR Genotype | mtDNA Haplotype | Metalaxyl Sensitivity | Race |
|-----------------|-------------|--------------|-----------------|-----------------------|------|
| 1T              | A2          | NI-1         | la              | nd                    | nd   |
| 2T              | A2          | NI-1         | la              | nd                    | nd   |
| 3T              | A2          | NI-1         | la              | nd                    | nd   |
| 4T              | A2          | NI-1         | la              | nd                    | nd   |
| 6T(**)          | A2          | NI-1         | la              | nd                    | nd   |
Mating type determination; (b) Simple sequence repeats (also known as microsatellites); and (c) mtDNA haplotyping were done in this study.

d) Metalaxyl sensitivity and (e) race determination was done in a previous study.

f) nd=not determined (unknown).

g) R=isolates resistant to the fungicide metalaxyl.

h) I=isolate with intermediate sensitivity to the fungicide metalaxyl.

(*) This isolate did not grow on potato leaflets, albeit weakly it grew on tomato leaflets.

(**) These isolates did not grow on potato leaflets, but they grew on tomato leaflets.

Table 3: Least square mean (LSMEAN) values of the aggressiveness components resulting from cross-inoculation tests with potato and tomato isolates of Phytophthora infestans in potato and tomato leaflets.

| ACa | Potato Isolates | Tomato Isolates | P>F | P>F |
|-----|-----------------|-----------------|-----|-----|
|     | Leaflet         | Leaflet         |     |     |
|     | Potato          | Tomato          |     |     |
|     | IP              | 2.01 (48)       | 1.90 (46) | 0.04 | 1.81 (43) | 1.78 (43) | 0.75 |
|     | LP              | 3.61 (87)       | 2.78 (67) | 0.0001 | 4.54 (109) | 2.75 (66) | 0.0001 |
|     | LA              | 1275            | 1128 | 0.05 | 1071 | 1076 | 0.95 |
|     | LGR             | 4.88(10^{-3})   | 4.52 (10^{-3}) | 0.01 | 4.06 (10^{-3}) | 4.24 (10^{-3}) | 0.33 |
|     | SP              | 2.51(10^{7})    | 3.9 (10^{7}) | 0.0001 | 2.9 (10^{7}) | 3.7 (10^{7}) | 0.05 |
|     | SA              | 124             | 13 | 0.003 | 11.3 | 12.9 | 0.0001 |

Aggressiveness components

(a) IP=Incubation period [time (days) after inoculation when necrotic spots appeared; in parenthesis is indicated the IP in hours];

(b) LP=Latency period [time (days) after inoculation when sporangia appeared; in parenthesis is indicated the LP in hours];

(d) LA=Lesion area (mm^2) including the sporulating annulus (divide by 10^6 to convert it to square meters);

(e) LGR=Lesion growth rate, measured in meters per day;

(f) SP=Spore production calculated by multiplying the sporangia concentration by the volume of a preservative solution [(0.04M copper sulfate, 0.2M sodium acetate/acetic acid, pH 5.4); Mizubuti and Fry [37]].

(g) SA=Sporulating area (mm^2), which is the difference between LA and the area before the LP (hours); divide by 106 to convert it to square meters.

(h) SR=Sporulation rate (sporangia per square meter per day), which is calculated using the equation SR=SP/SA;

(i) AI=Index of aggressiveness, calculated by the formula AI=ln(LA x SP x 1/LP) according to Montarry et al. [38].
**Figure 1:** Genotypes of *Phytophthora infestans* detected using simple sequence repeat (SSRs) markers and the percentage of potato (n=53) and tomato (n=19) isolates found in each genotype.

**Figure 2:** Aggressiveness parameters of *P. infestans* isolates from Nicaragua measured through cross-inoculation assays using detached potato and tomato leaflets. Vertical bars represent the standard deviation.
SSR fingerprinting and mtDNA haplotyping

Five SSR multilocus genotypes among 72 isolates of *P. infestans* from Nicaragua were detected and all 72 isolates sampled from potato and tomato fields were of the Ia mtDNA haplotype and A2 mating type. The most predominant was the genotype NI-1, found in 63 out of 72 isolates and reaching a frequency of 87.5%. The NI-1 genotype was common to 46 potato isolates and 17 tomato isolates. The frequency of the remaining four genotypes was very low (Figure 1). Variation in tomato isolates was found only in two isolates at loci 4B and Pi16 and in both cases they shared the same allele sizes with two potato isolates (Table 1). The potato and tomato isolates grouped in NI-2 and NI-4 genotypes were sampled from locations distant from one another 120 (NI-2) and 80 (NI-4) kilometers respectively. In contrast, potato isolates belonging to NI-3 and NI-5 genotypes were sampled from either the same field (NI-3) or neighboring fields (NI-5). In general, two kinds of variations were detected, namely, from heterozygosity to homogyzosity at loci 4B and G11 and from homogyzosity to heterozygosity at locus Pi16. The common trait of the five identified genotypes is that they belong to the A2 mating type and have the Ia mtDNA haplotype (Table 2). The 4B, G11 and Pi16 loci were the most variable, as they showed differences among tested isolates of *P. infestans*.

Discussion

This study also undertaken to determine whether there are differences in aggressiveness among potato and tomato isolates through reciprocal aggressiveness tests. A single potato isolates and four tomato isolates did not grow at all on potato leaflets, but they grew on tomato leaflets, indicating that there might be some kind of host preference of these isolates toward tomato. This is in line with findings of other studies in which it has been found host specificity of *P. infestans* isolates toward either potato or tomato [24,27-29]. It is noteworthy to point out that both potato and tomato isolates were kept on their hosts of origin before being used in the aggressiveness tests and the time between subculture of the isolates on potato or tomato leaflets and their use in the tests was relatively short, ten days at the most. Therefore, the reasons behind the loss of pathogenicity of the single potato isolate that could not infect potato leaflets remain unknown.

Some observations done in this study would support the hypothesis that tomato is a better host than potato due to the following:

i. the time elapsed between the end of the IP (appearance of small necrotic spots) and the beginning of the LP (appearance of sporangia) was shorter on tomato leaflets than on potato ones, that is, the LP was shorter than IP, showing that potato isolates displayed a biotrophic colonization phase on tomato leaflets as has been reported in other studies [3,8,42];

ii. the LP was shorter on tomato leaflets than on potato ones;

iii. the LA was greater on tomato leaflets, indicating that disease intensity is expected to be higher on tomato;

iv. The SP was 1.9 times greater on tomato leaflets than on potato ones;

v. The aggressiveness index was greater on tomato leaflets than on potato ones and was almost the same as the aggressiveness index of the tomato isolates on tomato leaflets, suggesting that potato and tomato isolates are equally aggressive on tomato.

Contrary to our initial hypothesis, tomato isolates performed better on tomato leaflets than on potato ones. This finding could be showing host-specificity of tomato isolates toward tomato. In general, tomato isolates were more aggressive on their host of origin, whereas potato isolates were more aggressive on the alternative host.

In a previous work, an SSR fingerprinting analysis showed that Nicaraguan population of *P. infestans* belonged to a single multilocus genotype referred to as NI-1. Nevertheless, in the same study two rare genotypes that showed one-step allelic differences at the loci Pi16 and G11 were detected (Blandón-Díaz et al., unpublished). In the present study, five SSR multilocus genotypes, four of which had not been previously described, were found among the 72 *P. infestans* isolates tested. The NI-1 single multilocus genotype continued to be dominant within the clonal lineage, comprising 46 potato isolates and 17 tomato isolates. This finding could indicate that there is neither host specificity nor population differentiation between this group of potato and tomato isolates as has been suggested in an earlier study (Blandón-Díaz et al., unpublished). Moreover, the movement of planting material (infected potato seed tubers and tomato seedlings) among and within production areas seems to be fostering a possible migration (genotype flow) of *P. infestans* strains between potato and tomato crops and consequently, preventing population differentiation and host specificity. Although at very low frequency, four new genotypes (NI-2, NI-3, NI-4 and NI-5) were detected as a result of variation observed at loci 4B, G11, and Pi16, which were also the most variable loci. Two isolates of NI-2, NI-3 and NI-4 genotypes were detected, and three isolates of NI-5 genotype were found. Two types of variations were observed. The first type of variation seen was in isolates with a loss of heterozygosity at loci 4B and G11. A difference of one allele from these loci that are heterozygous in the commonly found multilocus NI-1 genotype was detected. In a study conducted in Australia with *P. cinnamomi*, the loss of heterozygosity was attributed to a mitotic crossing over and not to a sexual reproduction or self-fertility [42]. Mitotic recombination was also thought to be responsible for loss of heterozygosity in *P. infestans* allozymes [43]. The second variation found was a two-step difference from homogyzosity (176/176) to heterozygosity (176/180) at locus Pi16 that showed one potato and one tomato isolate. These observed new variants could have arisen by mutation or mitotic recombination as has been stated in previous studies dealing with asexually reproducing populations of *P. infestans* [8,44] and characterization of the genetic diversity of *P. ramorum* populations from United States and Europe [45]. In a previous study conducted with *P. infestans* isolates from
Nicaragua, one step difference in allele sizes at loci G11 (from 132/156 to 132/154) and Pi16 (from 176/176 to 174/176) was shown (Blandón-Díaz et al., unpublished). Therefore, the observed differences in microsatellite allele sizes in the present study are in agreement with the microsatellite mutation processes that have been inferred in other studies [46].

The results from the multilocus analysis showed that Nicaraguan population of *P. infestans* is characteristically clonal in the distribution of genotypic variation, though novel genotypes at very low frequencies were detected. This conclusion is also supported by the predominance of only one mating type (A2). On the one hand, variation at loci 4B and Pi16 led to detection of two novel genotypes (NI-2 and NI-4) until recently unknown that were composed by one isolate of potato and one of tomato each. The isolates belonging to the NI-2 and NI-4 genotypes were sampled from locations that were separated from each other by almost a hundred kilometers. Therefore, a relationship at geographic scale between isolates of these two genotypes was not found. On the other hand, variation found at loci 4B and G11 revealed the presence of the NI-3 and NI-5 genotypes, which were exclusively potato isolates sampled from either the same field (NI-3 genotype) or neighboring potato fields (NI-5). The distribution of genetic variation among these isolates turned out to be found within either a single field or neighboring fields, that is, on a fine geographical scale. No variation was found for mtDNA markers because only the la haplotype was detected. Hence, it was confirmed that la is still the dominant mtDNA haplotype in Nicaraguan population of *P. infestans*. This result is in agreement with a previous study (Blandón-Díaz et al., unpublished) in which was suggested that the Ibb mtDNA haplotype had been completely replaced by the la haplotype. Moreover, it is believed that the la haplotype has also replaced the Ibb haplotype which was found in herbarium specimen from Nicaragua dating from 1956 [47].

So far, the NI-1 is still the most widely distributed and dominant genotype within the Nicaraguan clonal lineage of *P. infestans*. It is also known that this genotype is formed by non-host specific potato and tomato strains, which are resistant to metalaxyl and has a complex race structure (Blandón-Díaz et al., unpublished). However, the occurrence of new variants could pose a greater threat for potato and tomato crops in Nicaragua, especially, if these variants are equally or more pathogenic and more ecologically adapted than the predominant NI-1 genotype. Therefore, more extensive sampling at the sites from which isolates were recovered and genotyping of these new variants would be required to track the movement and diversification of these variants. Meaningful shifts in *P. infestans* populations have been occurred in UK for instance, where in just four years the prevalence of genotype 13_A2 or "Blue 13", rose to 79% of late blight outbreaks. The "Blue 13" genotype was first detected in the Netherlands in 2004 [48]. A similar situation has been experienced in the United States, where over a period of five years the US-8 genotype became the most widely distributed, dominant and troublesome genotype [10]. Nicaragua imports annually potato seed from the Netherlands, therefore the occurrence of the "Blue 13" genotype in the Netherlands could have very serious epidemiological implications for potato production in Nicaragua regardless of the fact that the "Blue 13" genotype appears to be better adapted to cooler temperatures [49].

Although SSR fingerprinting and mtDNA haplotyping showed no differentiation between potato and tomato isolates, aggressiveness tests revealed that tomato isolates showed a general, but not exclusively, host-specificity and were more aggressive on tomato. In contrast, potato isolates showed host-preference toward tomato detached leaflets and were more aggressive on them. Overall, both potato and tomato isolates were able to attack the alternative host with the abovementioned differences. All of the isolates (including potato and tomato isolates) tested for aggressiveness differentiation belonged to the dominant NI-1 genotype. Only one tomato isolate (NIC-96), grouped in the NI-4 genotype, had a differential host preference, since this isolate grew on tomato leaflets, but it did not grow on potato leaflets. However, other isolates with the NI-4 genotype were isolated from potato. This could mean that the host preference border is very tenuous (at any time the isolate NIC-96 could switch its preference to potato) or that the NI-4 genotype is heterogeneous and contains a mixture of individuals with varying degrees of adaptation.

**Conclusion**

The findings of this study provide useful information regarding late blight disease management on potato and tomato crops in Nicaragua. If potato and tomato are grown in the same geographical region or surrounding areas, then the take-home disease management message here would appear to be that potato and tomato growers must be ready to apply protectant or systemic (translaminar) fungicides to both potato and tomato, if late blight is found in either crop. Furthermore, in the near future, it is recommended to monitor more extensively and more systematically *P. infestans* populations from Nicaragua to detect possible population shifts due to the processes that govern pathogen evolution such as, mutation, migration (gene and genotype flow), genetic drift, mating system (reproduction) and selection.

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