

Genetic structure of *Phytophthora infestans sensu lato* assessed using microsatellite markers

Estructura genética de *Phytophthora infestans sensu lato* evaluada mediante marcadores microsatélites

Tharling Yadhannia Hernández-Díaz¹, Edison Martínez-Palacios^{1,2},
Claudia Elizabeth Salazar-González³, Luz Estela Lagos Mora¹

- ¹. Departamento de Biología, Universidad de Nariño, Pasto, Nariño, Colombia, tharling.bio@udenar.edu.co, <https://orcid.org/0009-0005-2506-6895>, (Correspondence); luzestela@udenar.edu.co, <https://orcid.org/0000-0002-5152-3413>; eddy2757@udenar.edu.co, <https://orcid.org/0000-0002-6654-7602>
². Facultad de Medicina, Universidad Cooperativa de Colombia, Pasto, Nariño, Colombia.
³. Departamento de Producción y Sanidad Vegetal, Universidad de Nariño, Pasto, Nariño, Colombia, claudiasalazarg@udenar.edu.co, <https://orcid.org/0000-0002-5461-2761>

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ABSTRACT

Phytophthora infestans sensu lato is a phytopathogenic oomycete that causes late blight in Solanaceae and has a significant global impact. Its rapid evolution requires population monitoring using polymorphic markers to detect new genotypes with greater biological efficacy. In this context, this study aimed to evaluate the genetic variability of isolates of *Phytophthora infestans sensu lato* obtained from different species of Solanaceae cultivated in southern Colombia. A total of 40 pathogen isolates were collected from different hosts, including *Solanum tuberosum*, *Solanum lycopersicum*, *Solanum muricatum*, and *Solanum betaceum*. Genomic DNA was extracted and amplified using 12 microsatellite markers, and the resulting fragments were visualized on 3% agarose gels. Amplification band sizes were measured using ImageJ, and data analysis was performed using the Poppr package in R. High marker polymorphism was observed, with more than four alleles amplified per locus, evidence of triploidy, and high genetic diversity compared to other regions of Colombia. Populations were structured according to their host, with genetic differentiation (Gst) values ranging from 0.24 to 0.65, likely influenced by host preference and the presence of alternative hosts. Linkage disequilibrium analysis suggested asexual reproduction in *Phytophthora betacei* populations and possible sexual reproduction in *P. infestans* populations from potato, sweet cucumber, and tomato, emphasizing the need for further investigation of these populations. The markers were highly informative in both the diversity and the reproductive form of the oomycete.

Keywords: genetic diversity; multilocus genotype; oomycete; population; Solanaceae; SSR.

RESUMEN

Phytophthora infestans es un oomiceto fitopatógeno que causa el tizón tardío en solanáceas, con alto impacto global. Su rápida evolución demanda el monitoreo poblacional con marcadores polimórficos para la detección de nuevos genotipos con mayor eficacia biológica. Por consiguiente, el objetivo de este estudio fue evaluar la variabilidad genética de aislamientos de *P. infestans sensu lato* obtenidos de diferentes especies de solanáceas cultivadas en el sur de Colombia. Para lo cual, se aislaron 40 muestras del patógeno obtenidas de *S. tuberosum*, *S. lycopersicum*, *S. muricatum* y *S. betaceum*. Se extrajo su ADN y se amplificó con 12 marcadores microsatélites, que se visualizaron en geles de agarosa al 3%. La medición de las bandas de amplificación se realizó con ImageJ y el análisis de datos con el paquete Poppr del software R. Se obtuvo alto polimorfismo de los marcadores con la amplificación de más de cuatro alelos por locus, presencia de triploidia y elevada diversidad genética, en comparación con otras regiones del país. Las poblaciones se estructuraron de acuerdo a su hospedero con una diferenciación genética (Gst) entre 0,24 y 0,65, probablemente por la preferencia de hospedero y el uso de hospederos alternativos. El desequilibrio

de ligamiento sugiere reproducción asexual en poblaciones de *P. betacei* y posible reproducción sexual en poblaciones de *P. infestans* provenientes de papa, pepino dulce y tomate, generando una alerta para continuar con la investigación de estas poblaciones. Los marcadores fueron altamente informativos tanto en la diversidad como en la forma de reproducción del oomiceto.

Palabras clave: diversidad genética; genotipo multilocus; oomiceto; población; solanáceas; SSR.

INTRODUCTION

Phytophthora infestans (Mont.) de Bary is a heterothallic phytopathogenic oomycete responsible for late blight, one of the most devastating plant diseases worldwide, leading to an estimated annual loss of approximately 7.2 billion dollars (Fry, 2020; Yuen, 2021). This pathogen mainly affects cultivated species of the Solanaceae family, such as potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), sweet cucumber (*Solanum muricatum* Aiton), and some wild species (Lindqvist-Kreuze *et al.*, 2020). In contrast, late blight in tree tomato (*Solanum betaceum* Cav.) is caused by *Phytophthora betacei*, a species described in Colombia (Mideros *et al.*, 2018). These species belong to clade 1c and form the *P. infestans sensu lato* complex.

Species within *P. infestans* and *P. betacei* (*P. infestans sensu lato*) present a high incidence of late blight and accelerated genetic changes in their populations, mainly due to mechanisms such as mutation, recombination, parasexuality, mitotic recombination, selection, and migration of new genotypes (Fry, 2020; Yuen, 2021). The emergence of fungicide-resistant strains and the occurrence of sexual reproduction are clear indicators of these population changes, allowing the establishment of more aggressive genotypes with greater reproductive success (Babarinde *et al.*, 2024; Ludwiczewska *et al.*, 2025). These dynamics underscore the need to investigate the biological, epidemiological, and genetic aspects of the pathogen. These population changes have been studied using various genotypic and phenotypic markers. Over the years, research has increasingly focused on inter- and intraspecific variability and differences among individual strains, highlighting the need for fully characterized molecular markers (Mathew *et al.*, 2020). One of the most widely used markers is simple sequence repeat markers (SSR) or microsatellites, which have been proven valuable in assessing genetic diversity, population structure, disease dynamics, evolutionary studies, and tracking pathogen migration (Chaves *et al.*, 2019; Mathew *et al.*, 2020).

SSR markers have been used to determine the intraspecific variability of *P. infestans sensu lato* populations in the departments of Nariño and Putumayo. Chaves *et al.* (2020) reported a high degree of genetic variability within the populations of *P. infestans* and *P. betacei* between 2012 and 2016. Additionally, the first isolates of *P. betacei* in tree tomato (*S. betaceum*) were recorded in these departments, leading Mideros *et al.* (2018) to suggest ecological speciation in allopatry due to the high levels of host specialization. These findings indicate that pathogen populations exhibit high genetic variability despite the asexual reproduction reported in Colombia. However, the presence of the A2 mating type in Ecuador (Oliva *et al.*, 2006) must be considered, as the uncontrolled movement of Solanaceae agricultural products across the Colombian-Ecuadorian border may facilitate sexual reproduction of the pathogen, further increasing genetic variability (Hernández-Díaz *et al.*, 2024).

Population analysis and continuous monitoring of the pathogen in Nariño and Putumayo are essential to provide valuable insights for developing effective late blight management strategies. However, the current genetic status of these populations in

Nariño and Putumayo remains unknown. Therefore, the objective of this study was to determine the genetic structure of *P. infestans* and *P. betacei* populations isolated from different Solanaceae species in municipalities across southern Colombia.

MATERIALS AND METHODS

Obtaining and culturing isolates

P. infestans and *P. betacei* isolates were obtained from the strain collection of the Genetics and Evolution of Tropical Organisms (GENPAT) research group of the University of Nariño, comprising samples collected between 2019 and 2022. Additional isolates were obtained through field sampling in the municipalities of Imues, San José de Albán, Pasto, Taminango, and San Lorenzo in Nariño, as well as Sibundoy in the department of Putumayo. Sampling was conducted in Solanaceae crops and orchards, including potato (*S. tuberosum*), tree tomato (*S. betaceum*), tomato (*S. lycopersicum*), and sweet cucumber (*S. muricatum*). Leaves and stems exhibiting wet necrotic spots and whitish hyphal growth were collected. Samples were transported to the Genetics and Evolution laboratory at the University of Nariño in properly labeled bags (Hernández-Díaz *et al.*, 2024).

A section of 0.5 to 1 cm² was taken from each lesion, including both necrotic and healthy tissue, and disinfected with 70% ethanol for 25 s, followed by 1.25% sodium hypochlorite for 15 s and sterile distilled water. The samples were then dried with sterile paper towels (Berdúo-Sandoval *et al.*, 2019; Mideros *et al.*, 2018) and cultured on tomato-pea medium supplemented with Rifampicin®. The inoculum was maintained at room temperature for eight days, and its identification was confirmed through microscopic and macroscopic observation (Shimelash & Dessie, 2020).

DNA extraction

Monosporic isolates were cultured in tomato-pea medium at room temperature in darkness for 15 days. The mycelium was macerated into a fine powder using liquid nitrogen. Genomic DNA was extracted following the protocol described by Griffith and Shaw (1998). The concentration and quality of the nucleic acid were assessed using a 1% agarose gel and quantified using a NanoDrop™ 2000 spectrophotometer.

Microsatellite region amplification

Microsatellite marker amplification was performed using PCR with the following markers: Pi63, Pi70, PinfSSR4, PinfSSR6, PinfSSR11, D13, G11, PI04, Pi4B, PinfSSR2, PinfSSR3, and PinfSSR8, all of which have been fully characterized in *P. infestans* (Li *et al.*, 2013). The first five markers were tested and analyzed by Hernández-Díaz *et al.* (2024), highlighting the need to increase the number of microsatellite markers to 12 and standardize the following reaction conditions: 1× Colorless GoTaq® Reaction Buffer, 0.2 mM dNTPs, 1.25 U GoTaq® DNA Polymerase (Promega), 1 mM MgCl₂, 0.5 μM of forward and reverse primers, and 10 ng/μL of template DNA.

The PCR conditions were as follows: initial denaturation at 95°C for 15 min, 30 cycles at 95°C for 20 s, 58°C for 90 s, 72°C for 60 s, and final extension at 72°C for 20 min (Li *et al.*, 2013). The presence of amplicons was confirmed using 1% agarose gel electrophoresis. To determine allele size in base pairs (bp), samples were run on 3% agarose gels at 85 V and 400 mA for 4 h 30 min, using the ExcelBand™ 50bp molecular weight marker (SMOBIO). A minimum of two gels per sample were run to confirm the amplification range, and the band measurements were performed in triplicate using ImageJ software (Schneider *et al.*, 2012).

Data analysis

Populations were defined according to their host using the R package “Poppr” 1.0.5 (Kamvar *et al.*, 2014). The following genetic parameters were estimated: allelic frequencies per locus, multilocus genotypes (MLG), Nei’s genetic diversity (Hexp), and diversity indices, including Shannon-Wiener (H), Stoddart and Taylor (G), and Simpson’s index (λ). Additionally, genetic differentiation (Gst) (Hedrick, 2005), the percentage of polymorphic loci, the number of unique alleles, the analysis of molecular variance (AMOVA), and the association index were assessed. Population structure was evaluated using a discriminant analysis of principal components (DAPC) (Jombart *et al.*, 2010).

RESULTS

A total of 30 *P. infestans* isolates and 10 *P. betacei* isolates associated with characteristic late blight symptoms were obtained, with 10 isolates corresponding to each evaluated host.

The loci Pi63, Pi70, SSR2, SSR3, Pi04, SSR8, and G11 were diploid in *P. infestans*, as were all loci in *P. betacei*, except Pi4B, which was triploid. In contrast, the markers SSR6, SSR11, SSR4, Pi4B, and D13 showed evidence of triploidy in isolates from *S. muricatum* and *S. tuberosum* (*P. infestans*).

Additionally, the bands obtained fell within the reported amplification range for each microsatellite and exhibited more than four alleles per locus in *P. infestans* and more than three alleles in *P. betacei*, depending on the host (Table 1).

Table 1. Summary of data by locus in *P. infestans* and *P. betacei* isolates depending on the host

Locus	<i>P. infestans</i>									<i>P. betacei</i>		
	<i>S. tuberosum</i>			<i>S. lycopersicum</i>			<i>S. muricatum</i>			<i>S. betaceum</i>		
	Alleles	1-D	Hexp	Alleles	1-D	Hexp	Alleles	1-D	Hexp	Alleles	1-D	Hexp
SSR6	4	0.7	0.74	7	0.79	0.83	8	0.83	0.87	9	0.86	0.91
SSR11	17	0.93	0.96	11	0.88	0.93	9	0.83	0.87	6	0.80	0.84
Pi63	4	0.48	0.51	8	0.84	0.89	7	0.82	0.86	6	0.80	0.84
SSR4	8	0.82	0.86	5	0.76	0.80	10	0.85	0.89	9	0.86	0.91
Pi70	6	0.70	0.74	7	0.82	0.86	6	0.8	0.84	4	0.64	0.67
SSR2	3	0.62	0.65	3	0.54	0.57	5	0.68	0.72	5	0.74	0.78
SSR3	7	0.82	0.86	6	0.7	0.73	7	0.82	0.86	8	0.86	0.91
Pi04	3	0.62	0.65	3	0.54	0.57	4	0.72	0.76	3	0.46	0.48
Pi4B	5	0.75	0.8	6	0.81	0.86	8	0.84	0.88	11	0.89	0.93
SSR8	2	0.49	0.53	7	0.82	0.86	7	0.82	0.86	4	0.72	0.76
G11	8	0.86	0.91	5	0.72	0.76	6	0.75	0.79	11	0.89	0.94
D13	10	0.86	0.91	12	0.90	0.95	13	0.89	0.93	4	0.58	0.61
mean	6.42	0.72	0.76	6.67	0.76	0.8	7.5	0.81	0.85	6.67	0.76	0.80

Note. Alleles-number of alleles observed at each locus; 1-D: Simpson’s diversity index; Hexp: Nei’s diversity index.

For *P. infestans*, the D13 locus was the most polymorphic in isolates obtained from *S. muricatum* and *S. lycopersicum*, while in *S. tuberosum* isolates, SSR11 exhibited the highest polymorphism (Table 1). The SSR6, SSR4, SSR2, SSR3, Pi70, and Pi04 loci contained at least one allele with a frequency higher than 0.5, whereas SSR11, Pi63, Pi4B, G11, SSR8, and D13 loci displayed lower-frequency alleles in most isolates from *S. lycopersicum*, *S. tuberosum*, and *S. muricatum*.

For *P. betacei*, the most polymorphic loci were Pi4B and G11, followed by SSR6 and SSR4, compared to the other microsatellites evaluated (Table 1). Additionally, unique alleles were detected at the SSR6, Pi63, SSR4, SSR3, Pi04, Pi4B, SSR8, and Pi70 loci in this population.

The high allelic variability observed is reflected in the high genetic diversity calculated based on the MLGs identified, which correspond to all isolated samples from both species (Table 2).

Table 2. Summary of genotypic diversity of *P. infestans* and *P. betacei*

Species	Host	N	MLG	H	G	λ	E.5	Hexp
<i>P. betacei</i>	<i>S.betaceum</i>	10	10	2.3	10	0.9	1	0.80
	<i>S.muricatum</i>	10	10	2.3	10	0.9	1	0.84
<i>P. infestans</i>	<i>S.tuberosum</i>	10	10	2.3	10	0.9	1	0.76
	<i>S.lycopersicum</i>	10	10	2.3	10	0.9	1	0.80

Note. N: number of isolates in the population; MLG: number of multilocus genotypes observed; H: Shannon-Wiener diversity index of multilocus genotypes; G: Stoddart and Taylor genotypic diversity index; λ : corrected Simpson index; E.5: uniformity index; Hexp: Nei's genetic diversity.

The Shannon-Wiener, Stoddart and Taylor and Simpson diversity indices are similar for both *P. infestans* and *P. betacei*, while Nei's genetic diversity (Hexp) ranges between 0.76 and 0.84, indicating high genetic diversity among isolates based on their host (Table 2).

Genetic differentiation (Gst) values calculated for *P. infestans* based on host specificity were 0.24 between potato and sweet cucumber, 0.62 between tomato and sweet cucumber, and 0.67 between potato and tomato. The analysis of molecular variance (AMOVA) indicated statistically significant differences among the *P. infestans* populations determined by host (Table 3). However, the highest percentage of genetic variation was observed within isolates rather than between host-defined populations (Table 3).

Table 3. Molecular variance analysis (AMOVA) of *P. infestans* isolates among and within their hosts

		Df	SSD	MSD	Sigma	Total (%)	p-value
Potato – Sweet Cucumber	Between hosts	1	5.62	5.62	0.22	6.10	0.05
	Within hosts	18	61.33	3.41	3.41	93.90	0.05
	Total	19	66.95	3.52	3.63	100.00	
Sweet Cucumber - Tomato	Between hosts	1	13.13	13.13	0.76	12.08	0.01
	Within hosts	18	99.53	5.53	5.53	87.92	0.01
	Total	19	112.66	5.93	6.29	100.00	
Potato - Tomato	Between hosts	1	14.26	14.26	0.93	15.71	0.01
	Within hosts	18	89.64	4.98	4.98	84.29	0.01
	Total	19	103.90	5.47	5.91	100.00	

Df: degrees of freedom; SSD: sum of square differences; MSD: mean square deviation; Sigma: variance.

In contrast, *P. betacei* populations did not exhibit significant genetic differentiation based on geographic origin ($p = 0.703$). However, 100% of the genetic variation was detected within the isolates from *S. betaceum*, suggesting genetic homogeneity within this host group due to gene flow.

The population structure of the isolates was characterized using discriminant analysis of principal components (DAPC), which separated the isolates into four well-defined groups based on their host. The *P. infestans* populations obtained from potato, tomato, and sweet cucumber were clearly differentiated from each other and from the *P. betacei* population (Fig. 1a). Overall, all *P. infestans* and *P. betacei* isolates were correctly grouped with their respective host, with a population assignment probability greater than 60%. However, some isolates exhibited affinities with a different host (Fig. 1b).

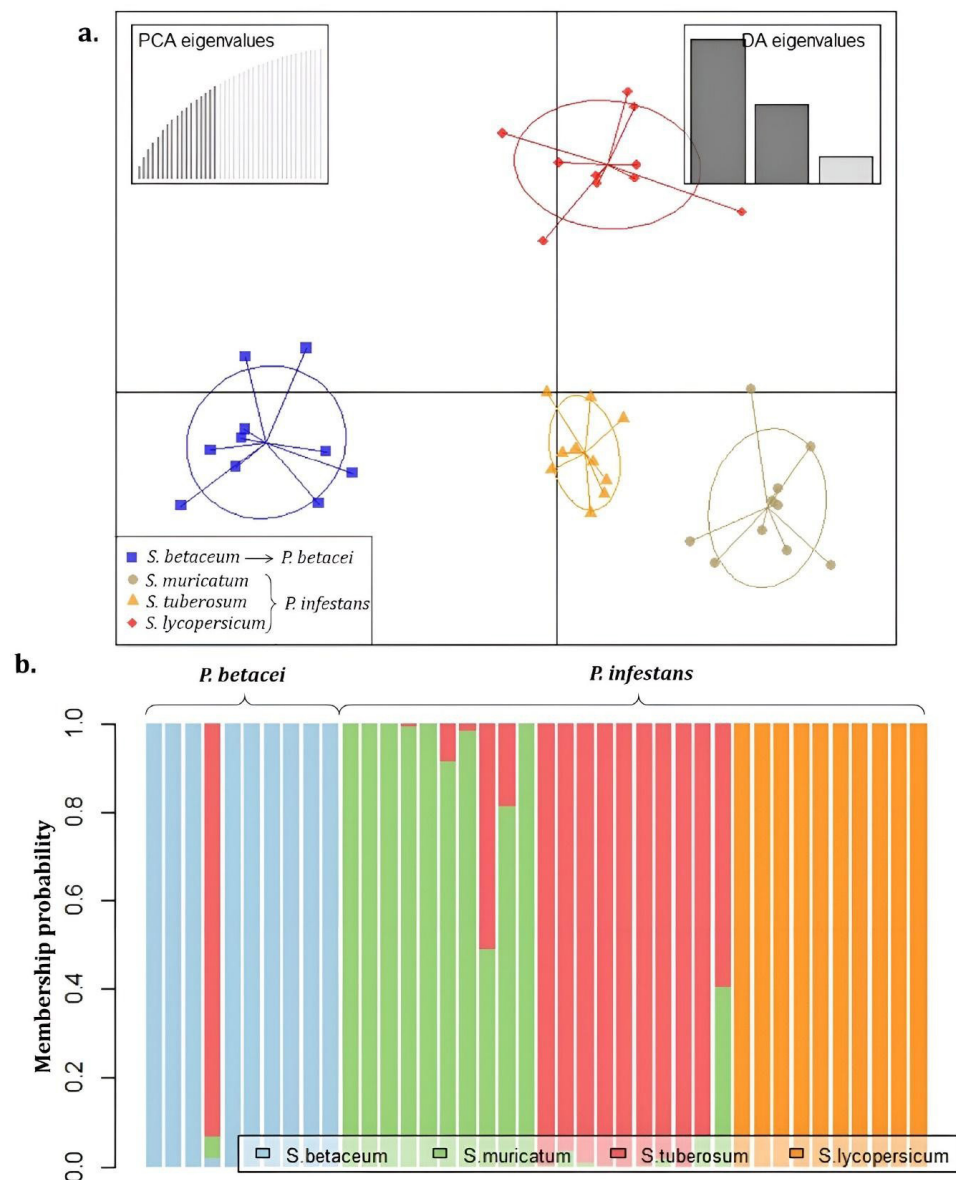


Figure 1. **a.** Discriminant Analysis of Principal Components (DAPC) detected for microsatellite analysis of *P. betacei* and *P. infestans* according to their host. Isolates are represented by dots and populations by ellipses. **b.** Genetic structure of *P. betacei* and *P. infestans* according to their host.

Analysis of both clone-corrected and uncorrected data rejected the null hypothesis in *P. betacei* populations, which states that the alleles at different loci should be unlinked in sexually reproduced populations (Kamvar *et al.*, 2014; Wharton *et al.*, 2023). The index of association ($\bar{r}_d$) values obtained were 0.0522 and 0.0713 ($p < 0.005$), placing them outside the expected distribution for linkage disequilibrium, further supporting a predominantly clonal population structure (Fig. 2a). In contrast, in *P. infestans* populations structured by host, the null hypothesis suggesting sexual reproduction was supported (Fig. 2b-d).

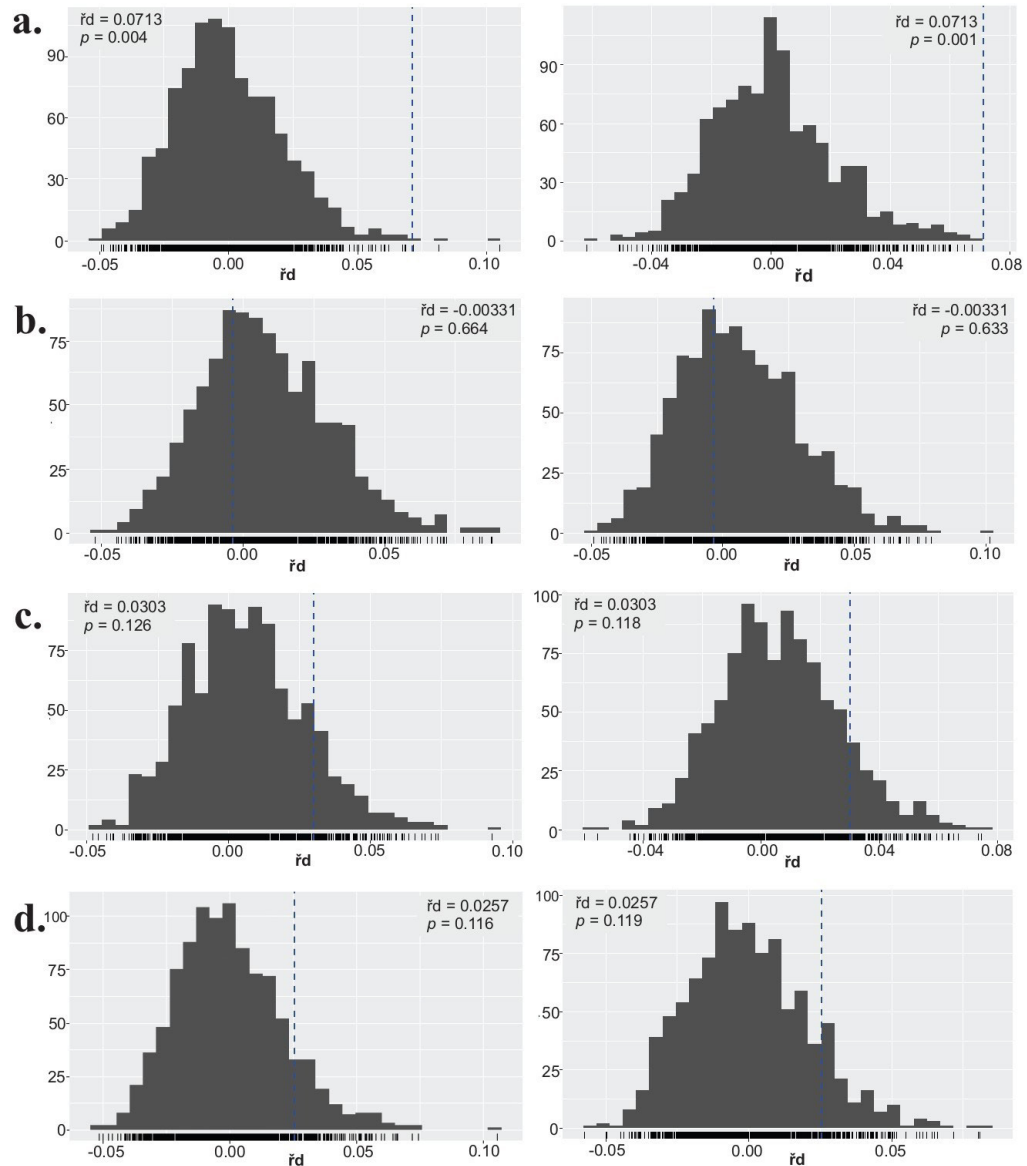


Figure 2. Standardized association index of **a.** *P. betacei* isolated from *S. betaceum*, and of *P. infestans* isolated from **b.** *S. tuberosum*, **c.** *S. muricatum*, and **d.** *S. lycopersicum*.

DISCUSSION

The presence of diploid and triploid isolates in *P. infestans* and *P. betacei* is consistent with findings in populations from Bangladesh (Wharton *et al.*, 2023), the United States (Martin *et al.*, 2019), and Colombia (Chaves *et al.*, 2020; Hernández-Díaz *et al.*, 2024). These differences in ploidy levels influence the number of alleles per locus and may enhance pathogen fitness by increasing heterozygosity, genetic diversity, and adaptive plasticity. Furthermore, ploidy variation has been associated with fungicide resistance in fungi and oomycetes (De Miccolis Angelini *et al.*, 2015; Knaus *et al.*, 2020).

The results shown in Table 1 indicate a high allelic diversity, uniformly represented with values close to one, as all loci were highly polymorphic and informative (Dangi *et al.*, 2021; Hernández-Díaz *et al.*, 2024; Olave-Achury *et al.*, 2022).

The D13 marker has been reported as one of the loci with the highest number of alleles, followed by SSR4 and G11 (Hassan *et al.*, 2020; Kanetis *et al.*, 2021), which is consistent with the results of this study. However, previous research has identified Pi04 (2 alleles), Pi70 (1 or 2 alleles), and SSR2 (2 alleles) as the least polymorphic loci (Göre *et al.*, 2021). In contrast, the present results indicate that Pi04, Pi70, and Pi63 exhibited high polymorphism, amplifying more than four alleles per locus in both species, despite showing the lowest allelic richness compared to the other loci analyzed (Table 2).

The high variability observed may be attributed to the presence of polyploidy and aneuploidy, which have been previously reported in the pathogen. Additionally, the evaluated loci exhibit a high mutation rate, as they are located in non-coding regions with long repeat units, leading to polymorphisms in SSR regions and an increased number of alleles (Hernández-Díaz *et al.*, 2024; Li *et al.*, 2017).

Furthermore, Li *et al.* (2017) suggest that different markers may exhibit varying levels of polymorphism depending on the region and population analyzed, highlighting that allelic diversity is dynamic and constantly changing in response to population dynamics. In this study, a significant increase in allelic diversity was observed in *P. infestans sensu lato* populations from southern Colombia.

The high diversity of MLGs identified may be attributed to polyploidy, observed in three of the evaluated loci, as it increases heterozygosity and allelic variability (Dangi *et al.*, 2021). Olave-Achury *et al.* (2022) reported 50 MLGs in *P. infestans* isolated from potato and tomato in Colombia's central region. In Nariño and Putumayo, Chaves *et al.* (2020) identified 31 MLGs for *P. infestans* and seven MLGs for *P. betacei*, which is consistent with the findings of this study. Additionally, these authors reported that genotypic diversity was higher in southern Colombia compared to the central region, where only 15 MLGs for *P. infestans* and three for *P. betacei* were found.

One of the key factors contributing to the observed variability in pathogen populations is selection pressure from frequent fungicide application (De Miccolis Angelini *et al.*, 2015). Field surveys revealed that hosts in both crop fields and orchards were fumigated every 4 to 10 days upon detection of disease symptoms, potentially influencing the genetic diversity of these populations (Hernández-Díaz *et al.*, 2024).

The high diversity calculated by the Shannon-Wiener, Stoddart and Taylor, Simpson and Nei indices arises from various factors, such as gene flow, sexual recombination, parasexuality, mitotic recombination, and mutation (Fry, 2020). However, in Colombia, there is only one reported case of the A2 mating type in *P. infestans* (Vargas *et al.*, 2009), suggesting a low likelihood of sexual reproduction in this pathogen. Consequently, asexual recombination and mutation have been proposed as the primary mechanisms

driving genetic changes and diversity in Colombian *P. infestans* populations (Chaves *et al.*, 2020; Fry, 2020).

It is important to note that the genetic variation detected in a population is also influenced by the markers used. The high mutation rate of microsatellites, along with their codominant inheritance mechanism, makes them valuable markers in population genetics, as they detect additional polymorphisms that allow for broader differentiation in *Phytophthora* isolates, even within the same clonal lineage identified by conventional markers (Hernández-Díaz *et al.*, 2024; Kanetis *et al.*, 2021).

The genetic differentiation values calculated by *Gst* and AMOVA (Table 3) indicate that *P. infestans sensu lato* populations are structured according to the host of origin, as migration and gene flow between hosts appear restricted (Hernández-Díaz *et al.*, 2024). This pattern has also been observed in clonal populations of tomato and potato in Bangladesh (Wharton *et al.*, 2023) and potato and tree tomato in Colombia (Chaves *et al.*, 2020; Mideros *et al.*, 2018), supporting the hypothesis of species differentiation within this pathogen complex.

The population structure observed in Figures 1a and 1b is consistent with the host preference previously reported for these pathogens. Although *P. infestans* and *P. betacei* can infect other Solanaceae species, their virulence, sporulation rate, and aggressiveness are generally higher in their primary host, demonstrating greater pathogenic fitness (Kröner *et al.*, 2017; Olave-Achury *et al.*, 2022).

These adaptive pathogenic traits, which are primarily influenced by the pathogen's fitness rather than its ability to initiate infection, are crucial as they enable the pathogen to utilize alternative hosts when its primary host is unavailable or when environmental conditions are unfavorable for survival (Lindqvist-Kreuzer *et al.*, 2020). This situation may explain why one isolate obtained from tree tomato and one from sweet cucumber exhibited population assignment probabilities of approximately 80% and 50% to potato, respectively (Fig. 1b).

Despite the high diversity observed, similar to that found in populations with sexual reproduction, such as those in some regions of Europe (Ludwiczewska *et al.*, 2025; Runno-Paurson *et al.*, 2022), where the percentage of unique MLGs is high, this study found no evidence of sexual reproduction in *P. betacei* (Fig. 2a). However, in *P. infestans* populations, sexual reproduction is suggested (Fig. 2b-d).

Notably, 90% of isolates obtained from *S. tuberosum* and 100% of *S. muricatum* were triploid. Knaus *et al.* (2020) and Li *et al.* (2017) reported that *P. infestans* strains in sexual populations are predominantly diploid, whereas clonal populations exhibit triploidy, which limits sexual reproduction due to the unequal number of chromosomes. Furthermore, the mating type of *P. infestans* in Colombia has been identified exclusively as A1, with no reports of self-fertile isolates (Gómez-González *et al.*, 2020; Olave-Achury *et al.*, 2022), reducing the likelihood of sexual reproduction. However, the A2 mating type has been reported in Ecuador (Oliva *et al.*, 2006; Oliva *et al.*, 2010), highlighting the need for additional analyses, such as mating type determination in *P. infestans* populations in southern regions of Colombia.

CONCLUSIONS

Microsatellite markers were polymorphic and informative for both *P. infestans* and *P. batecei*, allowing for genetic characterization of the populations. *P. infestans sensu lato* isolates were structured according to their host, driven by allelic richness and

differences, demonstrating a high degree of genetic diversity in their populations, with a unique multilocus genotype for each isolate. Furthermore, additional analysis of the pathogen's reproduction in southern Colombia is required, as linkage disequilibrium analysis raises concerns about sexual reproduction in *P. infestans*, a scenario that would require the implementation of stricter management and control strategies.

AUTHOR CONTRIBUTIONS

Conceptualization, L.E.L.M. and C.E.S.G.; Methodology, T.Y.H.D., E.M.P., C.E.S.G. and L.E.L.M.; Software, T.Y.H.D.; Validation, T.Y.H.D., E.M.P., C.E.S.G. and L.E.L.M.; Formal Analysis, T.Y.H.D., E.M.P. and L.E.L.M.; Investigation, T.Y.H.D.; Resources, T.Y.H.D., E.M.P. and L.E.L.M.; Data Curation, T.Y.H.D.; Writing—Original Draft Preparation, T.Y.H.D.; Writing—Review & Editing, E.M.P., C.E.S.G. and L.E.L.M.; Visualization, T.Y.H.D., E.M.P., C.E.S.G. and L.E.L.M.; Supervision, L.E.L.M. and C.E.S.G.; Project Administration, L.E.L.M. and C.E.S.G.; Funding Acquisition, L.E.L.M. and C.E.S.G.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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