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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS* ASSOCIATED WITH POTATO LATE BLIGHT IN PAKISTAN

ARSH BIBI[1], IRSHAD ULLAH[2], UMER IQBAL[3], IRFAN ULLAH[4], ALI SHAHWAIZ[5] AND DENNIS HALTERMAN[6]*

[1]Agricultural Biotechnology Division, National Institute for Biotechnology and Genetic Engineering, Constituent College Pakistan Institute of Engineering and Applied Sciences, Faisalabad, Pakistan
[2]Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan
[3]Plant Genetic Resources Institute, National Agriculture Research Center, Islamabad, Pakistan
[4]Department of Biotechnology and Genetic Engineering Hazara University, KP Pakistan
[5]Department of Biotechnology Government College University Faisalabad (GCUF), Pakistan
[6]Vegetable Crops Research Unit USDA, Madison -Wisconsin, U.S.A

*Corresponding author's email: dennis.halterman@usda.gov

Abstract

Phytophthora infestans, the source of potato late blight, poses a danger to the world's potato crop, resulting in significant financial losses in contemporary agriculture and impairing farming in developing countries. In this work, isolates of *P. infestans* obtained from different fields at the National Agriculture Research Center (NARC) in Islamabad during the 2023 growing season were identified and characterized. *Phytophthora infestans* was isolated using pea agar and rye-A agar medium. For this research, seventy-two isolates of late blight were collected and separated from Potato (*Solanum tuberosum*) leaves. The pathogenicity of isolates was determined by their capacity to induce disease and symptoms including yellowing, wilting, or plant death. Both morphological and molecular techniques were used in the identification procedure, including PCR directed at the ITS and PINF regions. Morphological analysis revealed characteristics such as terminal, semi-papillate sporangia that were caducous with short pedicels and non-proliferating sporangiophores, indicating a heterothallic nature. Molecular identification was confirmed using the IDphy online resource and the NCBI database, where the ITS sequences of the isolates showed a 99-100% match with known sequences of *P. infestans* in clade b. This research confirmed the presence of *P. infestans* in Pakistan for the first time using these methods and highlighted the pathogen's high genetic similarity to global strains. The research suggests the need for further studies to explore the variety of *P. infestans* populations within Pakistan further.

Key words: Late blight; Molecular identification; Pakistan; *Phytophthora infestans;* Potato

Introduction

Phytophthora infestans, the source of potato late blight disease, poses a danger to the world's potato crop, resulting in significant financial losses in contemporary agriculture and limiting existence farming in under-developing countries (Kamoun & Smart, 2005; Fry, 2008). *P. infestans* functions similarly to a biotroph in nature but can also be cultured in laboratories (Hammond-Kosack & Parker, 2003). *Phytophthora* species undergo various cellular stages during their infection cycle. The initial dispersal stages include uninucleate, motile zoospores and multinucleate sporangia. Sporangia of *P. infestans* may be dispersed into a new host by splashing water or wind, where they can either germinate or release zoospores to start an infection. Once released, zoospores shed their flagella and form a cyst by synthesizing cell walls. Within hours, the cyst germinates and penetrates host tissue either through stomata or by developing an appressorium-like swollen germ tube. From this primary infection vesicle, spherical hyphae emerge and spread throughout the plant tissue (Avrova *et al.*, 2008). There are different specialized cell types and transition between them is regulated from one stage to next as shown in Fig. 1. If potato tubers are infected, they may become fully damaged while being stored before consumption. Diseased seed tubers may harbor spores that travel to other parts of the plant, to other plants in the field, and even, via international trade, to distant fields. The entire plant may sometimes dry out and die in a few weeks (Widmark, 2010; Castañeda-Álvarez *et al.*, 2015). The pathogen can cause yield losses ranging from 20-100% depending on variety susceptibility, climatic conditions, and control measures implemented (Jimoh and Oluwayomi, 2023). In Pakistan specifically, late blight can induce 100% yield losses under epidemic conditions (Nadeem Ahmed *et al.*, 2015).

Late blight caused by *P. infestans* was reported in Pakistan during the 1980s, with early observations from the Swat valleys (Kalam & Malam Jabba). Subsequent studies confirmed the presence of two mating types in Punjab (Ahmad &Mirza, 1995; Batool *et al.*, 1999). Further investigation was reported on the type A2 mating in potato-growing regions of Punjab and Khyber Pakhtoon Khwa (KPK) in Pakistan over three seasons between 1997-2000, while the type A1 mating was reported in 1999-2000 (Ahmad *et al.*, 2002). One hundred seventy-eight isolates of *P.*

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infestans were investigated against metalxyl sensitivity to minimize potato yield loss in Punjab, the lower valley of KPK , upper Swat valley, khagan, and Azad Jammu Kashmir (AJK) in 2004 (Ahmad *et al.*, 2008). From Punjab region, one hundred forty-nine isolates were acquired from six host potato cultivars ; 73% were identified as A1, 23% as A2 type mating, and 4% as self-sterile (Raza *et al.*, 2021). However, variation in the population structure of *P. infestans* was evaluated based on mtDNA haplotype, RFLP, peptidase, mating variety, glucose-6-phosphate isomerase (Pep), and SSR genetic markers (Cooke & Lees, 2004; Pule *et al.*, 2013). In developing countries like Pakistan, such techniques are either unavailable or expensive. Additionally, conventional isolation and identification of *P. infestans* would be time-consuming and challenging. It is becoming increasingly clear that reliable and comprehensive oomycete characterization and verification can only be achieved through an elaborative method. For accurate results it is essential to combine both conventional methods and modern molecular biological methods (Gautam *et al.*, 2022). Polymerase chain reaction (PCR) has an advantage over the conventional methods for diagnosing disease as it is a sensitive, rapid, and versatile technique (Trout *et al.*, 1997). Hence, a simple and cost-friendly method like PCR might help rapidly detect *P. infestans*. The prime objectives of this study were to identify isolates of *P. infestans* based on morphological and molecular identification, and detection through a PCR method using primers reported by Trout *et al.*, (1997). In Pakistan, the characterization of *P. infestans* populations remains poorly understood. To the best of our understanding, previous literature on *P. infestans* have focused primarily on appearance and exterior manifestation of late blight disease symptoms, as well as the pathogen's mating type (Ahmad & Mirza, 1995; Ahmad *et al.*, 2008; Raza *et al.*, 2021).

Materials and Methods

Study area and samples collection: Potato leaves exhibiting distinct symptoms of late blight disease were gathered during the 2023 growing season. The infected samples were gathered from various areas within the National Agriculture Research Center (NARC), Islamabad. Fresh, actively sporulating lesions were selected from five fields, with two leaves taken from each plant. The collected samples were transported to the lab at (4˚C) in sterile clear polythene bags. In all, 72 leaf samples were collected from the five fields, mostly from the variety Lady Rosetta, though other varieties were also included.

Isolation of *Phytophthora infestans*: Parts from infected leaves and healthy tissue were cut into slices, surface sterilized with 0.1% mercuric chloride solution for 1 minute and rinsed thrice with sterilized autoclaved water. The surface was dried over sterilized filter paper and plated on rye agar media , and the pea agar media (Gamboa *et al.*, 2019) was amended with antibiotics Rifampicin 75µg/mL, vancomycin 100µg/mL, and anti-fungi nystatin 75µg/mL and pentachloronitrobenzene 100µg/mL. It was placed in the dark at 18 ± 1˚C for 10 to 15 days. Mycelia were examined under a microscope to check for the accurate type of sporangiophore.

Morphological identification: To determine the *P. infestans* specimens based on their morphology, the protocols of Drenth and Sendall and Martin *et al.*, (Drenth & Sendall, 2001; Martin *et al.*, 2012) were utilized. Important morphological features such as colony morphology, the shape of the sporangium, and hyphae were noticed. For analysis of morphological characters, a seven-day-old culture was used. The sporangia were dislodged in 10 µL of lactophenol cotton blue (20 mL lactic acid, 40 mL glycerol, 20g phenol crystal, 0.05g aniline blue and 20mL of distilled water). The coverslip was placed on the top of lactophenol cotton blue carefully to avoid any bubble formation. Slides were observed under a compound microscope at 40x and 100x. The reading of sporangia shape and size was noted for identification.

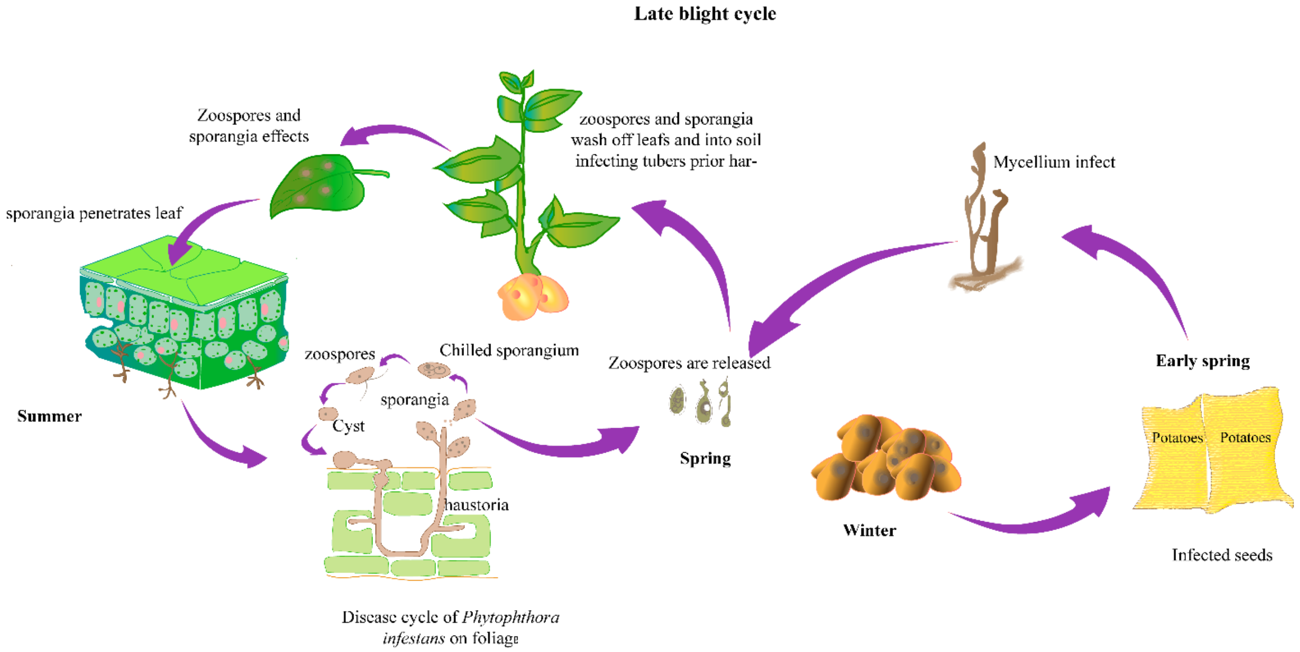

Fig. 1. Diagrammatic representation of life cycle of *Phytophthora infestans*. The life cycle begins with infected tubers or plant debris in early spring. Sporangia form and release zoospores that infect foliage, leading to rapid disease spread during summer. The pathogen invades stems and tubers, which serve as inoculum for the next season. In winter, it overwinters in infected tubers, completing the cycle.

Genomic DNA extraction: Sporangia and mycelia from *P. infestans* cultures aged 10–15 days were harvested using a scalpel. A 10 mg tissue sample was placed into a 1.5mL tube with 200 µL of extraction buffer containing 0.1 mM Tris-HCl (pH = 8), 0.5 mM of EDTA, 3% of SDS, and 1 M of NaCl. The tissue was homogenized using a sterile plastic micropipette. Subsequently, 200 µL of 24 parts (191.7 µL) of chloroform and 1 part (8.33 µL) of isoamyl alcohol were added and incubated at 65°C for 5 minutes. The samples were placed at room temperature and then centrifuged (12000 rpm) at 4°C for 5 minutes. An equal proportion of isopropanol was added, and the supernatant was placed into a new sterile 1.5mL tube. The solution was gently mixed and incubated for 20 minutes at – 20°C. After incubation, it was centrifuged (10,000g) for 5 min at 4°C. The supernatant was carefully discarded, and the DNA pellet was washed with 70% ethanol. Finally, the DNA pellet was centrifuged at 12,000 rpm for 1–2 minutes. The pellet was then air-dried and kept at room temperature for time of 10 minutes and resuspended in nuclease-free water (20–30 µL). The concentration of the DNA samples was quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific) and working dilutions of 50 ng/µL were prepared. DNA was stored at – 20 °C for further processing.

Molecular analysis through PCR: Twenty µL of PCR volume was used for the PCR analysis and the confirmation of *P. infestans* isolates. About 50 ng of DNA was used in the PCR mixture of *P. infestans* shown in Table 1. The 32X thermocycling conditions included an initial step of denaturation at 95°C for 2 minutes, denaturation at 95°C for 1 minute, annealing for O8 and AE7 primers at 52°C, and 55°C for PINF2-ITS3 and PINF-ITS5 primer for 30 seconds, initial extension at 72°C for 1 minute and then final extension at 72°C for 5 minutes was performed using thermal cycler (Bio Rad, USA). The primer sequences are listed in Table 2. The reaction holding temperature was 4°C. Negative control was used to monitor for PCR contamination. A 1% of agarose gel was utilized to visualize the targeted bands on gel-documentation system (UV based) and 1 kb DNA ladder (ThermoFisher Scientific) was utilized as a reference.

Table 1. PCR master mix composition for *Phytophthora infestans* detection.

Reagents used	Total volume (20 µL)
DNA (50 ng)	1 µL
FIREPOL Master Mix (Solis BioDyne, Europe) 2X	4 µL
Forward primer (10 pmol/µL)	1 µL
Reverse (10 pmol/µL)	1 µL
PCR-grade water	13 µL

Table 2. Primers utilized for molecular identification of *Phytophthora infestans*.

Primer name	Primer sequence (5' to 3')	Size (bp)
AE7-1	GCCGCCGACATATTGAAT	171
AE7-2	CAAATCTGCGAACGAGACAT	
O81	AAGATGATGTTGGATGATTG	245
O82	TGCCTGATTTCTACCTTCT	
O83	GAAAGGCATAGAAGGTAGA	258
O84	TAACCGACCAAGTAGTAAA	
PINF2	CGATTCAAATGCCAAGCTAAAG	456
ITS3	GCATCGATGAAGAACGCAGC	
PINF	CTCGCTACAATAGGAGGGTC	600
ITS5	GGAAGTAAAAGTCGTAACAAGG	

DNA sequencing: The amplified products from *P. infestans* isolates, using the *PINF 2* and *ITS 3*-primer set, were purified with the DNA Cleanup Micro Kit (K0832) (Thermo Fisher Scientific, USA) following the protocol directed by manufacturers. The purified PCR products were then sent to Macrogen, Korea, for DNA sequencing

Sequence analysis and alignment: De novo sequence assembly was performed utilizing BioEdit soft 7.7.1 to generate contiguous sequences from paired reads. Sequence homology analysis was subsequently conducted via BLAST queries against nucleotide databases curated by the NCBI. Species-level identification was inferred by phylogenetic comparison of the generated contigs with representative sequences deposited to the NCBI GenBank database. Following species confirmation, the generated sequences were submitted to the GenBank database within the International Nucleotide Sequence Database (INSDC) guidelines, and unique accession numbers were assigned. Multiple sequence alignment (MSA) was conducted utilizing the Clustal W algorithm implemented in Molecular Evolutionary Genetics Analysis (MEGA) soft 11.0.13.

Evolutionary relationships of taxa: Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987). A bootstrap consensus tree, derived via 1000 bootstrap replicates, was constructed to visualize the evolutionary relationships among the analyzed taxa. Branches below 50% bootstrap values collapsed to resolve polytomies. Bootstrap support values, representing the percentage of replicate trees (out of 1000) in which the associated taxa clustered together, are displayed adjacent to the corresponding branches (Felsenstein, 1985). Evolutionary distances were computed using the Jukes-Cantor (JC) model (Jukes & Cantor, 1969), expressed as the number of nucleotide substitutions per site. The phylogenetic analysis encompassed 27 nucleotide sequences, with all unknown base calls eliminated pairwise utilizing the full delete option. The final aligned dataset comprised 423 nucleotide positions. All phylogenetic analyses were conducted using MEGA11 software (Tamura *et al.*, 2021).

Pathogenicity assay: The isolated pathogenicity was confirmed by applying Koch's postulates for this purpose a detached leaf bioassay was performed using (Karki *et al.*, 2021) with a modification. Healthy potato leaves were disengaged from 4–6-week-old plants grown in the greenhouse, leaves were sterilized using Clorox, washed with autoclaved distilled water for 10 minutes, and placed on sterilized filter paper to remove moisture. The isolate was grown on Rye A agar media for 10-14 days at 18°C. To release zoospores, 5mL of sterile cold water at 4˚C was poured into plates, sporangia were mixed using a spreader while avoiding scratching media. After removing mycelia with two layers of cheesecloth, zoospores were collected and diluted in 20 milliliters of ice-cold, sterile water and transferred into 50 falcon tubes that were kept at 4°C for one to four hours to promote sporulation. Zoospore concentration was measured using a hemocytometer under a compound light microscope and the concentration was adjusted to 10,000 zoospores/mL. One leaf was placed on a Petri plate with moistened sterilized filter paper, 2-4 of 10 µL droplets of inoculum per leaf were kept on the abaxial side of every leaflet with Eppendorf. Plates were covered with their lids and kept at 21°C in a room with natural light. Pathogen was re-isolated and examined microscopically, results found to be similar when compared with natural environment.

Results

Symptoms of potato late blight: Under favorable conditions, the late blight pathogen establishes itself and can rapidly devastate the entire potato plant. Initial detection of *P. infestans* infection is based on visual examination of typical signs, including water-soaked, black to brown spots and the presence of white cottony on the abaxial layer of the leaves. Stems may also develop black to dark brown spots accompanied by sporulation (Fig. 2). A survey of potato late blight disease was conducted across multiple fields at the National Agriculture Research Center (NARC), Islamabad, during December 2023 growing season. Seventy-two leaves from five different fields were collected and analyzed in this study (Table 3).

Morphological identification: Morphological characteristics of *P. infestans* were assessed following a 2–3-week incubation period at 18°C under conditions of complete darkness. The colonies exhibited a petaloid shape with white coloration, and the mycelia were aseptate. Examination of the sporangial morphology revealed terminal, semi-papillate, and caducous sporangia with short pedicels. The sporangia were non-proliferating, ovoid in shape, and the sporangiophores were compound sympodial. The organism was identified as heterothallic based on these features (Fig. 3 A to H).

Molecular identification and confirmation of *Phytophthora infestans*: To ensure the rapid and specific identification of *Phytophthora infestans*, a combination of high-copy-number primer sets, and sequence-based molecular tools was employed. The O8 and *AE7* primer families, originally designed by Judelson & Tooley (2000), were utilized due to their high sensitivity in detecting *P. infestans* DNA. These primers *O8-1/O8-2*, *O8-3/O8-4*, and *AE7-1/AE7-2* were able to amplify target DNA with a 100-fold higher sensitivity compared to traditional ITS-based assays. Strong amplification was observed with minimal nonspecific binding to other *Phytophthora* species or plant DNA, confirming their specificity. These results provided reliable molecular confirmation for the field isolates Arsh5PI2023 and Arsh9PI2023.

Further confirmation was carried out using standard molecular identification protocols, including sequencing of the *internal transcribed spacer* (*ITS*) and *ribosomal RNA* (*PINF*) regions. Sequences were analyzed using IDTool.org and compared against the NCBI GenBank database. BLAST analysis revealed 99–100% identity with known *P. infestans* sequences, placing the isolates confidently within clade 1b. The sequences were subsequently submitted to GenBank and assigned accession numbers PP733163 (Arsh5PI2023) and PP733162 (Arsh9PI2023). Phylogenetic analysis supported these findings, showing clear clustering of the isolates within clade 1b of *Phytophthora*, further validating their taxonomic position (Figs. 4 and 5).

Evolutionary relationships among taxa: Phylogenetic relationships were reconstructed using the Neighbor-Joining (NJ) method (Saitou & Nei, 1987), A bootstrap consensus tree, generated from 1000 bootstrap replicates, was used to infer the evolutionary relationships among the analyzed taxa (Fig. 5). Branches with below 50 % bootstrap values collapsed to resolve polytomies. Bootstrap support values, representing the percentage of replicate trees in which associated taxa clustered together during the bootstrap analysis, are displayed adjacent to the corresponding branches (Felsenstein, 1985) Evolutionary distances were computed using the Jukes-Cantor (JC) model (Jukes & Cantor, 1969) and are expressed as the number of nucleotide substitutions per site. 27 nucleotide sequences were included in the examination, and pairwise deletion of doubtful base calls was done utilizing the full delete option. The last final aligned dataset comprised 423 nucleotide positions. All phylogenetic examined was done utilizing software MEGA11 (Tamura *et al.*, 2021).

DNA barcode and QR code of isolated samples: The obtained nucleotide sequences were further used to convert the DNA sequence data into codes such as (DNA barcode) and QR code (quick response code) for conservation purposes and future studies (Fig. 6; Table 4).

Table 4. Descriptive information of DNA barcode sequences of both query sequences along with references database sequences in molecular and evolutionary analysis.

Total characters	Conserved	Variable	Parsimony informative	Singleton
423	414	7	1	6

Pathogenicity assay and confirmation through Koch's postulates: Koch's postulates were fulfilled and *Phytophthora infestans* isolates pathogenicity were confirmed under controlled laboratory environment. Healthy, disease-free potato plants were selected, sterilized and inoculated with sporangial suspensions (1 × 10^{10} sporangia/mL) derived from pure cultures of representative isolates Arsh5PI2023 and Arsh9PI2023. Plants were kept at 18°C with over 90% humidity to replicate favorable disease conditions. Within 4-5 days post-inoculation, water-soaked lesions and white sporulation on the abaxial leaf surfaces consistent with late blight disease were observed (Fig. 7). Pathogen re-isolation from symptomatic tissues was accomplished and the re-cultured isolates were morphologically and molecularly identical to the original isolates. The isolates were confirmed to induce late blight disease and thus fulfilled Koch's postulates.

Table 3. *Phytophthora infestans* isolates collected from NARC, Islamabad during the 2023 sampling season.

Isolate name	Place	No of samples	Sampling year
Arsh5PI2023	NARC, Islamabad	27	2023
Arsh9PI2023	NARC, Islamabad	45	2023

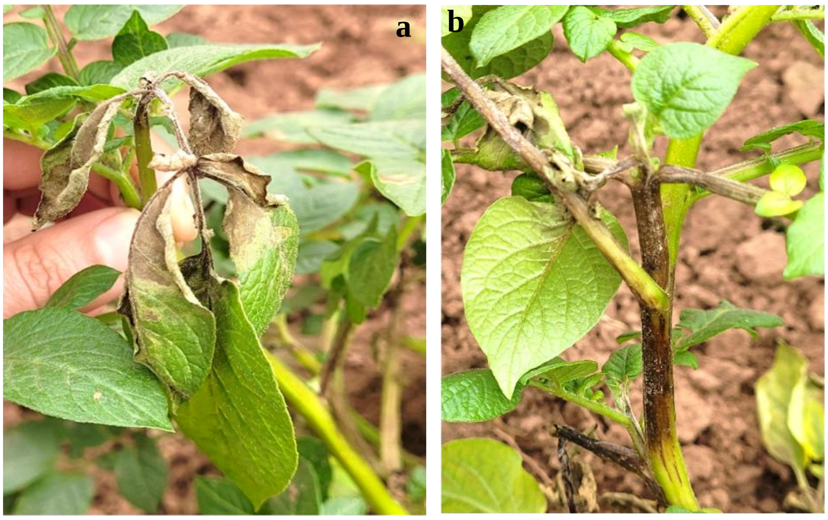

Fig. 2. Typical stem lesions caused by *Phytophthora infestans* on potatoes. The dark brown to black necrotic region is a characteristic symptom of late blight infection, often accompanied by water-soaked margins, leading to collapse of the affected tissues. b) Foliar symptoms of late blight on potato leaves. The image shows wilted and necrotic leaf tissue with dark brown lesions, indicating active *Phytophthora infestans* infection under humid conditions.

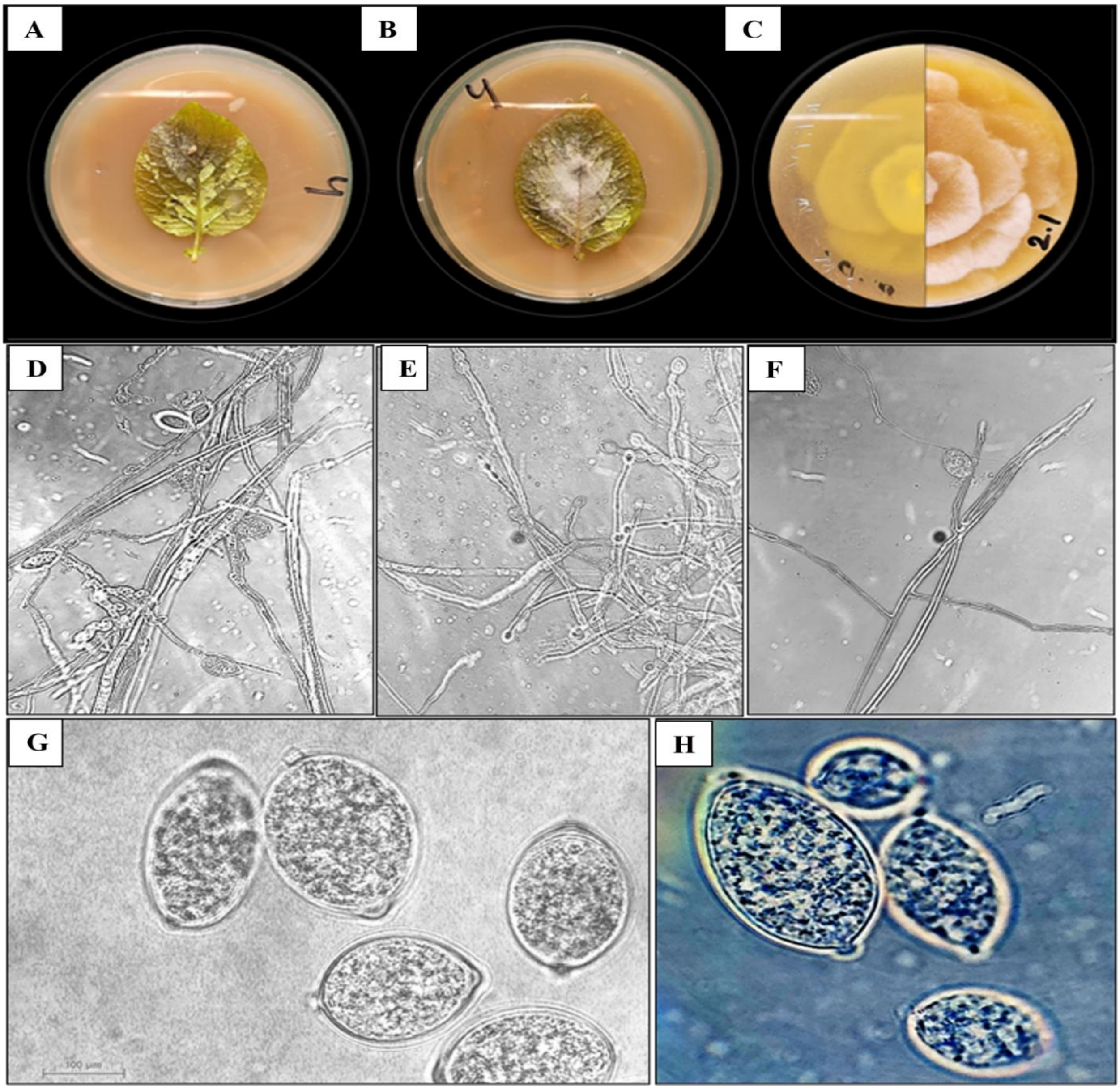

Fig. 3. Morphology and developmental stages of *Phytophthora infestans*. (a–b) Growth and development of *P. infestans* on detached potato leaves placed on rye agar, showing sporulation and lesion formation. (c) Front and reverse view of a mature *P. infestans* culture on Rye- agar, exhibiting characteristic white fluffy mycelium. (d–f) Microscopic images showing extensive, branching hyphal networks of *P. infestans* isolates. (g–h) Sporangia at 100× magnification, displaying their ovoid shape and internal contents. These structures are responsible for producing zoospores and play a crucial role in rapid disease spread under moist conditions.

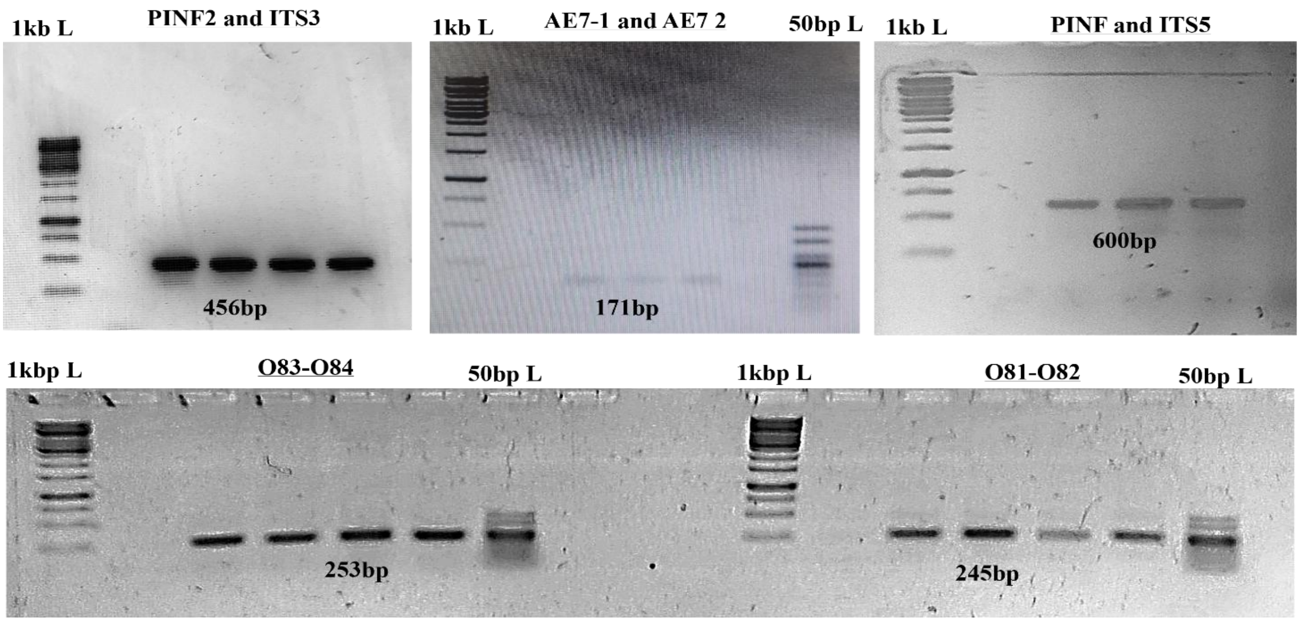

Fig. 4. PCR amplification of *P. infestans* using different primers for identification. Distinct amplicons of expected sizes were observed for each primer pair: 456 bp with *PINF2* and *ITS3*, 171 bp with *AE7-1* and *AE7-2*, 600 bp with *PINF* and *ITS5*, 253 bp with *O83-O84*, and 245 bp with *O81-O82*. Clear and specific bands indicate successful amplification of target regions, confirming the presence of *P. infestans* DNA. A 1 kb DNA ladder (ThermoFisher Scientific) was used as a molecular size reference; a negative control was run adjacent to the DNA ladder.

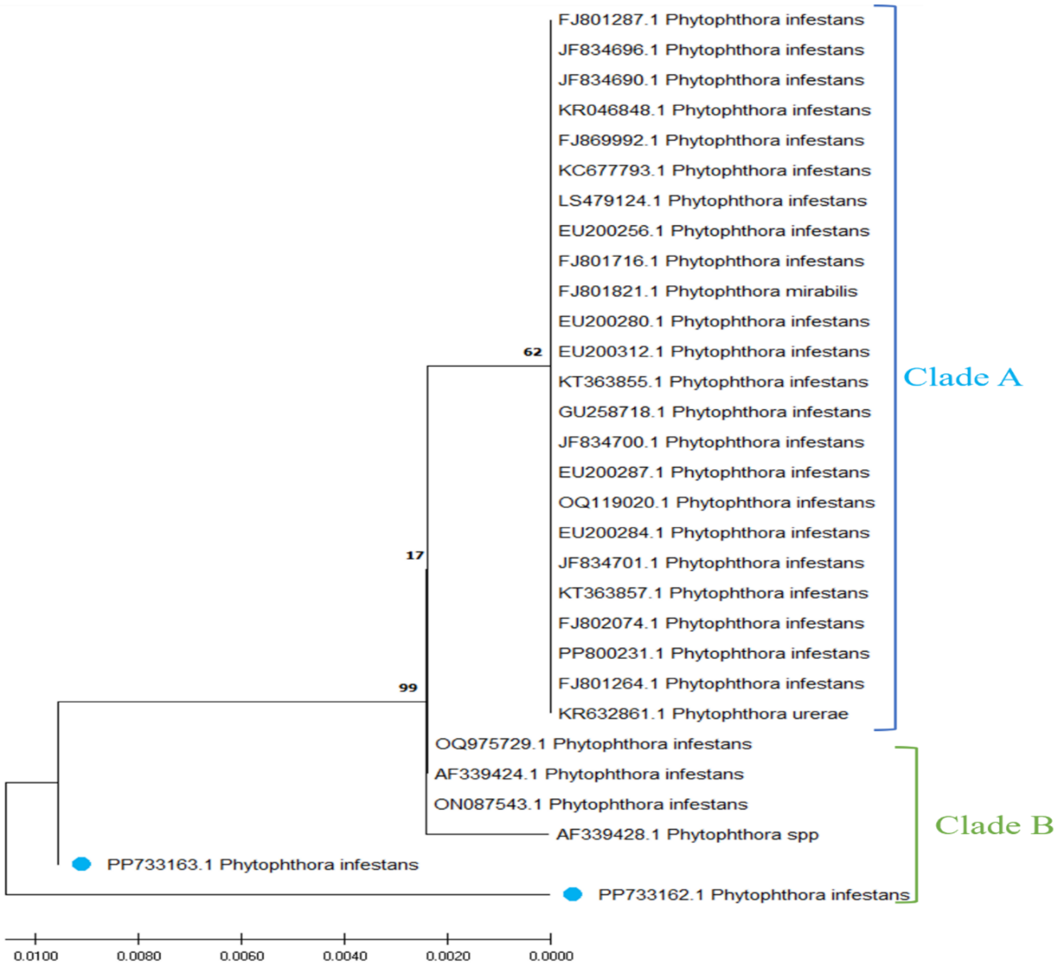

Fig. 5. Phylogenetic tree showing the evolutionary relationships among 27 *Phytophthora* isolates based on partial gene sequences. The tree was generated using the Neighbor-Joining method with Jukes-Cantor distances. Bootstrap values (1000 replicates) are shown at each node. The tree highlights genetic divergence among isolates and supports species-level differentiation. The final alignment included 423 nucleotide positions, and all analyses were performed using MEGA11.

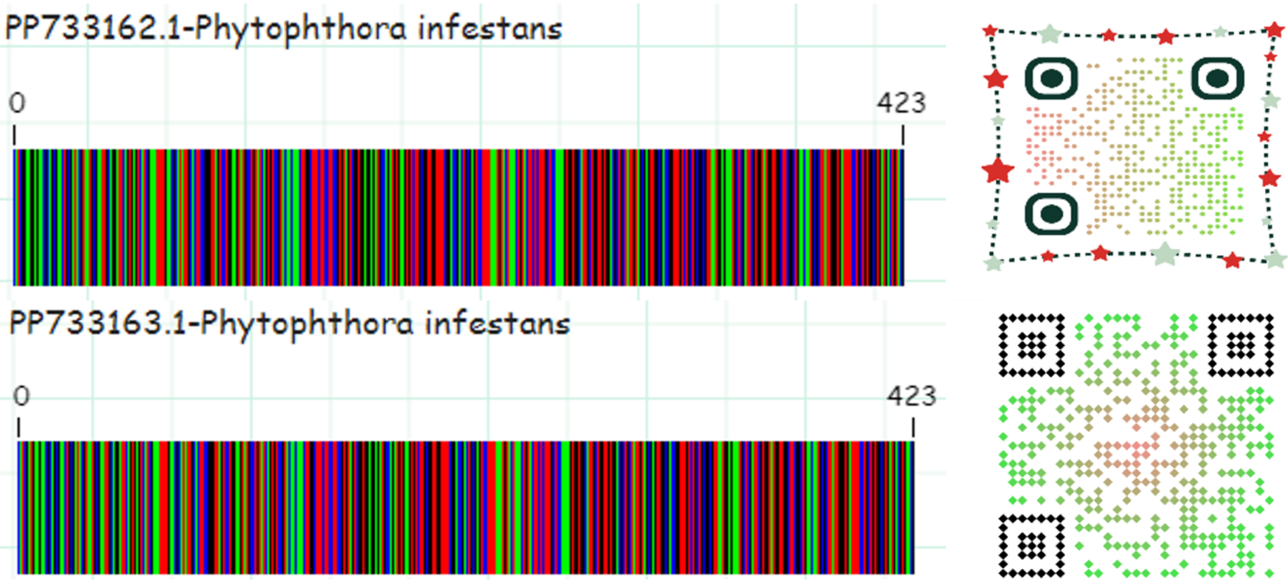

Fig. 6. DNA barcode and QR code of ITs marker for the query samples.

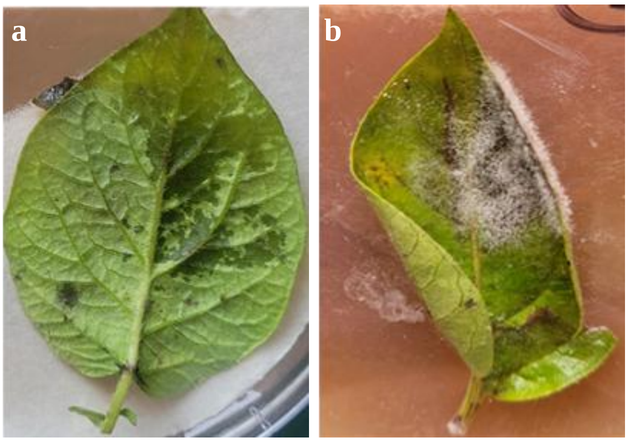

Fig. 7. Early signs of infection: (a) water-soaked lesions (b) *P. infestans* sporulation: white cottony sporangia on the underside of the leaf.

Discussion

This research presents the first holistic morphological and molecular description of *Phytophthora infestans* isolates Arsh5PI2023 and Arsh9PI2023 obtained from infected potato plants at the National Agriculture Research Center (NARC), Islamabad. *Phytophthora infestans* causing late blight is still among the most damaging potato crop diseases globally, frequently resulting in extensive yield loss and heavy economic losses. In this study, 72 isolates were isolated from symptomatic leaves in the 2023 crop season. The pathogen was cultured on both pea agar and rye-A agar. The morphological characteristics were indicative of typical *P. infestans* features, such as semi-papillate, terminal, caducous sporangia with short pedicels and non-proliferating sporangiophores. These results are in good harmony with the traditional descriptions of *P. infestans* sporulation and growth pattern on artificial media, as presented by (Erwin & Ribeiro, 1996), and reaffirmed in recent molecular research (Azimova *et al.*, 2024).

Molecular analysis with *PINF* and *ITS* primers was done for species confirmation. PCR amplification of the internal transcribed spacer (*ITS*) regions followed by sequencing showed 99–100% identity with known *P. infestans* strains from clade b, using both the NCBI and IDphy databases. ITS-based species identification is a widely used and effective method for the detection of *P. infestans*, and species-specific primers targeting *ITS1* and *ITS2* regions have been successfully applied for rapid diagnosis in several studies (Trout *et al.*, 1997; Judelson & Randall, 1998). However, the *ITS* region alone has certain limitations, particularly due to the heterogeneity of nucleotide sequences in oomycetes (Azimova *et al.*, 2024; Bibi *et al.*, 2024). As a result, several other genetic markers have been evaluated to improve resolution, including *cox1, cox2, β-tubulin (β-tub), EF1-α, enolase,* heat shock protein 90, *28S rDNA*, and *tigA* gene fusion protein. These additional loci have shown promising results in distinguishing closely related *Phytophthora* species (Robideau *et al.*, 2011; Choi *et al.*, 2015; Azimova *et al.*, 2024).

In Uzbekistan, sequencing of both *ITS* and *cox2* genes allowed for successful identification of the *P. infestans* isolate TVKT-1. Notably, single nucleotide polymorphisms (SNPs) were observed in both regions, which reflect localized genetic divergence. Such mutations could result from the impact of environmental factors, such as the unique eco-biocenosis and changing climatic conditions of Central Asia (Azimova *et al.*, 2024). This finding parallels earlier work suggesting that the *Phytophthora* genome is highly plastic and capable of rapid evolutionary change due to the presence of mobile genetic elements (Choi *et al.*, 2015). Considering the similarities in agro-ecological conditions, such evolutionary potential is highly relevant to Pakistan and supports the need for deeper population genetic studies of *P. infestans* isolates in the country.

The pathogenic potential of Pakistani isolates was confirmed by inoculation assays, which reproduced typical late blight symptoms, including necrotic lesions, leaf chlorosis, and plant wilting. Similar aggressive pathogenicity was documented in the Yemeni isolate Pi Alharethi YEM2021, which produced classic symptoms on detached potato leaves during Koch's postulate testing (Al Harethi *et* *al.*, 2023). Moreover, microscopic analyses from that study identified key features such as coenocytic, multinucleated, aseptate mycelium and papillate lemoniform sporangia (Erwin & Ribeiro, 1996), all of which are consistent with the morphological traits observed in our isolates. Interestingly, the Yemeni study also reported the formation of chlamydospores (Al Harethi *et al.*, 2023), which act as overwintering propagules that survive in the soil and serve as inoculum sources for subsequent cropping seasons. Although chlamydospores were not seen in the present Pakistani isolates, their presence in Yemen indicates that this possible survival mechanism needs to be explored under Pakistani agro-climatic conditions.

The reproductive biology of *P. infestans* is responsible for its adaptability as well as genetic diversity. The pathogen has two mating types, A1 and A2, and in the same population can reproduce sexually and produce thick-walled oospores. These oospores are responsible for long-term survival and enhanced pathogen virulence (Fry *et al.*, 1992; Elansky *et al.*, 2015). Under Yemen conditions, oospore formation was noted in single-pure cultures and documented the presence of homothallic isolates (Al Harethi *et al.*, 2023). Self-fertile strains are a new epidemiological risk factor because they will increase on their own, causing more genotypic variation, more aggressiveness, and more fungicide resistance than the common A1-only populations (Zhu *et al.*, 2016). Homothallic *P. infestans* isolates also appeared in China (Han *et al.*, 2013; Guo *et al.*, 2022), Mexico (Orona *et al.*, 2013), and Egypt (El-Ganainy *et al.*, 2022), showing the world spread of more pathogenic pathogen strains. A comparison of the regions indicated that A2 mating type was predominant (70%) in Algeria, whereas A1 was predominant in Egypt (Rekad *et al.*, 2017; Arafa *et al.*, 2020). These differences highlight the need to determine the mating type structure of Pakistan's native *P. infestans* populations in order to understand their epidemic potential better.

This research's findings are in agreement with other recent publications in Central and West Asia and present new molecular and morphological information about *P. infestans* in Pakistan. The isolates' high genetic compatibility with global populations indicates the potential for pathogen introduction by seed trade or climatic-mediated dispersal. Simultaneously, data from research in Uzbekistan and Yemen show continuing evolution and adaptation of *P. infestans* populations within regions because of local environmental stresses and gene development like SNP mutations and homothallic strains (Azimova *et al.*, 2024). This requires ongoing molecular surveillance, mating type detection, and diversity screening to allow for region-level late blight management programs.

Conclusion

A total of 72 late blight isolates were collected and purified from symptomatic potato foliage during this study. Pathogenicity was confirmed by assessing the isolates' capacity to induce disease symptoms, including chlorosis, wilting, and/or plant necrosis. Morphological characterization was performed both on the host plant Potato (*Solanum tuberosum*) and on artificial culture media to aid in pathogen identification. Molecular analysis, employing PCR replicates of the internal transcribed spacer (*ITS*) and *PINF* regions, identified the causal agent as *Phytophthora infestans*. The resulting sequences were deposited in GenBank under accession numbers PP733163 and PP733162. While this study provides a preliminary identification and characterization of *P. infestans* in Pakistan, further investigation is necessary to elucidate the genetic diversity of *P. infestans* populations within the region over an extended temporal scale.

Availability of Data or Accessibility to Data: The data sets created and examined during this research have been deposited to the NCBI. The ITS and PINF markers were used to get nucleotide sequences. Are accessible in GenBank under accession numbers PP733163.1 and PP733162.1.

Conflict of Interest: The Authors declare that there is no conflict of interest

Authors' Contribution Statements: Conceptualization: Arsh Bibi, Dennis Halterman and Omer Iqbal **Data curation:** Arsh Bibi, Irshad Ullah, Irfan Ullah and Ali Shahwaiz. **Formal analysis**: Arsh Bibi and Irfan Ullah **Funding acquisition:** Dennis Halterman and Omer Iqbal**. Investigation:** Arsh Bibi and Irfan Ullah **Methodology:** Arsh Bibi **Project administration:** Dennis Halterman**. Resources, Software:** Arsh Bibi, Irfan Ullah and Irshad Ullah. **Validation:** Dennis Halterman and Omer Iqbal**. Visualization:** Arsh Bibi and Ali Shahwaiz. **Writing original draft:** Arsh Bibi. **Writing review and editing:** Irfan Ullah and Irshad Ullah. **Supervision:** Dennis Halterman and Omer Iqbal.

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