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# A COMPARISON OF METHODS FOR THE DETECTION OF *PHYTOPHTHORA INFESTANS* ON POTATOES IN MAURITIUS

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**Abstract:** Late blight, a disease caused by oomycota, *Phytophthora infestans*, is a greater threat to the potato crop than any other disease in Mauritius. This disease remains the most challenging to manage once symptoms have appeared, thus requiring rapid detection for effective disease management. The aim of this study was to compare different methods for early detection of the causal agent of potato late blight. Conventional culture-based methods involved the direct isolation of *P. infestans* from infected leaves on Carrot Piece Agar (CPA), Carrot Sucrose Agar (CSA), Commercial Potato Dextrose Agar (CPDA), Fresh Potato Dextrose Agar (FPDA-1 and FPDA-2), Oatmeal Agar (OMA), Pea Sucrose Agar (PSA) and Water Agar (WA) without antibiotic supplementation. Mycelial growth on agar was subsequently identified using molecular techniques. A culture-independent method was also attempted whereby total genomic DNA was directly extracted from symptomatic leaves with mycelial growth followed by PCR amplification with ITS5/ITS4 primers and sequencing. The different media ranked in the following decreasing order of performance: PSA >>> CSA ~ FPDA-1 > CPA ~ CPDA ~ OMA, with growth appearing on PSA within 7 days without contamination. DNA sequencing confirmed the identity of the agent recovered from PSA and from diseased leaves to be *P. infestans*. Findings of this study point to an optimum nutritive medium for recovering and culturing *P. infestans* from leaves with foliar blight without the use of antibiotics. Alternatively, a culture-independent method can be used for rapid detection and identification during routine disease surveillance.

**Key words:** late blight, morphology, pea sucrose agar, sequencing.

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## Introduction

Potato, *Solanum tuberosum* L. is the world's fourth most significant crop after rice, wheat, and maize and the first among the non-grain crops (Campos and Ortiz, 2020). Potatoes are widely considered as a "one-stop-shop" for human nutrition (Stewart and Mcdougall, 2012). Moreover, the potato crop is known for its relative ease of cultivation compared to cereals (Gebru et al., 2017; Campos and Ortiz, 2020). Potato is an important strategic commodity for Mauritius (Ministry of Agro-Industry and Food Security, 2016). In 2018, the production and per capita consumption of potatoes were 17,033 tonnes (FAOSTAT, 2019) and 16.41 kg/year (Statistics Mauritius, 2018), respectively in Mauritius. The main cultivars of potatoes planted are 'Spunta', 'Delaware', 'Belle Isle' (FAREI, 2021) and a newly released cultivar 'Vigora' (Ramdhin, personal communication). However, similar to other *Solanaceous* crops, potatoes are susceptible to a variety of diseases (Amsel and Bishop, 2008), especially late blight, which can have devastating consequences for its cultivation (Sparks et al., 2014).

Potato late blight is caused by oomycota, *Phytophthora infestans* (Mont.) de Bary. It has been reported that *Phytophthora* spp. can affect both cultivated and wild plants, thus causing major losses in agriculture and disrupting the natural forest ecosystem (Ho, 2018). Environmental factors such as temperature, relative humidity and rainfall strongly influence *P. infestans* infection and disease development on potato plants (Arora et al., 2014). According to Whisson et al. (2016), sporangia of *P. infestans* on leaves can spread rapidly in growing areas by water and/or wind. In general, ideal conditions for infection and late blight development include night temperatures of 10–16$^{o}$C accompanied by light showers, followed by several days of temperatures of 13–16$^{o}$C with a relative humidity of above 90% (Platt, 2008; Kirk, 2009; Kirk et al., 2013). Relative humidity of >90% and temperatures of 16–21$^{o}$C can be classified as favourable for spore production and sporangial germination (Platt, 2008). According to the Mauritius Meteorological Services (MMS), average night minimum temperatures can drop to 16.4$^{o}$C during the coolest months of the island (July and August) (MMS, 2021). During the potato production season, local climatic conditions significantly contribute to the spore's dissemination and germination.

Late blight is by far the most important potato disease worldwide (Platt, 2008; Kirk et al., 2013) as well as for Mauritius (Ibrahim and Taleb-Hossenkhan, 2017). Potato late blight occurs annually in many potato production areas worldwide and can result in 100% crop losses (Platt, 2008). In 2004, an outbreak of late blight occurred in Mauritius, and significant yield losses were recorded for the cultivar 'Spunta', previously reported as relatively tolerant (FARC, 2004; Neeliah et al., 2006). Similarly, in June 2020, another potato late blight outbreak was reported in Mauritius (Ponnappa-Naiken, 2020). Given that late blight constitutes a great threat

to potato production in Mauritius, there is a need for early detection of the pathogen for effective management of the disease.

Conventional culture-based diagnoses are well-known, although they are time-consuming, labour-intensive and technically challenging despite being very sensitive and accurate (Drenth et al., 2006; Khan et al., 2017). *P. infestans* attacks mainly living or freshly injured plant material making isolation of the pathogen from necrotic tissue difficult due to the presence of saprobes, which quickly overgrow the pathogen (Drenth and Sendall, 2001). Considering the very high reproductive potential and the rapid rate of spread of *P. infestans*, (Khan et al., 2017), the management of late blight is inherently more difficult than other diseases. Management strategies depend primarily on the early diagnosis and detection of the causal agent in the potato fields. Compared to traditional methods, PCR is rapid and accurate for specific identification of *P. infestans* in plant material (Hussain et al., 2013; Khan et al., 2017). The objective of this study was to compare the effectiveness and turnaround time of culture-dependent and culture-independent detection and identification of the causal agent of late blight affecting potato plants.

## Material and Methods

Site visit and sample collection

In August 2020, an open potato field of the Spunta cultivar, located in Vacoas (20°19'38.9"S 57°29'38.3"E) in the super-humid agro-climatic zone of Mauritius, was surveyed for late blight symptoms. Disease incidence was estimated by counting the number of plants with disease symptoms from 100 random plants and repeated 4 times. In addition, a total of 25 samples of symptomatic leaves were collected, labelled and transported to the Microbiology laboratory of the University of Mauritius.

Microscopic examination of infected leaves

The infected leaves were surface-sterilised by rubbing with cotton wool, moistened with 70% ethyl alcohol, and incubated in humid chambers at 15±3°C for 24 h. After incubation, some mycelia were taken at the sporulating lesion using a sterile needle and placed on a drop of cotton blue lactophenol dye on a clean microscope slide. A cover slip was placed over the dye drop and examined under 400x magnification using a bright-field compound microscope (Euromex, The Netherlands).

Preparation of culture media

A total of eight different culture media were compared in terms of their ability to recover and isolate the pathogen. Carrot Piece Agar (CPA) (Werres et al., 2001),

Carrot Sucrose Agar (CSA) (Hussain and Hussain, 2016; Kumbar, 2017), Fresh Potato Dextrose Agar (FPDA), Oatmeal Agar (OMA) (Hussain and Hussain, 2016) and Pea Sucrose Agar (PSA) (Stammler, 2006), were all prepared from freshly available ingredients. FPDA was prepared by boiling fresh potatoes in distilled water until completely softened (Fresh Potato Dextrose Agar Version 1 [FPDA-1]) (Hussain and Hussain, 2016) or still firm (FPDA-2) (Kiraly et al., 1970). The ready-to-use commercially available media used included Potato Dextrose Agar (CPDA, HiMedia, India) and Water Agar (WA, HiMedia, India) (*Appendix*).

Comparison of techniques for recovery of *Phytophthora infestans* from diseased tissue

The direct transfer of mycelial growth to media

After overnight incubation of symptomatic potato leaves in humid chambers, any visible growth of the pathogen was aseptically transferred onto the differently prepared media (CPA, CPDA, CSA, FPDA-1, FPDA-2, OMA, PSA and WA) using a method adapted from Tumwine et al. (2000). Briefly, mycelia were carefully picked up using a sterile needle and placed on each medium without touching the leaf tissue. The plates were then incubated at 15±3$^{o}$C in the dark for 3–7 days, depending on the growth rate of *P. infestans* on the medium. Three independent replicates were carried out for each medium.

Transfer of infected leaves onto healthy potato tubers

Within this method, two approaches were attempted. Firstly, healthy tubers were surface-sterilised by wiping with 70% ethanol and aseptically cut into 5-mm thick slices. These slices were then placed in 90-mm sterile Petri dishes onto which small pieces of infected leaves, cut at the sporulating border of the lesion, were placed and covered with lids. The dishes were then incubated at 15±3$^{o}$C for 14 days, until sporulation around the slices was observed. The sporangia and hyphae were carefully picked and transferred onto the different media prepared as described above (Tumwine et al., 2000; Gamboa et al., 2019).

The second approach involved the sandwich method. A healthy potato tuber was washed with running water, followed by surface sterilisation by wiping with 70% alcohol. The tuber was cut aseptically into two halves between which the infected leaf was placed. The whole sandwich was secured with an elastic band and subsequently incubated at 15±3$^{o}$C for 4-9 days, followed by transferring of any visible mycelial growth from the tubers onto each medium as described above (Sobkowiak and Śliwka, 2017).

Isolation from sporangial suspension

A sporangial suspension of the causal agent was prepared by gently washing each infected leaf with ca. 1 ml of deionised water into a sterile glass Petri dish to dislodge the sporangia. This suspension was used in two different steps. Firstly, an aliquot of 100 µl of the suspension was spread-plated on the different media mentioned above and plates were incubated at 15±3°C for up to 7 days with the daily examination.

Secondly, some healthy leaves were artificially wounded using a sterile needle. They were then inoculated with 20 µl of the sporangial suspension and incubated in humid chambers at 15±3°C (Harrison et al., 1990; Lees et al., 2012). Any visible growth on inoculated leaves was then isolated and sub-cultured onto each medium as described in the first section.

Sub-culturing and microscopic examination of cultures

After 7–21 days, intermittent sub-culturing was done by transferring actively growing hyphae at the tips of the cultures onto fresh media (Tumwine et al., 2000). Sub-cultured plates were incubated at 15±3°C in the dark for another 20 days. The 20 day-old cultures were then examined under the microscope by picking the mycelial growth using a sterile needle, and similar procedures were conducted as described in the previous section.

Molecular identification of *Phytophthora infestans*

After 27–41 days of incubation, putative isolates of *P. infestans* on PSA were selected for molecular identification since growth was more prolific on PSA compared to the other media. Briefly, mycelial growth was scraped from the plate, weighed, and ground with liquid nitrogen prior to extraction using the Cetyl Trimethyl Ammonium Bromide (CTAB) method. For the detection purposes, a culture-independent method was included, in which total genomic DNA (leaf and causal agent) was directly extracted from the ground infected leaf tissue with liquid nitrogen (Khan et al., 2017; Riit et al., 2016), followed by DNA extraction using the CTAB method (Ranghoo and Hyde, 2000).

The ITS regions of rDNA were amplified using the ITS primers, ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCGCTTATTGATATGC-3') (Ristaino et al., 1998). The thermal cycling parameters comprised initial denaturation at 95°C for 3 min followed by 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s followed by a final extension at 72°C for 5 min. DNA sequencing reactions were done using a Big Dye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) following the protocol outlined by the manufacturers. Sequencing reaction products were purified by the ExoSAP method and were directly sequenced in both directions using an automated sequencer (ABI

3500 DNA sequencer (Applied Biosystems) at Inqaba Biotechnical Industries (Pty) Ltd, South Africa, using the same primers as for amplification. Forward and reverse sequences were assembled and edited using CLC Main Workbench Version 7.6 (https://www.qiagenbioinformatics.com/). Consensus sequences were computed using the ClustalW (Thompson et al., 1994), integrated in MEGA6 software (Tamura et al., 2013), and deposited in GenBank (http://www.ncbi.nlm.nih.gov). All generated sequences were compared with each other by calculating nucleotide (nt) similarities, as well as with previously deposited *Phytophthora* spp. isolates available in GenBank, using the similarity search tool BLAST.

## Results and Discussion

In-field disease incidence and symptomatology

The estimated disease incidence of late blight in the potato field was ca. 40%, and the disease was found to spread over a moderate distance, as shown in Figure 1A. The foliage of the potato plants infected by late blight was observed to have numerous characteristic dark lesions near the tips and margins, progressing to a pale green colour at the sporulating border (circled yellow) (Figure 1B).

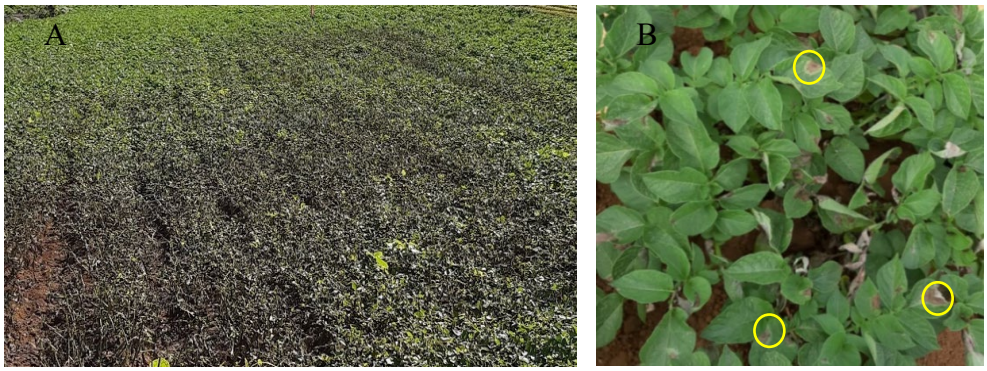


Figure 1. A: Late blight in a potato field at Vacoas, Mauritius, B: large dark lesions on leaves of potato plants with late blight.

Morphological identification

The microscopic examination of infected leaves (Figure 2A) revealed the presence of lemon-shaped ovoid and semi-papillate sporangia (Figure 2B), with an average length:width ratio of sporangia being 1.9. As for the sporangiophores observed, they were branched with swelling just below the sporangium (Figure 2B) and had an average width and length of 4.4 µm (n=50) and 622.8 µm (n=50), respectively. These morphological characteristics were similar to those described

by other researchers such as Drenth and Sendall (2001) and Sobkowiak and Sliwka (2017) and were tentatively identified as *P. infestans.*

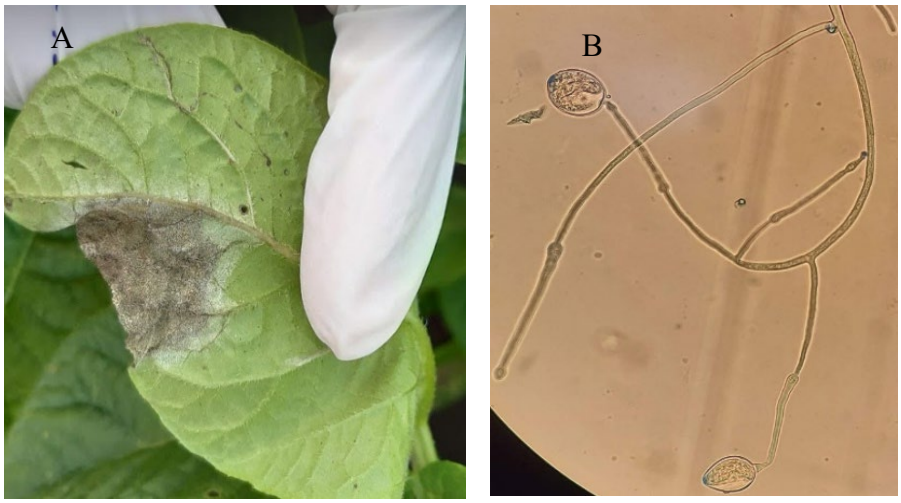


Figure 2. A: Visible mycelial mass of *Phytophthora infestans* on the abaxial surface of leaves, B: ovoid, semi-papillate sporangia on sympodial sporangiophores observed under a bright-field microscope (400x magnification).

Comparison of techniques for recovery of *Phytophthora infestans* from diseased tissue

*P. infestans* was isolated using different techniques and media to determine the optimum condition for the recovery of the agent. Indeed, optimisation techniques are important for extending the life of samples collected without risking the loss of the agent of interest (Tumwine et al., 2000). Our findings revealed that isolation of *P. infestans* was rendered difficult due to contamination by secondary pathogens present on the leaves or tubers, despite the use of aseptic techniques. In fact, no growth or occasional outgrowth by background contaminants was observed on incubated plates. The contamination could be attributed to the absence of antibiotics in the media, possibly favouring the growth of bacterial contaminants, as reported in the study done by Sarker et al. (2020). Equally, using "healthy" leaves or tubers for recovery of *P. infestans* could present further risks of contamination by other microorganisms. Indeed, the contamination of plates has been attributed to the growth of background microflora on the host tissue (Tumwine et al., 2000) or delayed sub-culturing of hyphal tips onto media (Sarker et al., 2020). On the other hand, the direct transfer of mycelial growth from infected leaves onto media was successful in this study, as demonstrated by the onset of growth after 7 days of incubation. In addition, the risk of contamination of the

culture was minimised by intermittent sub-culturing. Tumwine et al. (2000) also emphasised the importance of sterile techniques and regular sub-culturing.

Performance comparison of different culture media

The direct transfer of mycelial growth from infected leaves to media resulted in white, slightly fluffy, irregular colony growth on PSA after 7–20 days of incubation, while much slower growth was observed on CSA and FPDA-1. Observation of PSA plates under a microscope revealed lemon-shaped sporangia together with sporangiospores. Based on the colony morphology and characteristics of sporangia, the pathogen was tentatively identified as *Phytophthora infestans*. Hence, *P. infestans* could be successfully recovered on PSA from freshly infected potato leaves using a simple and cost-effective method, namely the direct transfer of mycelial growth from infected leaves to PSA without antibiotics.

In addition, it is worth mentioning that no antibiotics were used in the preparation of the various media, making it a very cost-effective method for analyses of samples collected during routine screening. Moreover, compared to PSA, CSA and FPDA-1, other media (CPA, CPDA, FPDA-2, OMA and WA) did not promote the growth of the pathogen since microscopic examination of the plates revealed the presence of sporangia, which were initially transferred from the infected leaves, without sporangiophores. The absence of sporangiophores could be attributed to the lack of one or more nutrients needed for the development of *P. infestans* (Kumbar, 2017; Sarker et al., 2020). For instance, Kumbar (2017) noted that the absence of sucrose in CPA inhibited the sporangial germination of *P. infestans*.

Molecular identification of *Phytophthora infestans* from culture and total genomic DNA

The sequence of the representative isolates obtained from the culture-dependent approach (Accession No. MW794194) was compared with those available in GenBank. It was found to share 99–100% nucleotide similarity with over 100 *P. infestans* isolates, 100% with an isolate from *Solanaceous* crops, Accession No. EU200296 (Vargas et al., 2009). Similarly, the sequence of the PCR product obtained from the total DNA from the culture-independent route (Accession No. MZ504994) also shared 99.50% nucleotide similarity with over 100 *P. infestans* isolates, with 95.50% *P. infestans* isolate (MK507866) from the tomato from the United Kingdom (Pettitt et al., 2019). The isolates included in this study are the first molecularly characterised *P. infestans* isolates from Mauritius.

However, the culture-dependent method took around 30 days (Figure 3) and proved to be very time-consuming, as also noted by other researchers (Hussain et al., 2005; Hussain et al., 2013). Since sporangia can germinate within a few hours under favourable conditions, early diagnosis and detection of the causal agent in

infected potato fields are thus necessary for successful disease control (Khan et al., 2017), and this has prompted the development of faster PCR-based methods (Judelson and Tooley, 2000; Hussain et al., 2005; Haverkort et al., 2009; Lees et al., 2012).

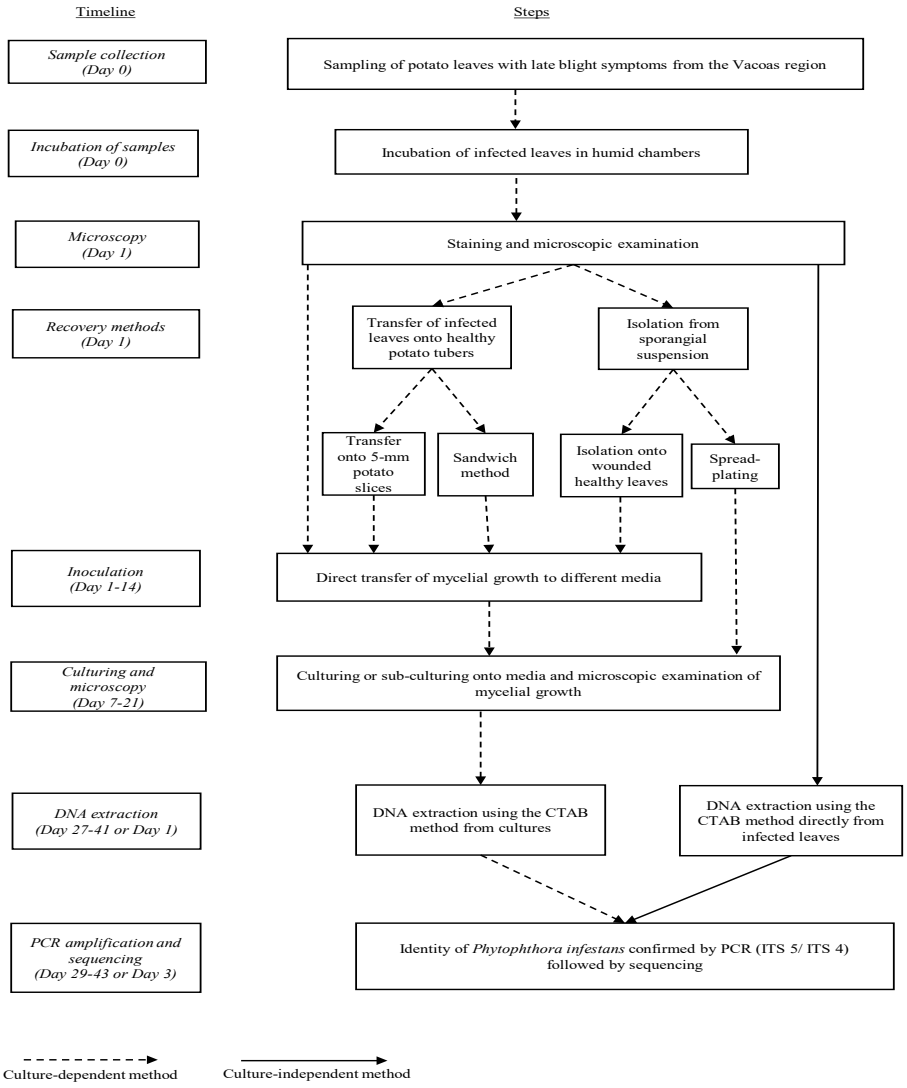


Figure 3. A flow diagram showing the different steps for the culture-dependent and culture-independent methods for the identification of the causative agent from diseased potato leaves.

Extraction of the total genomic DNA from infected leaves followed by PCR and sequencing enabled accurate confirmation of the identity of the etiological agent of foliar blight within 3 days of sample collection (Figure 3). Molecular techniques are being increasingly deployed for the diagnosis of foliar late blight (Judelson and Tooley, 2000; Drenth and Sendall, 2001; Hussain et al., 2005; Haverkort et al., 2009; Lees et al., 2012; Khan et al., 2017) since the symptoms are similar to other fungal diseases, abiotic disorders, or fungicide injuries (Pscheidt, 1991). The primers ITS5/ITS4 were found reliable for rapid taxonomic identification of *Phytophthora* species, as also reported by Ristaino et al. (1998). In our study, direct microscopic observation of diseased leaves for morphological characteristics typical of *P. infestans* coupled with molecular confirmation by the culture-independent approach enabled fast and accurate pathogen detection.

A flow diagram showing the different steps for the culture-dependent and culture-independent methods is shown in Figure 3.

## Conclusion

The culture-based approach for isolation of *P. infestans* on PSA without the use of antibiotics followed by molecular identification proved to be both cost-effective and successful although it was more time-consuming compared to the culture-independent method. *In situ* microscopic examination of symptomatic leaf tissues coupled with total genomic DNA extraction, PCR using ITS5/ITS4 primers and sequencing, resulted in the quick and accurate identification of the pathogen. Besides being considerably faster and semi-specific, the culture-independent method is particularly useful in laboratories with limited financial resources and personnel.

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## References

Amsel, N., & Bishop, E. (2008). Potatoes. College Seminar 235 Food for Thought: The Science, Culture, & Politics of Food Spring; 2008. Retrieved March 14, 2020, from https://academics.hamilton.edu/foodforthought/foodforthoughtsylspr08.pdf.

Arora, R.K., Sharma, S., & Singh, B.P. (2014). Late Blight Disease of Potato and Its Management. *Potato Journal, 41* (1), 16-40.

Campos, H., & Ortiz, O. (2020). *The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind.* Switzerland: Springer Nature Switzerland AG.

Drenth, A., & Sendall, B. (2001). Practical guide to detection and identification of *Phytophthora*. Australia: *CRC for Tropical Plant Protection*. Retrieved December 23, 2020, from https://docplayer.net/17681728-Practical-guide-to-detection-and-identification-of-phytophthora.html.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., & Irvine, G. (2006). Development of a DNA-based method for detection and identification of *Phytophthora* species. *Australian Plant Pathology, 35*, 147-159.

FAOSTAT (2019). Crops. Retrieved June 13, 2020, from http://www.fao.org/faostat/en/#data/QC.

FARC (2004). Food and Agricultural Biotechnology Research & Development Priorities in the Non-Sugar Agricultural Sector (1999-2007) Priority areas 2003-2007. Report of working group on solanaceous crops. *Food and Agricultural Research Council, Mauritius*. Retrieved June 19, 2020, from http://farc.govmu.org/English/Policy/Pages/R-and-D-Policy-and-Planning.aspx.

FAREI (2021). Le Guide Agricole. Retrieved June 25, 2021, from http://farei.mu/apmis/publications/guide/guide-interactif/main.htm.

Gamboa, S., Perez, W., Andrade-Piedra, J.L., & Forbes, G. (2019). *Laboratory manual for Phytophthora infestans work at CIP.* International Potato Center.

Gebru, H., Mohammed, A., Dechassa, N. & Belew, D. (2017). Assessment of production practices of smallholder potato (*Solanum tuberosum* L.) farmers in Wolaita zone, southern Ethiopia. *Agriculture and Food Security, 6* (31), 1-11.

Harrison, J.G., Barker, H., Lowe, R., & Rees, E.A. (1990). Estimation of amounts of *Phytophthora infestans* mycelium in leaf tissue by enzyme- linked immunosorbent assay. *Plant Pathology, 39* (2), 274-277.

Haverkort, A.J., Struik, P.C., Visser, R.G.F., & Jacobsen, E. (2009). Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Research,* 52 (3), 249-264.

Ho, H.H. (2018). The taxonomy and biology of *Phytophthora* and *Pythium*. *Bacteriology & Mycology, 6* (1), 40-45.

Hussain, M., & Hussain, T. (2016). Physiological parameters influence mycelium growth and sporangium production of *Phytophthora infestans*. *Agrica, 5*, 42-46.

Hussain, S., Lees, A.K., Duncan, J.M., & Cooke, D.E.L. (2005). Development of a species-specific and sensitive detection assay for *Phytophthora infestans* and its application for monitoring of inoculum in tubers and soil. *Plant Pathology, 54* (3), 373-382.

Hussain, T., Singh, B.P., & Anwar, F. (2013). 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*, 380-386.

Ibrahim, A., & Taleb-Hossenkhan, N. (2017). Genotypic Characterization of *Phytophthora infestans* from Mauritius using Random Amplified Polymorphic DNA (RAPD), Mitochondrial Haplotyping and Mating Type Analysis. *Plant Pathology Journal, 16*, 121-129.

Judelson, H.S., & Tooley, P.W. (2000). Enhanced polymerase chain reaction methods for detecting and quantifying *Phytophthora infestans* in plants. *Phytopathology.* 90 (10), 1112-1119.

Khan, M., Li, B., Jiang, Y., Weng, Q., & Chen, Q. (2017). Evaluation of different PCR-based assays and LAMP method for rapid detection of *Phytophthora infestans* by targeting the Ypt1 gene. *Frontiers in Microbiology, 8*, 1-11.

Kiraly, Z., Klement, Z., Solymosy, F., & Voros, J**.** (1970). Methods in Plant Pathology. In: Z. Kiraly (Eds). (pp. 237-477). Budapest.

Kirk, W. (2009). Potato Late Blight Alert for the Midwest. Field Crop Advisory Team Alert Curent News Articles.

Kirk, W., Wharton, P., Hammerschmidt, R., Abu-el Samen, F., & Douches, D. (2013). Late Blight. Retrieved June 13, 2020, from http://www.potatodiseases.org/lateblight.html.

Kumbar, B. (2017). Standardization of Specific Media for *Phytophthora infestans*. *Global Journal of Bio-Science and Biotechnology, 6,* 374-376.

Lees, A.K., Sullivan, L., Lynott, J.S., & Cullen, D.W. (2012). Development of a quantitative real-time PCR assay for *Phytophthora infestans* and its applicability to leaf, tuber and soil samples. *Plant Pathology, 61* (5), 867-876.

Mauritius Meteorological Services (MMS). 2021. Climate of Mauritius. Retrieved March 28, 2021, from http://metservice.intnet.mu/climate-services/climate-of-mauritius.php.

Ministry of Agro-Industry And Food Security. (2016). Retrieved June 13, 2020, from http://agriculture.govmu.org/English/Documents/Book%20Final.pdf.

Neeliah, H., Rajkomar, B., Dookun-Saumtully, A., & Ramkissoon, J. (2006). National Policy Regarding Commercial Gm Trade and The Potential Farm-Level Impact of Adopting Gm Crops: *Mauritius. Food Agriculture and Natural Resources Policy Analysis Network (FANRPAN)*. Retrieved June 19, 2020, from www.fanrpan.org.

Pettitt, T.R., Keane, G.J., John, S.O.L., Cooke, D.E.L., & Žerjav, M. (2019). Atypical late blight symptoms following first recorded infections by *Phytophthora infestans* genotype EU_39_A1 in UK vine tomatoes. *New Disease Reports, 39*, 16.

Platt, H.W. (2008). Fungal and Fungal- like diseases, Late Blight. In: S.Wale, H.W. Platt & W. Cattlin W, (Eds.), *Diseases, Pests and Disorders of Potatoes*. (pp. 49-52). London, UK: Manson Publishing.

Ponnappa-Naiken, M. (2020). "Pomme de terre: combattre le *Phytophthora infestans*". *Mauritius Broadcasting Coorperation (MBC).* Retrieved June 18, 2020, from https://mbcradio.tv/article/vid%C3%A9o-pomme-de-terre-combattre-le-phytophthora-infestans.

Pscheidt, J.W. (1991). Diagnosis and Control of *Phytophthora* Diseases. *Idaho: A Pacific Northwest Extension Publication*. Retrieved December 09, 2020, from, https://pnwhandbooks.org/plantdisease/pathogen-articles/common/oomycetes/diagnosis-control-phytophthora-diseases.

Ranghoo, V.M., & Hyde, K.D. (2000). *Ascominuta lignicola*, a new Ioculoascomycete from submerged wood in Hong Kong. *Mycoscience, 41*, 1-5.

Riit, T., Tedersoo, L., Drenkhan, R., Runno-Paurson, E., Kokko, H., & Anslan, S. (2016). Oomycete-specific ITS primers for identification and metabarcoding. *MycoKeys, 14*, 17-30.

Ristaino, J.B., Madritch, M., Trout, C.L., & Parra, G. (1998). PCR amplification of ribosomal DNA for species identification in the plant pathogen genus *Phytophthora*. *Applied And Environmental Microbiology, 64* (3), 948-954.

Sarker, S.R., McComb, J., Burgess, T.I., & Hardy, G.E.S.J. (2020). Antimicrobials in *Phytophthora* isolation media and the growth of *Phytophthora* species. *Plant Pathology, 69* (8), 1426-1436.

Sobkowiak, S., & Śliwka, J. (2017). *Phytophthora Infestans*: Isolation of Pure Cultures, Storage and Inoculum Preparation. *Plant Breeding and Seed Science, 76* (1), 10-15.

Sparks, A.H., Forbes, G.A., Hijmans, R.J., & Garrett, K.A. (2014). Climate change may have limited effect on global risk of potato late blight. *Global Change Biology, 20* (12), 3621-3631.

Stammler, G. (2006). *Phytophthora infestans*. Fungicide Resistance Action Committee (FRAC).

Statistics Mauritius. (2018). Digest of Agricultural Statistics 2017. Retrieved June 13, 2020, From http://statsmauritius.govmu.org/English/StatsbySubj/Documents/Digest/Agriculture/Digest_Agri_Yr17.pdf.

Stewart, D., & Mcdougall, G. (2012). Potato; A nutritious, tasty but often maligned staple food. Food & Health Innovation Service (FHIS). Retrieved March 10, 2021, from https://www.hutton.ac.uk/webfm_send/743.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology Evolution*, 30, 2725-2729.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680.

Tumwine, J., Frinking, H.D., & Jeger, M.J. (2000). Isolation techniques and cultural media for *Phytophthora infestans* from tomatoes. *Mycologist, 14*, 137-139.

Vargas, A.M., Ocampo, L.M.Q., Céspedes, M.C., Carreño, N., González, A., Rojas, A., Zuluaga, A.P., Myers, K., Fry, W.E., Jiménez, P., Bernal, A.J. & Restrepo, S. (2009). Characterization of *Phytophthora infestans* Populations in Colombia: First Report of the A2 Mating Type. *Phytopathology, 99* (1), 82-88.

Werres, S., Marwitz, R., Man i't Veld, W.A., de Cock, A.W.A.M., Bonants, P.J.M., De Weerdt, M., Themann, K., Ilieva, E., & Baayen, R.P. (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research,105* (10), 1155-1165.

Whisson, S.C., Boevink, P.C., Wang, S., & Birch, P.R.J. (2016). The cell biology of late blight disease. *Current Opinion in Microbiology, 34*, 127-135.

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## Appendix

*Carrot Piece Agar (CPA)* (Werres et al., 2001): carrots 50 g; agar 20 g; distilled water 1000 ml.

Grate the fresh carrots. Place the grated carrots directly in a conical flask and add the agar, followed by the distilled water. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Carrot Sucrose Agar (CSA)* (Hussain and Hussain, 2016; Kumbar, 2017): carrots 220 g; distilled water 1000 ml; sucrose 20 g; agar 9 g.

Cut the fresh carrots into pieces. Boil the cut carrots in 500 mL distilled water until cooked. Comminute the warm carrots in a blender for 1 min at high speed. Filter through a 3–4 layered muslin cloth and squeeze out the juice from the residue. Make up the filtrate to 1 L and add the sucrose and agar. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Commercial Potato Dextrose Agar (CPDA)*: PDA (HiMedia) 39.1 g; distilled water 1000 ml.

Add PDA to water, autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Fresh Potato Dextrose Agar Version 1 (FPDA-1)* (Hussain and Hussain, 2016): potatoes 225 g; 1000 ml distilled water; 20 g dextrose; 20 g agar.

Cut the peeled and washed potatoes into pieces. Boil the cut potatoes in distilled water until fully cooked. Strain the solution and keep the filtrate for use. Make up the filtrate to 1 L and add the dextrose and agar. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Fresh Potato Dextrose Agar Version 2 (FPDA-2)* (Kiraly et al., 1970): potatoes 200 g; distilled water 1000 ml; dextrose 20 g; agar 20 g.

Cut the peeled and washed potatoes into pieces. Boil the cut potatoes in distilled water for 10–15 min and make sure that the boiled potatoes remain firm. Strain the solution and keep the filtrate for use. Make up the filtrate to 1 L and add the dextrose and agar. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Oatmeal Agar (OMA)* (Hussain and Hussain, 2016): oats 20 g; distilled water 1000 ml; agar 20 g.

Mix oats with distilled water. Boil the mixture for 10 min. Filter the solution through a muslin cloth. Make up the filtrate to 1 L and add the agar. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Pea Sucrose Agar (PSA)* (Stammler, 2006): frozen peas 150 g; distilled water 1000 ml; glucose 5 g; agar 20 g.

Boil the frozen peas in distilled water until fully cooked. Filter the homogenised mixture through 3–4 layers of a muslin cloth. Make up the filtrate to 1 L and add the glucose and agar. Autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

*Water Agar* (*WA)* (Hussain and Hussain, 2016): agar 20 g; distilled water 1000 ml.

Add agar to water, autoclave the mixture at 121°C for 20 min and then cool to 50°C in a water bath.

# UPOREDNO OCENJIVANJE METODA ZA DETEKCIJU PATOGENA KROMPIRA *PHYTOPHTHORA INFESTANS* NA MAURICIJUSU

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R e z i m e

Plamenjača, bolest koju izaziva oomiceta *Phytophthora infestans* predstavlja veću opasnost za usev krompira nego bilo koja druga bolest na Mauricijusu. Nakon pojave simptoma, suzbijanje je vrlo teško, što ukazuje da je neophodno ustanoviti način brze detekcije u cilju obezbeđenja primene efikasnih mera suzbijanja. Osnovni cilj ovih istraživanja bio je da se uporede različite metode za ranu detekciju prouzrokovača plamenjače krompira. Metode zasnovane na konvencionalnim metodama uključile su direktnu izolaciju *P. infestans* iz zaraženih listova bez dodavanja antibiotika na podlogu od komadića mrkve (CPA), podlogu od mrkve i saharoze (CSA), komercijalni krompir dektrozni agar (CPDA), svež krompir dekstrozni agar (FPDA-1 and FPDA-2), podlogu od ovsa (OMA), podlogu od graška i saharoze (PSA) i vodeni agar (WA). Porast micelije na podlogama identifikovan je korišćenjem molekularnih metoda. Direktna detekcja bez prethodne izolacije takođe je primenjena. Ukupna genomna DNA direktno je ekstrahovana iz listova sa simptomima i sporulacijom, nakon čega je PCR amplifikacija i sekvenciranje obavljeno primenom prajmera ITS5/ITS4. Po opadajućoj pogodnosti, podloge se mogu poređati na sledeći način: PSA >>> CSA ~ FPDA-1 > CPA ~ CPDA ~ OMA, pri čemu je porast na PSA bio bez kontaminacija i vidljiv nakon sedam dana. Sekvenciranje je potvrdilo identitet *P. infestans* kako sa PSA tako i direktno iz zaraženih listova. Dobijeni rezultati ukazuju na značaj izbora optimalne hranljive podloge bez antibiotika za izolaciju i gajenje *P. infestans* direktno iz listova sa plamenjačom. S druge strane, metoda direktnog dokazivanja bez izolacije može da se koristi za brzu detekciju i identifikaciju tokom rutinskog nadzora nad pojavom bolesti.

**Ključne reči:** plamenjača, morfologija, podloga od graška i saharoze, sekvenciranje.

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