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Morphological and Molecular characterization of *Phytophthora infestans* causing Potato Late Blight Disease

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ABSTRACT: Potatoes are an important vegetable crop of India, grown throughout the year under moist and humid conditions, which makes them more prone to diseases. Among the potato diseases, late blight caused by *Phytophthora infestans* is the most devastating. This paper describes the morphological and molecular characterization of four isolates of potato late blight pathogen *P. infestans* collected from different potato-growing areas in the Nilgiris district in Tamil Nadu. The four isolates were varied with respect to their pathogenic and morphological features, such as growth rate, colony colour, colony pattern, and sporangial production. The maximum mycelial growth rate of *P. infestans* was observed on clarified V8 juice agar and carrot agar after 5-7 days of incubation, while glucose as paragine agar showed minimal mycelial growth. The maximum sporangial production was seen when diluted tomato juice broth was used. Molecular analysis of ITS-PCR confirmed the identification of the isolates as *P. infestans*.

Keywords: Potato, *Phytophthora infestans*, isolation, late blight, morphology, characterization.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most popular vegetables in the world, belonging to the Solanaceae family. There are nearly 200 different wild potato species, each with its own unique flavour and nutritional value. Potatoes are the fourth most commonly grown food crop in the world, after maize, wheat and rice. They are available throughout the year, and can help to meet nutritional needs in people who are deficient in these nutrients. It is also largely rich in antioxidants such as vitamin A, B1, B6, C and carotenoids (White *et al*., 2009). *Phytophthora infestans* (Anton de Bary) is the most destructive oomycete pathogen that causes late blight disease in potato and was responsible for the Irish famine in the 1840s (Montarry *et al*., 2008; Chowdappa *et al*., 2015). This pathogen is a heterothallic and hemibiotrophic in nature. The pathogen is sensitive and short-lived, and it destroys the plants it infects within two weeks under favourable conditions (Fry *et al.,* 2008). The pathogenesis comparatively sensitive, short lived in infected potato leaf tissue, and the pathogen seems to possess a limited competitive saprophytic ability. In these aspects, the isolation and culturing of pathogen in artificial medium is relatively difficult. Several culture media have been used in various countries to isolate and maintain *P. infestans* isolates. It is very essential to supplement the necessary elements and compounds required for the growth and metabolism of the pathogen in the medium (Padmaja *et al.,* 2015). Thus, different synthetic media were used in this study to see which was the best medium for the growth of *P. infestans.*

Symptoms of the disease appear near the leaf tips as water-soaked irregular pale green lesions, which rapidly grow into large brown to black necrotic spots on the upper surface of leaves, and white hyphae growth can be seen on the lower surface of infected leaves (Mhatre *et al.,* 2021). The infection on the stem appears as a brown lesion around the stem, eventually leading to collapse. In severe cases, the disease can affect the entire crop in a week and transform it into a dark blighted crop. The disease also affects tubers, making them hard, dry and rigid, resulting in rotting in the field and in storage (Agrios, 2005; Arora *et al*., 2014). In India prior to 2006, late blight was seen as a frequent threat to potato cultivation in the North Indian states (Chowdappa *et al*., 2011). Since 2008, severe outbreaks of the disease have been seen in South Indian potato crops, resulting in total crop loss (Jain *et al.,* 2019).

In addition, many diagnostic laboratories are not familiar with oomycete culture procedures, and the

rapid growth of bacteria from leaf samples with secondary bacterial infection usually limits the isolation of *P. infestans* (George *et al.,* 2017). Plant pathogenic oomycetes can be rapidly and efficiently isolated from infected plant tissues using antibiotic-enriched selective media following proper laboratory procedures. The selective media used for the isolation of oomycetes pathogen often contain antibacterial agents such as penicillin, ampicillin and rifampicin which prevent the development of bacterial growth and fungal growth. The molecular characterization of *P. infestans* by using the internal transcribed spacer regions (ITS) of rDNA may be helpful for easy detection and confirmation of species and the assessment of genetic variability among isolates. The main focus of this study is to i) isolate the pathogen from the infected leaf tissues, and ii) characterize the isolates of *P. infestans* with respect to their morphological and molecular features.

MATERIALS AND METHODS

Survey and sample collection. A roving field survey was conducted in major potato growing areas such as, Muthorai, Nanjanad, Wellington, Emerald and Palada in the Nilgiris district, Indiaduring *Rabi* 2021-22 to assess the severity and incidence of late blight disease in potato. Two fields were selected in each place. Totally, ten fields were surveyed at the time of survey, late blight infected leaves and stem samples were collected for pathogen isolation and characterization. The disease incidence in each field was determined by visually inspecting the plants and cutting two diagonal transects across the field (number of infected plants and healthy plants along each diagonal).

Isolation of *P. infestans* and pure culture maintenance. The plants exhibiting typical symptoms of late blight were collected from the field during the survey and brought to the laboratory. Tissue segment method (Vasudeva *et al.,* 1958) was employed for isolation of *P. infestans* on clarified V8 PARP medium supplemented with antibiotics such as pimaricin (400µl), penicillin (50µg/ml), ampicillin (250mg/ml), and rifampicin (100mg/ml) to suppress the growth of other microbes (Jadesha *et al.,* 2020). Liquid soap was used to gently remove all soil-associated surface impurities from infected leaf samples. Small pieces (5×5 mm) of infected leaf tissues from the sporulating border of young lesions including a little bit of healthy leaf tissue was dissected using sterile scalpel. Leaf bits were placed in 70% ethanol for 1 minute followed by 4% sodium hypochlorite solution for 60 seconds for surface sterilizing the tissue then rinsed thrice with sterile distilled water in laminar airflow chamber. The excess moisture was blotted with sterile Whatman filter papers. The sterile leaf bits were placed in half plate containing selective medium, clarified V8 PARP supplemented with combination of antibiotics. Inoculated plates were incubated at 20°C for 7-10 days under 12 hours of photoperiod in BOD (Martin *et al.,* 2012).

Phytophthora infestans was initially identified based on mycelial and morphological characteristics using standard mycological keys. The pure fungus culture was obtained using the single hyphal tip method (Tutte *et al*., 1969), which involved transferring the upper portion of actively growing mycelium from a 10-day-old culture into a 1.5ml microfuge tube containing 1 ml of sterile water. On the surface of V8 juice agar plates, three to five drops of suspension were added. The single hyphal bit was forced to ramify through the agar using this technique. Plates were examined under a dissecting microscope at 10x magnification after 5 days, and a single hyphal branch was transferred to V8 juice agar plates. On the surface of V8 juice agar plates, three to five drops of suspension were added. The single hyphal bit was forced to ramify through the agar using this technique. Plates were examined under a dissecting microscope at 10x magnification after 5 days, and a single hyphal branch was transferred to V8 juice agar plates.

Pathogenicity assay under controlled conditions. Pathogenicity study of all four isolates (PPI1, PPI2, PPI3 and PPI4) was performed under controlled conditions on the highly susceptible cultivar Kufri Swarna. Inoculation of *P. infestans* four isolates were done by spraying zoospore suspensions (Becktell *et al*., 2005). Five 7mm mycelia discs of each isolate were placed in 90mm Petri dishes containing 15-20ml of diluted tomato juice broth. The cultures were allowed to grow for 24-48 hours at 20°C under continuous light conditions (Widmer *et al*., 2009). Zoospores were released from the sporangia after the cultures were refrigerated for 30 minutes and then incubated at room temperature. The released zoospores were diluted to the final concentrations. The motility of zoospores was verified under compound microscope.

All the potted potato plants were inoculated by spraying a zoospore suspension (10,000-20,000 sporangia/ml) with handheld sprayer. Spraying of sterile water serves as control kept separately (Wang *et al.,* 2020). The plants were watered daily for 3-4 times at 3hours interval. Each isolate containing 3 potted was considered as one replication and was replicated five times. Initial symptoms were observed after 48-72 hours after inoculation (Pande *et al.,* 2011).

Disease assessment. Late blight incidence was assessed using the formula (Ahmed *et al.*, 2015) as mentioned below.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

The pathogen was re-isolated from the artificially inoculated potato plant shown typical late blight symptoms and the culture was confirmed with the original culture for morphology and colony characters. To evaluate the virulence of four isolates, per cent

disease incidence was calculated and virulent isolate was used throughout the research.

Morphological characterization

Sporangial induction and zoospores production. Six day old culture of *P.infestans* (PPI3) was taken for induction of sporangia and zoospores. Mycelial discs of 9mm diameter of *P. infestans* were cut using sterile cork borer from the edge of 5 days old culture grown on clarified V8 juice agar and inoculated into 90mm diameter to different Petri dishes flooded with 10-15 ml of 1% non sterile soil extract, tap water, distilled water, rain water, pond water (Malvi *et al.,* 2022) and diluted tomato juice broth (Jadesha *et al.,* 2020). The Petri dishes were kept for 48-72 hours at 19-22°C under light conditions. Consistent light condition and non-sterile liquids may promote the sporangial formation. Then, the plates were observed under stereo zoom microscope Induction of sporangia was initiated and abundant production was observed within 48-72 hours primarily towards the margin of the colony. The sporangial suspension was incubated at 4°C for 1 to 2 hours to release of zoospores from the sporangia (Widmer *et al*., 2009).

Micro morphology. All the four isolates were isolated aseptically on the V8 juice agar plates and incubated at 21±2°C for 7 days and cultural characteristics such as colony pattern, growth rate and colony color were observed after 15 days of incubation (Vanegas *et al.,* 2022). Mycelial characters and fungal morphology as described in were observed under LEICA DM2000 phase contrast microscope at 40X magnification of objective lens.

Macro morphology

Colony pattern. Twelve different culture media *viz*., Carrot agar, Clarified V8 juice agar, Corn meal agar, Czapek's Dox agar Glucose asparagine agar, Potato carrot agar Oat meal agar, Potato dextrose agar, Potato leaf extract agar, Richard's synthetic agar, Saboraud dextrose agar, Tomato juice agar were prepared in accordance with the manufacturer's (HiMedia, India) specifications and compositions (Dhingra and Sinclair, 1985). Mycelial discs of 7mm diameter were taken from ten days old pre-cultured Petri plates by using sterile cork borer and placed in all the Petri dishes containing twelve different media. Three replications in each media were maintained and the mean mycelial growth was recorded. The plates were incubated at 19±2°C in 12 hours dark and 12 hours light conditions for 7-10 days. This study was conducted for evaluating the best culture media for the radial growth of *P. infestans* using the twelve different solid media (Rangaswami *et al*., 1958). The average colony diameter of the fungal growth was measured in millimeters (Jadesha *et al*., 2020).

Molecular characterization

DNA extraction. Isolates of *P. infestans* were cultured on oat meal agar for 7-10 days. Mycelia were scrapped from the plates and ground at liquid nitrogen. Extraction of DNA is done by cetyl trimethyl ammonium bromide (CTAB) procedure (Wangsomboondee *et al*., 2002). The frozen mycelia were added with 750µl CTAB buffer and transferred the extract into 2ml centrifuge tubes. Then the tubes were vortexed and incubated in water bath at 65°C for 30 minutes. After incubation, 750µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each tube and centrifuged at 13000 rpm for 15 minutes at room temperature. The uppermost aqueous phase was removed to a new tube and ice cold isopropan-2-ol (600µl) was added and tubes were incubated at -20°C for overnight incubation. After incubation, tubes were centrifuged at 13000 rpm for 15 minutes. The supernatant was discarded and pellets were washed with 70% ethanol, then air dried. DNA was suspended in 30µl TE buffer and stored in -20°C. DNA concentration was checked with 0.8% agarose gel for one hour.

PCR amplification. The identification of *Phytophthora* was confirmed later by performing ITS-PCR assay. Employing the universal primer pairs ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), amplified the ITS regions between small nuclear 18S rDNA and the larger nuclear 28S rDNA, including 5.8S rDNA (Hussain *et al.,* 2014). PCR was performed in a volume of 25µl, containing 10mM of oligonucleotide primer (1 µl), 20mM of each of the four deoxynucleotide triphosphates (0.75 µl) 25mM $MgCl_2$ (1.0 µl), 0.35 µl of Taq DNA polymerase, 10 × Assay buffer (2.5 µl) and 1.0 µl template DNA (60 ng/µl) (Nath *et al.,* 2015). All PCR reactions were carried out using Mastercycler® Nexus gradient X2 PCR cycler (MA, USA) with the following PCR conditions, initial denaturation at 94°C for 3 min, 35 cycles of amplification with denaturation 94°C for 30 sec, annealing at 56°C for 1 min and 72°C for 2 min for extension with final extension 72°C for 10 min (George *et al.,* 2017) and the PCR products were checked for amplification by 1.0 percent agarose gel electrophoresis. The UVITEC Gel doc EZ Imaging system was used to view the size of amplicons and sanger dideoxy sequencing was done to analyze the nucleotide sequence of DNA. The resulted high quality sequences were analyzed with NCBI BLASTn to confirm the authenticity of isolates.

Statistical analysis. The obtained data were statistically analyzed by IBM SPSS Statistics 22. Duncan's multiple range test and post hoc tests were performed at 5% level of significance (Gomez *et al.,* 1984).

RESULTS AND DISCUSSION

Survey and assessment of potato late blight incidence

The survey results have shown that the disease was prevalent in all the surveyed areas with different

magnitude of infection. The small circular to irregular-shaped water-soaked spots were observed on leaf surface with whitish mildew-appearing area is visible at the edge of the lesions or along petioles. The maximum disease incidence was recorded in Muthorai (95.63%) followed by Palada (87.29%) and Emerald (82.56%). The minimum disease incidence was recorded in Nanjanad (59.37%) followed by Wellington (69.01%) have shown in Fig 1.

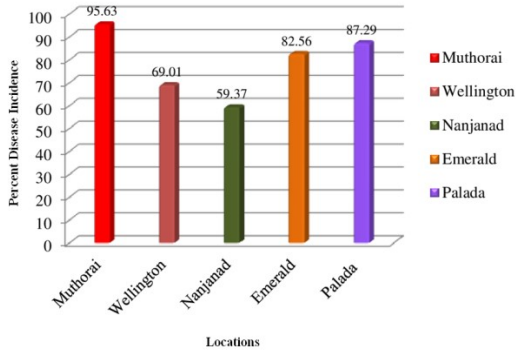


Fig. 1. Incidence of potato late blight in the Nilgiris during *Rabi* 2021-22.

Isolation of *P. infestans*

Mostly *Phytophthora* spp. was hard to isolate, especially from infected potato leaf tissues due to the hasty growth rate of other saprophytic fungi according to Henricot *et al.* (2014). Successful isolation of *Phytophthora* spp. can be accomplished by the use of selective media that either impedes the development of other fungus and bacteria. Five different media *viz.,* potato dextrose agar, corn meal agar, carrot agar, lima bean agar, and potato leaf extract agar were screened for isolation of *P. infestans* from the infected leaf bits (Fatima *et al.,* 2015). In the present study, among all the media tested, V8 PARP (V8 juice amended with pimaricin, ampicillin, rifampicin, penicillin) medium was found effective in supporting the growth of *P. infestans* which minimized the bacterial contamination by the presence of antibiotics supplemented in the medium. The results of the present study was in compliance with findings of (Jadesha *et al*., 2020). Totally, four isolates (PPI1, PPI2, PPI3 and PPI4) were identified form this study (Table 1).

Table 1: Survey and isolation of *P. infestans.*

Sr. No.	Isolate	Cultivar	Location of isolation	GPS Co-ordinates	Accession number
1.	PPI1	Kufri Jyoti	Wellington	11.3789° N 76.7743° E	ON705717
2.	PPI2	Kufri Himalini	Nanjanad	10.5544° N 77.6879° E	ON705718
3.	PPI3	Kufri Jyoti	Muthorai	11.3866° N 76.6710° E	ON705719
4.	PPI4	Kufri Swarna	Emerald	11.3323° N 76.6196° E	ON705720

Morphological characterization

Evaluation of different media on the growth of *P.infestans.* The suitable culture media and physiological conditions for mycelial growth was evaluated by placing of 7 mm mycelial discs into twelve different culture media (Table 2). Among twelve different media evaluated against *P. infestans*, the maximum mycelial growth of pathogen was observed in clarified V8 juice agar (90 mm) followed by carrot agar (89 mm), potato dextrose agar (88 mm), potato carrot agar (79.30 mm), and potato leaf extract agar (70.30 mm). The results are in agreement with George *et al,* (2017) who found the suitability ofV8 juice agar for culturing the *P. infestans*. The minimum mycelial growth was noticed in glucose asparagine agar of 22.70 mm followed by Richard's synthetic agar of 31 mm was recorded. The obtained results were similar to findings of (Henricot *et al.,* 2014) that recorded maximum growth of *P. pachyplura* was noticed in V8 juice agar of 90mm and in potato dextrose agar (84 mm). The least mycelial growth was recorded in glucose asparagine (41.80 mm) was observed in *P. parasitica* (Prasad *et al.,* 2017) which is similar to that of our results.

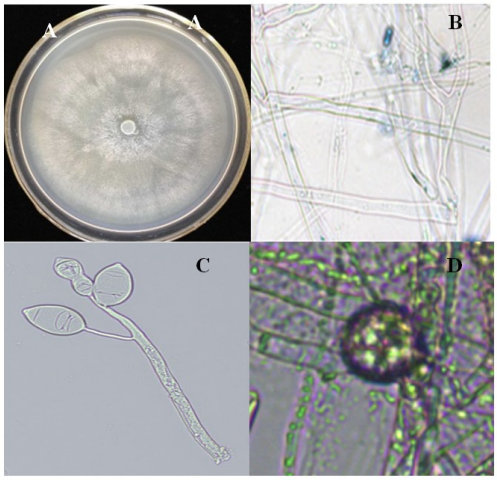
Colony pattern. In the present study, four isolates were obtained from the infected potato leaf and stem samples collected from the Nilgiris district. The colony colour of *P. infestans* was observed cottony white to dull white with rosette pattern. Mycelium was long, aseptate, hyaline, sympodial branching pattern, uniform in diameter. The mycelium was too sensitive and had a watery nature. The pathogen growth was achieved in transparent media. So, thin film of media is enough for establishment of the pathogen under artificial conditions. The sporulation was not observed in culture media. The sporangia were observed in 1% soil suspension with mycelial discs which were incubated for 48-72 hours (Mehmood *et al.,* 2022). The lemoni form sporangium with semi papilla was seen under stereo zoom microscope. The limitations in availability of nutrients and temperature might be the reason for lack of sporulation in *Phytophthora* spp. On the basis of the study of cultural and morphological characteristics, it was concluded that the species closely resembled to *P. infestans* (Prasad *et al.,* 2017). However sporulation was not observed in any of the medium tested.

Table 2: Radial growth and micromorphological characteristics of *P. infestans* (PPI3) on different media.

Sr. No.	Medium	Mean mycelial diameter (mm)	Colony margin	Colony color	Aerial mycelium
1.	Carrot agar	89.00[a]	Uniform	Dull white	Sparsely raised
2.	Clarified V8 juice agar	90.00[a]	Uniform	Dull white	Densely raised
3.	Corn meal agar	57.00[g]	Rosette	Cottony white	Sparsely raised
4.	Czapek's Dox agar	48.70[h]	Wavy	Cottony white	Sparse
5.	Glucose asparagine agar	22.70[j]	Circular	White	sparse
6.	Oat meal agar	76.00[c]	Daisy	Cottony white	Cottony cushion densely raised
7.	Potato carrot agar	79.30[b]	Smooth/wavy	Dull white	Undulated slightly raised
8.	Potato dextrose agar	88.00[a]	Wavy/smooth	Dull white	Densely Raised
9.	Potato leaf extract agar	70.30[d]	Irregular/smooth	Cottony white	Cottony cushion sparsely raised
10.	Richard's synthetic agar	31.00[k]	Irregular	Transparent	Sparse
11.	Saboraud dextrose agar	63.70[i]	Wavy	Transparent	Sparse
12.	Tomato juice agar	67.00[e]	Rosette	Dull white	Sparsely raised

Means for groups in homogenous subsets are displayed.
Means followed by a common letter is significantly different at 5% level by DMRT

Sporulation of *P. infestans.* The study showed that different methods given by research workers were tried for induction of sporangia of *P. infestans* and results were given in Table 3. The result revealed that out of six methods tried, diluted tomato juice broth produced more amounts of lemon shaped sporangia with semi papilla and oospores also observed under stereo zoom microscope. The obtained results were similar to that of Jadesha *et al.* (2020). The sporangial production was formed in a lesser amount in 15 ml of rain water and river water. Non-sterilized liquids and non-sterile soil extracts may induce sporangial development in *P. infestans* (Fig. 2).



A. Mycelial colony on V-8 agar plate; B. *P.infestans* asexual phase: aseptate, hyaline mycelium; C. Lemon shaped sporangium produced at specialized branching structure; D. *P. infestans* sexual phase: oospore

Fig. 2. Morphological features of *P. infestans* (PPI3)

Molecular characterization of *P. infestans.* The internal transcribed spacer (ITS) regions of 18S rDNA were amplified by employing primer pair ITS1 and ITS4 (Cespedes *et al*., 2013). The initial identification was confirmed by the amplicon size of 850 base pairs in all the four isolates (Fig. 3). Similarly, George *et al.* (2017) obtained the amplicon size of 850 base pairs in *Phytophthora* isolates. BLASTn analysis revealed that the sequence of ITS regions of all the four isolates were shown 100% sequence homology with sequences of *P. infestans* in GenBank, NCBI and the nucleotide sequences were submitted and accession numbers were assigned by NCBI GenBank.

Pathogenicity test. Twenty five days old potato plants of highly susceptible variety (Kufri Swarna) was artificially inoculated with *P. infestans* by spraying zoospore suspension method. The first symptom of late blight disease was recorded during the pathogenicity test was almost identical to the symptoms at open field conditions. Initially, a small water soaked lesion of late blight disease was appeared on upper leaf surface during third day after inoculations. The infection progressed and lesion became enlarged with whitish downy growth on its lower surface after fifteen days from inoculation. The fungus was re-isolated from the infected leaves were compared with original culture of *P. infestans.* Jadesha *et al.* (2020) observed the symptom development in redgram after 48 hours of inoculation with *P. infestans* pathogen.

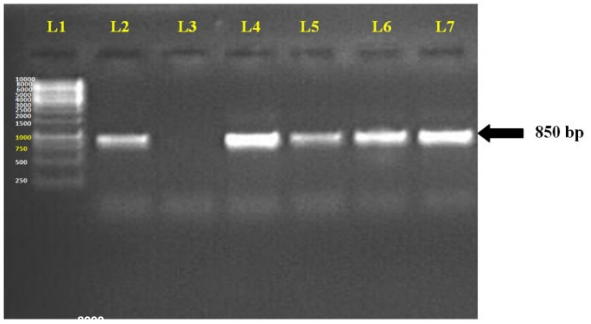
Table 3: Sporulation of *P. infestans* (PPI3) in different source.

Sr. No.	Method	Asexual spores	Sexual spore
1.	River water	+	+
2.	Sterile water	-	-
3.	1% Soil extract	-	-
4.	Pond water	-	-
5.	Rain water	+	-
6.	Diluted tomato juice broth	++	++

- : Absent
+ : Present in lesser amount
++ : Present in abundant quantity

Virulence of *P. infestans* isolates

Artificial inoculation was employed for assessing the virulence of four isolates *viz.,* PPI1, PPI2, PPI3, PPI4 in potato plants (Kufri Swarna) and the results were presented in Table 4. It was found that PPI3 was most virulent with disease incidence of 95.30 percent on 20th day after inoculation followed by PPI1 (81.05 percent). The least virulent with disease incidence was found in PPI4 and PPI3 with percent disease incidence of 73.14 and 66.30 percent, respectively. Hence, the isolate PPI3 was used throughout the study. The infective potential of zoospores and concentrations were observed more in PPI3 when compared to other isolates. The results obtained from this study were similar to that of Widmer *et al.* (2009).



L1-1KB Ladder; L2-Positive Control; L3-Negative Control; L4-PPI 1; L5- PPI 2; L6-PPI 3; L7- PPI 4

Fig. 3. ITS-PCR amplification of *P. infestans* isolates.

Table 4: Pathogenic and morphological characteristics of four different isolates *P. infestans.*

Isolate No.	Shape of sporangia	Papilla	Chlamydospores	Sporangial dimensions (µm)		Pedicel length	Shape and size of oospore	Pathogenicity potential (PDI)
				Length	Breadth			
PPI1	Ellipsoidal	Semipapillate	Absent	53.271	32.475	Medium	Spherical; 30 µm	81.05
PPI2	Ovoid	Semipapillate	Absent	52.059	30.718	Short	Spherical; 30 µm	73.14
PPI3	Lemoniform	Semipapillate	Absent	63.495	42.840	Medium	Globose; 32 µm	95.30
PPI4	Obpyriform	Slightly papillate	Present	42.843	29.718	Long	Spherical; 27 µm	66.30

CONCLUSION

The pathogen, *P. infestans* causes significant yield loss to potato farmers worldwide. This study revealed information on the morphological and molecular characteristics of *P. infestans* from the Nilgiris district of Tamil Nadu. This is important for understanding host-pathogen interactions and developing disease control strategies.

FUTURE SCOPE

The past decade has seen significant progress in molecular approaches to the species-specific identification and diagnosis of *P. infestans*. However, there are still many untapped areas where further advances could enhance research capabilities. This includes developing advanced diagnostic techniques that eliminate the time required to process samples. Many oomycete pathogens, especially *P. infestans* play a magical role in the ecosystem. Therefore, these techniques can be used to determine the interactions between *P. infestans* and potato at the molecular level.

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Conflict of Interest: None.

REFERENCES

Ahmed, N., Khan, M. A., Khan, N. A. & Ali, M. A. (2015). Prediction of potato late blight disease based upon environmental factors in Faisalabad. *Pakistan Journal of Plant Pathology and Microbiological Studies*, *3*.

Cespedes, M. C., Cárdenas, M. E., Vargas, A. M., Rojas, A., Morales, J. G., Jiménez, P. & Restrepo, S. (2013). Physiological and molecular characterization of *Phytophthora infestans* isolates from the Central Colombian Andean Region. *Revista Iberoamericana de Micologia*, *30*(2), 81-87.

Chowdappa, P., Mohan Kumar, S. P., Sanjeev, S. & Singh, B. P. (2011). Integrated management of early and late blight of potato and tomato. *ORP on leaf spot diseases series*, 17.

Chowdappa, P., Nirmal Kumar, B. J., Madhura, S., Mohan Kumar, S. P., Myers, K. L., Fry, W. E. & Cooke, D. E. L. (2015). Severe outbreaks of late blight on potato and tomato in South India caused by recent changes in the *Phytophthora infestans* population. *Plant Pathology, 64*(1), 191-199.

Dhingra, O. D. & Sinclair, J. B. (1985). Culture media and their formulas. *Basic Plant Pathology Methods. O. Dhingra and J. Sinclair, eds. CRC Press: Boca Raton, FL*, 285-315.

Gomez, K. A. & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & sons.

Fatima, K., Noureddine, K., Henni, J. E. & Mabrouk, K. (2015). Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-west of Algeria. *International Journal of Agronomy and Agricultural Research, 6*(4), 44-53.

Fry, W. (2008). *Phytophthora infestans*: the plant (and R gene) destroyer. *Molecular plant pathology. 9*(3). 385-402.

Henricot, B., Pérez Sierra, A. & Jung, T. (2014). *Phytophthora pachypleura* sp. nov., a new species causing root rot of *Aucuba japonica* and other ornamentals in the United Kingdom. *Plant Pathology*, *63*(5), 1095-1109.

Hussain, T., Singh, B. P. & Anwar, F. (2014). A quantitative Real Time PCR based method for the detection of *Phytophthora infestans* causing Late blight of potato, in infested soil. *Saudi journal of biological sciences, 21*(4), 380-386.

Jadesha, G., Sharma, M., & Reddy, P. N. (2020). Illustration of Key Morphological Characteristics of *Phytophthora cajani*-Pathogen of Phytophthora Blight of Pigeonpea. *Legume Research-An International Journal*, *1*, 7.

Malvi, S., Pali, P., Kharte, S. & Sinha, S. (2022). Chapter-8 Detection, Diagnosis and Identification of *Phytophthora* species and their management. *Advanced Research in Crop Diseases and their Management,* 112.

Martin, F. N., Abad, Z. G., Balci, Y. & Ivors, K. (2012). Identification and detection of Phytophthora: reviewing our progress, identifying our needs. *Plant disease, 96*(8), 1080-1103.

Mehmood, B., Azad, A., Rahim, N., Arif, S., Khan, M. R., Hussain, A. & Jamil, M. (2022). Management of Late Blight of Potato caused by *Phytophthora infestans* through Botanical Aqueous Extracts. *International Journal of Phytopathology, 11*(1), 35-42.

Montarry, J., Glais, I., Corbiere, R. & Andrivon, D. (2008). Adaptation to the most abundant host genotype in an agricultural plant–pathogen system–potato late blight. *Journal of evolutionary biology, 21*(5), 1397-1407.

Naroei, K. & Salari, M. (2015). Reliable detection of the fungal pathogen, molecular detection and identification *Fusarium oxysporum*. In *Biological Forum 7*(2), 473-492.

Nath, V. S., Hegde, V. M., Jeeva, M. L., Misra, R. S., Veena, S. S., Raj, M. & Sankar, D. S. (2015). Morphological, pathological and molecular characterization of *Phytophthora colocasiae* responsible for taro leaf blight disease in India. *Phytoparasitica, 43*(1), 21-35.

Padmaja, G., Uma Devi, G., Kanaka Mahalakshmi, B. & Sridevi, D. (2015). Compatibility of phylloplane microflora of taro and fungicides in managing *Phytophthora colocasiae*. *Society for Scientific Development in Agriculture and Technology Meerut (UP) INDIA and Progressive Research–An International Journal, 10*, 1437-1440.

Pande, S., Sharma, M., Mangla, U. N., Ghosh, R. & Sundaresan, G. (2011). Phytophthora blight of Pigeonpea [*Cajanus cajan* (L.) Millsp.]: An updating review of biology, pathogenicity and disease management. *Crop Protection, 30*(8), 951-957.

Rangaswami, G. (1958). An agar blocks technique for isolating soil microorganisms with special reference to Pythiaceous fungi. Science and Culture. 24: 85.

Robin, C., Smith, I. & Hansen, E. M. (2012). *Phytophthora cinnamomi*. *Forest Phytophthoras. 2*(1).

Tutte, J. (1969). Plant pathological methods Fungi and bacteria. Burgess publishing company. Minnesota. USA. 229

Vanegas, D. M., Navarro, R. A., Alfanador, L., Gutierrez, J. A., Morales, J. G. & Gaviria, B. M. (2022). Characterization of *Phytophthora nicotianae* isolates from tobacco plants (*Nicotiana tabacum*) in Colombia. *Revista Ceres, 69*, 78-91.

Vasudeva, R. S., Subbaiah, T. V., Sastry, M. L. N., Rangaswamy, G. & Iyengar, M. R. S. (1958). 'Bulbiformin', an antibiotic produced by Bacillus subtilis. *Annals of Applied Biology, 46*(3), 336-345.

Wang, E. S., Kieu, N. P., Lenman, M. & Andreasson, E. (2020). Tissue culture and refreshment techniques for improvement of transformation in local tetraploid and diploid potato with late blight resistance as an example. *Plants, 9*(6), 695.

Wangsomboondee, T. & Ristaino, J. B. (2002). Optimization of sample size and DNA extraction methods to improve PCR detection of different propagules of *Phytophthora infestans*. *Plant Disease, 86*(3), 247-253.

White, P. J., Bradshaw, J. E., Finlay, M., Dale, B., Ramsay, G., Hammond, J. P. & Broadley, M. R. (2009). Relationships between yield and mineral concentrations in potato tubers. *Hort Science, 44*(1), 6-11.

Widmer, T. L. (2009). Infective potential of sporangia and zoospores of *Phytophthora ramorum*. *Plant Disease, 93*(1), 30-35.

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