

Detection of *Globodera pallida* directly from soil sample using mt-COI region based LAMP assay

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Abstract

Potato cyst nematodes (PCN), *Globodera rostochiensis* (Golden/yellow) and *G. pallida* (White), are economically important and relatively specialized pest of potato (*Solanum tuberosum* L.). Both the species are being identified based on cyst colour after 55-60 days after planting (DAP) however, after 65 DAP, we cannot differentiate based on cyst colour as both species turns brown. Moreover, the molecular techniques available to detect the PCN at species level is laborious, time consuming and costly. Therefore, development of rapid, accurate and economically cheap technique for detection of PCN at species level from the field is important to devise effective management strategies for sustainable potato production. Accordingly, in the first instance, loop-mediated isothermal amplification (LAMP) assay was developed to detect *G. pallida* directly from soil by using the mitochondrial (mt-COI) gene specific primer. The LAMP assay was completed within 60 min at 60 °C isothermal conditions and the primer, efficiently detects the *G. pallida* without any cross reaction with *G. rostochiensis*, *Meloidogyne incognita*, *M. javanica*, *Heterodera avenae*, *H. carotae*, and *Cactodera* spp. In analytical sensitivity tests, the assay was able to detect *G. pallida* with 1000 times less DNA concentration (10 fg/μl) as compared to conventional PCR (10 pg/μl) and the LAMP product was visualized by using SYBR Gold nucleic acid dye and the assay can be highly useful in detection of *G. pallida*.

Introduction

Globodera pallida (Stone 1973) and *G. rostochiensis* (Wollenweber 1923) are major biotic limitations in sustainable potato production with quarantine nature and has become a serious endemic pest worldwide causing an average of 12.2% yield losses in potato (Urwin et al. 2001). However, in case of dense inoculum of both the PCN species may cause yield loss up to 80% (Zasada and Dandurand 2018). White potato cyst nematode (*G. pallida*) alone can reduce up to 80% tuber yield (Talavera et al. 1998). In India, potato cyst nematode (PCN) was first reported from Udthagamandalam, The Nilgiri, Tamil Nadu (Jones 1961). Later on, its presence was noticed from Kodaikanal hills of Tamil Nadu, adjoining hills of Karnataka and Idukki District in Western Ghats of Kerala (Aarti et al. 2020b). Recently, it has been reported from some parts of Himachal Pradesh, Jammu & Kashmir and Uttarakhand hills (Aarti et al. 2020a). To find out the PCN population dynamics traditionally PCN species are identified based on different morphological characters, however, it takes long time and laborious as well. Therefore, PCR based approaches have been used to identify PCN species which produce specific and accurate results. Among the PCR based techniques, Random amplified polymorphic DNA (RAPD), Restriction fragment length polymorphism (RFLP), Amplified fragment length polymorphism (AFLP), Internal transcribed spacer (ITS1 and ITS2), ribosomal deoxyribonucleic acid (rDNA) and mitochondrial deoxyribonucleic acid (mt-DNA) have become popular for identification of PCN species (Aarti et al. 2017). But, these techniques require expensive thermo-cyclers as well as imaging systems and it cannot be used directly under field conditions. Recently, Loop-mediated isothermal amplification (LAMP) have emerged promising and alternate to PCR due to its simplicity, rapidity, specificity, sensitivity and cost effectiveness. Above all only a heating block or water bath are needed that could maintain a constant temperature

(Notomi et al. 2000; Tomita et al. 2008; Verma et al. 2019). In view of these advantages, this technology has been used commercially in a variety of pathogens detection kits (Mori and Notomi 2009).

In the field of plant nematology, LAMP assays have been developed for the detection of parasitic worms of pine wood *Bursaphelenchus xylophilus* (Kikuchi et al. 2009; Meng et al. 2018), coconut red ring worm *B. cocophilus* (Ide et al. 2017), burrowing worm *Radopholus similis* (Peng et al. 2012), citrus root worm *Tylenchulus semipenetrans* (Song et al. 2017), grass parasitic worm *Anguina wevelli* (Yu et al. 2018) and tropical root-knot worm *Meloidogyne incognita*, *M. enterolobii* (Niu et al. 2011; Niu et al. 2012) as well as the temperate root-knot worm *M. hapla* (Peng et al. 2017), and apple root-knot worm *M. mali* (Zhou et al. 2017). Quick and specific detection of *G. pallida* in the soil is imperative for development of management strategy against the particular species. As of now, no attempts have been made for the detection of *G. pallida* directly from soil sample using LAMP assay hence, we have made an effort to develop the same.

Materials And Methods

Nematode populations

Pure nematode populations (*G. pallida* and *G. rostochiensis*) were maintained on susceptible potato cultivar Kufri Jyoti under glass house conditions at ICAR-CPRS, Kufri, Himachal Pradesh. To check the cross reactivity of LAMP primers, DNA of plant parasitic nematodes like *M. incognita*, *M. javanica*, *Heterodera carotae*, *H. avenae*, and *Cactodera* spp. were obtained from the Department of Nematology, TNAU, Coimbatore and *G. pallida* from ICAR-CPRS, Muthorai, Udthagamandalam.

Genomic DNA extraction

The genomic DNA of *G. pallida* and *G. rostochiensis* was extracted repeatedly ten times from single cyst/female following the protocol standardized by Aarti et al. (2019). The quality and quantity of the purified DNA sample was determined using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Characterization of mt-COI region of G. pallida

Primers were designed using FastPCR software (Table 1) of nucleotide sequence from GeneBank (Accession numbers DQ631912) of mitochondrial-cytochrome oxidase subunit I gene (mt-COI) of *G. pallida* (Fig. 1 : Table 1). PCR reaction was carried out with 20 µl reaction mixture containing genomic DNA (50ng/µl) 1.0 µl, primer mix 1.0 µl each [FP (Forward primer) and BP (Backward primer) 10 pmol], Red Taq DNA polymerase (8 U) (Genei) 1.0 µl, Taq buffer A 2.0 µl, 2.5 mM dNTP mix 1 µl and double distilled water (DDH₂O) 13 µl. The PCR reaction was set at 95 °C initial denaturation for 1 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min in a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems™, Thermo Fisher Scientific Inc.). The PCR products were visualized on 2% agarose gel and eluted using

QIAquick Gel Extraction Kit (Quiagen). Further, DNA sequencing was performed using BigDye Direct terminator cycle sequencing kit (Applied Biosystems, UK). Automated DNA sequencer (Genetic Analyzer 3500, Applied Biosystems) was used to perform the sequencing analysis. Traces were aligned and visualized using the Sequence Scanner Software version 2.0 for Windows (Applied Biosystems 2012). The obtained nucleotide sequences were subjected to BLAST in blastn programme in NCBI to confirm its identity before utilizing it for designing *G. pallida* specific LAMP primers.

LAMP primer design

Based on the nucleotide sequence obtained using the Primer explorer V4 software program (<http://primerexplorer.jp/e/>), the LAMP primers were designed (Fig. 1). A total of four primers were designed: F3 (Forward outer primer), B3 (Backward outer primer), FIP [F1c–F2] (Forward inner primer), and BIP [B1c–B2] (Backward inner primer) (Table 1).

Standardization of LAMP reaction and conditions

In 0.2 ml micro centrifuge tubes, LAMP reaction mixture (25 µl) containing template DNA (50ng/µl) 2 µl, 30 pico moles of FIP and BIP primer each 1 µl, 10 pico moles of F3 and B3 primer each 0.5 µl, *Bst* DNA polymerase (8 U) 1.0 µl, 10× isothermal reaction buffer 2.5 µl, 10 mM dNTP mix 3.5 µl, 100 mM MgSO₄ 1.5 µl, and DDH₂O 11.5 µl was used for carrying out LAMP reaction. In negative control, the template DNA was replaced with DDH₂O.

Thermal gradient PCR was performed for 60 min at 54, 56, 58, 60, 62, and 64 °C to find out the optimum temperature. After temperature optimization, LAMP assays were performed at various durations (15, 30, 40, 50 and 60 min) to find out the optimal assay time. Finally, the reactions were ended at 80 °C for 5 min. LAMP PCR were checked on agarose gel (2%) stained with ethidium bromide and also visually examined by using 2 µl SYBR Gold nucleic acid (Invitrogen) (1:10 diluted) in 8 µl LAMP product.

Sensitivity assay for PCR and LAMP

LAMP sensitivity assay was determined by 10-fold serial dilutions of genomic DNA isolated from single cyst of *G. pallida* with initial concentration of 10 ng/µl. In addition, conventional PCR reaction was carried out with 20 µl reaction mixture containing genomic DNA 1.0 µl, primer mix 1.0 µl each [5 pmol of F3 (FP)/ and B3 (RP)], Red Taq DNA polymerase (8 U) (Genei) 1.0 µl, Taq buffer A 2.0 µl, 2.5 mM dNTP mix 1 µl and DDH₂O 13 µl. The PCR reaction was set as described above.

Specificity assay for PCR and LAMP

The specificity was checked with standardized assay conditions using genomic DNA of important plant parasitic nematode i.e. *G. pallida*, *G. rostochiensis*, *H. avenae*, *M. incognita*, *M. javanica* *H. carotae*, and *Cactodera* spp. The results were visualized by SYBR Gold nucleic acid dye as well as agarose gel (2%) stained with ethidium bromide.

Optimization of LAMP assay directly from nematode inoculated soil

To check the feasibility of field diagnostics LAMP assay, we concentrated optimizing LAMP primers for diagnosis of *G. pallida* directly from soil samples. Accordingly, as a proof of concept soil samples (250 mg) were artificially inoculated with 1, 3, 5 and 10 cysts. DNA template of *G. pallida* from 250 mg soil samples was isolated using a NucleoSpin soil kit (Macherey-Nagel, GmbH & Co. KG, Germany) by following the manufactures protocol. This time we performed the LAMP reaction in a water bath (maintained at 60 °C) and the products were analyzed as described earlier and the reactions were repeated thrice. Here, DDH₂O used as negative control.

Assessment of LAMP assay with soil samples collected from PCN sick field

Soil samples collected from PCN infested area of Himachal Pradesh (Kufri, Fagu, and Jubbal) and The Nilgiris (Muthorai, Appokodu and Porthyhada), Tamilnadu, India were used to check the efficacy of LAMP assay developed. For validation, DNA isolated from single cyst of *G. pallida* used as standard positive control. DNA from healthy soil used as no template control (NTC) and DDH₂O as a control.

Results

LAMP primer design

PCR primers were designed which successfully amplified 225 bp region (Fig. 2A) and the sequenced product in NCBI BLAST analysis, shared 99.14% nucleotide identity to the reference sequences of mt-COI region of *G. pallida*.

Standardization reaction and conditions for LAMP assay

At 60-64 °C, sharp bands of LAMP products were observed on 2.0% agarose gel whereas, almost no bands at 54 °C (Fig. 2B), therefore for further LAMP assay 60 °C temperature was set for amplification. While determining the optimal reaction time, maximum amplification of the LAMP products was noted at 60 min run and no clear detectable signal was obtained at 30-40 min run (Fig. 2C). LAMP products of *G. pallida* resulted visual change of colour from orange to green after addition of SYBR Gold nucleic acid dye. However, there were no sharp bands as well as colour change after the addition of SYBR Gold nucleic acid dye in the non template water control.

Comparative sensitivity assay

The LAMP assay was about 1000 times more sensitive than the conventional PCR as in the sensitivity test, conventional PCR was able to detect up to 10 picogram (pg)/ μ l genomic DNA whereas, LAMP assay produced positive results both in gel electrophoresis and SYBR Gold nucleic acid dye to the minimum genomic DNA concentration of 10 femtogram (fg)/ μ l (Fig. 3).

Specificity assay

In the specificity assay, LAMP primers amplified the DNA of *G. pallida* and not the DNA of other tested nematodes such as *G. rostochiensis*, *M. incognita*, *M. javanica*, *H. avenae*, *H. carotae*, and *Cactodera* spp. both in gel electrophoresis and SYBR Gold nucleic acid dye based detection of LAMP products (Fig. 4). Therefore, it has been concluded that LAMP primers were very specific to *G. pallida*.

Standardization of LAMP assay for G. pallida

The results of our study showed amplification as well as green fluorescence in SYBR Gold nucleic acid dye in genomic DNA extracted from soil inoculated with different *G. pallida* populations (Fig. 5A). Here, all the six soil samples collected from different PCN infested fields were found positive to *G. pallida* whereas, healthy soil (NTC) and water control did not exhibit amplification and fluorescence in SYBR Gold nucleic acid dye (Fig. 5B).

Discussion

White cyst nematode, *G. pallida* is a very specialized potato pest having very narrow host range of *Solanaceae* family crops. Worldwide it has been reported in 61 countries belonging to 5 continents and causes economic yield losses including India (EPPO 2018). It is an important quarantine pest which is mainly restricted to cooler/hilly potato growing regions. However, till date this pest at species level is being identified using morphological characters which are time consuming. Therefore, precise diagnosis method at field level is need of the day. All the molecular techniques developed till date for the identification at species require sophisticated equipments as well as expertise whereas, does not require the same and it is a precise amplification method that can be adopted in the field level diagnosing. In our study, primers designed for LAMP assay successfully detected the *G. pallida* directly from soil samples. In addition, the results were visualized through naked eye by adding SYBR Gold nucleic acid dye. Above all LAMP technique is friendly to people and the environment, the detection process does not require the use of Ethidium bromide and other toxic agents. Many researchers developed LAMP assays for the detection of many plant parasitic nematodes such as tropical root-knot nematodes (*Meloidogyne incognita* and *M. enterolobii*) (Niu et al, 2011; Niu et al. 2012), burrowing nematode (*Radopholus similis*) (Peng et al. 2012), pine wood or pine wilt nematode (*Bursaphelenchus xylophilu*) (Kikuchi et al, 2009; Kang et al. 2015; Meng et al. 2018), citrus root nematode (*Tylenchulus semipenetrans*) (Lin et al. 2016; Song et al. 2017), red ring nematode *B. cocophilus* (Ide et al. 2017), temperate root-knot nematode *M. hapla* (Peng et al. 2017), apple root-knot nematode *M. mali* (Zhou et al. 2017), grass nematode (*Anguina wevelli*) (Yu et al. 2018), *M. chitwoodi*, and *M. fallax* (Zhang and Gleason 2019).

In our study, the most efficient ladder like banding pattern was recorded at 60 °C incubation temperature for 60 min. However, Zhang and Gleason (2019) reported LAMP reaction for *M. chitwoodi* and *M. fallax* at 68 °C for 45 minutes. The LAMP reaction was performed at 63 °C for 45 min *Radopholus similis* (Peng et al. 2012). Rapid detection of *Meloidogyne* spp. by LAMP assay in soil and roots were obtained at 63 °C for 60 min (Niu et al. 2011). For the detection of *Anguina wevelli* LAMP assay resulted after 45 min at 63 °C (Yu et al. 2018).

We found that LAMP assay showed 1000 times more sensitive than the conventional PCR as the LAMP assay produced positive results to the least genomic DNA concentration 10 fg/μl whereas, the conventional PCR was up to 100 pg/μl. Interestingly, Zhang and Gleason (2019) found more sensitivity of LAMP assay while detection of potato nematodes *M. chitwoodi* and *M. fallax* as compared to conventional PCR. LAMP assay was found 10-100 times more sensitive as compared to conventional PCR for the detection of *R. similis* (Peng et al. 2012). During the detection of different RKN species and *M. incognita*, more or less same sensitivity of LAMP assay was observed by Niu et al. (2012) as compared to conventional PCR. Our results also corroborate with earlier reports of many researchers (Notomi et al. 2000; Kikuchi et al. 2009; Njiru et al. 2010; McKenna et al. 2011).

In the present study, the LAMP primers developed were very specific to *G. pallida* and there was no false reaction with the DNA of closely related species *G. rostochiensis* as well as other nematode species. Niu et al. (2011) also observed no cross reactivity of LAMP assay with DNA of other plant nematodes while detecting familiar *Meloidogyne* species and *M. incognita* populations having several geographical origins. Specificity of LAMP primers were also reported by Zhang and Gleason (2019) for the detection of *M. chitwoodi* and the closely-related species *M. fallax*.

Conclusion

In conclusion, the primers developed for LAMP assay specifically detected *G. pallida* with less assay time as compared to conventional PCR. Our study proposes that the assay developed very first time targeting mt-COI gene combined with SYBR Gold nucleic acid dye found to be highly reliable to detect *G. pallida* directly from the infested soil samples in the field conditions. Therefore, the LAMP assay for the diagnosis of *G. pallida* has remarkable practical field application in quarantine areas with respect to export and import of seed potato tubers.

Declarations

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Aarti Bairwa, Gaurav Verma and E. P. Venkatasalam planned and designed the research. Material preparation, data collection and analysis were performed by Bhawna Dipta, Aarti Bairwa, E. P. Venkatasalam, Kailash Naga and H. M. Priyank. The first draft of the manuscript was written by Aarti Bairwa and A. Shanthy, A. Jeevalatha and Sanjeev Sharma commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Aarti, B., Kailash, C.N., Dipta, B., Sharma, S., Venkatasalam, E.P. & Mhatre, P.H. (2019). Standardization of DNA extraction procedure from single potato cyst nematode (*Globodera* spp.) and whitefly (*Bemisia tabaci*). *In: ICAR-CPRI, News Letter, 76:1-2.*
- Aarti, B., Sharma, S., Venkatasalam, E.P., Sharma, A.K., Mhatre, P.H., Singh, R.K. & Chakrabarti, S.K. (2020a). Decontamination of cyst (*Globodera* spp.) infested potato seed tubers with sodium hypochlorite (NaOCl). *The Indian Journal of Agricultural Sciences, 90, 1345-1347.*
- Aarti, B., Venkatasalam, E.P., Jeevalatha, A., Mhatre, P.H., Buckseth, T., Jenifer, A., Sharma, S., Singh, R.K. & Chakrabarti, S.K. (2020b). Morphological and molecular characterization of potato cyst nematode populations from the Nilgiris. *The Indian Journal of Agricultural Sciences, 90, 273-278.*
- Aarti, B., Venkatasalam, E.P., [Sudha, R.](#), [Umamaheswari, R.](#) & [Singh, B.P.](#) (2017). Techniques for characterization and eradication of potato cyst nematode: a review. *Journal of Parasitic Diseases, 41, 607-620.* <https://doi.org/10.1007/s12639-016-0873-3>.

- EPPO (2018). Distribution maps for quarantine pests for Europe *Globodera rostochiensis* *Globodera pallida*. https://www.eppo.int/ACTIVITIES/plant_quarantine/A2_list.
- Ide, T., Kanzaki, N., Giraldo, P.P.P. & Giblin-Davis, R.M. (2017). Loop-mediated isothermal amplification (LAMP) for detection of the red ring nematode. *Nematology*, *19*, 559-565. <https://doi.org/10.1163/15685411-00003069>.
- Jones, F.G.W. (1961). The potato root eelworm, *Heterodera rostochiensis* in India. *Current Science*, *30*, 187.
- Kang, J. S., Kim, A. Y., Han, H. R., Moon, Y. S. & Koh, Y. H. (2015). Development of two alternative Loop mediated isothermal amplification tools for detecting pathogenic pine wood nematodes. *Forest Pathology*, *45*, 127-133. <https://doi.org/10.1111/efp.12147>.
- Kikuchi, T., Aikawa, T., Oeda, Y., Karim, N. & Kanzaki, N. (2009). A rapid and precise diagnostic method for detecting the pinewood nematode *Bursaphelenchus xylophilus* by loop-mediated isothermal amplification. *Phytopathology*, *99*, 1365-1369. <https://doi.org/10.1094/PHYTO-99-12-1365>.
- Lin, B., Wang, H., Zhuo, K. & Liao, J. (2016). Loop-mediated isothermal amplification for the detection in soil. *Plant Disease*, *100*, 877-883. <http://doi.org/10.1094/PDIS-07-15-0801-RE>.
- Mc Kenna, J.P., Fairley, D.J., Shields, M.D., Cosby, S.L., Wyatt, D.E., Mc Caughey, C. & Coyle, P.V. (2011). Development and clinical validation of a loop-mediated isothermal amplification method for the rapid detection of *Neisseria meningitidis*. *Diagnostic Microbiology and Infectious Disease*, *69*, 137-144. <https://doi.org/10.1016/j.diagmicrobio.2010.10.008>.
- Meng, F., Wang, X., Wang, L., Gou, D., Liu, H., Wang, Y. & Piao, C. (2018). A loop-mediated isothermal amplification-based method for detecting *Bursaphelenchus xylophilus* from *Monochamus alternatus*. *Forest Pathology*, *48*, e12404, <https://doi.org/10.1111/efp.12404>.
- Mori, Y. & Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *The Journal of Infection and Chemotherapy*, *15*, 62-69. <https://doi.org/10.1007/s10156-009-0669-9>.
- Niu, J.H., Guo, Q.X., Jian, H., Chen, C.L., Yang, D., Liu, Q. & Guo, Y.D. (2011). Rapid detection of *Meloidogyne* spp. by LAMP assay in soil and roots. *Crop Protection*, *30*, 1063-1069. <https://doi.org/10.1016/j.cropro.2011.03.028>.
- Niu, J.H., Jian, H., Guo, Q.X., Chen, C.L., Wang, X.Y., Liu, Q. & Guo, Y.D. (2012). Evaluation of loop mediated isothermal amplification (LAMP) assays based on 5S rDNA-S2 regions for detecting *Meloidogyne enterolobii*. *Plant Pathology*, *61*, 809-819. <https://doi.org/10.1111/j.1365-3059.2011.02562.x>.
- Njiru, Z.K., Ouma, J.O., Enyaru, J.C. & Dargantes, A.P. (2010). Loop-mediated Isothermal Amplification (LAMP) test for detection of *Trypanosoma evansi* strain B. *Experimental Parasitology*, *125*, 196-201.

[https:// doi.org/10.1016/j.exppara.2010.01.017](https://doi.org/10.1016/j.exppara.2010.01.017).

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, *28*, E63. <https://doi.org/10.1093/nar/28.12.e63>.

Peng, H., Long, H., Huang, W., Liu, J., Cui, J., Kong, L., Hu, X., Gu, J. & Peng, D. (2017). Rapid, simple and direct detection of *Meloidogyne hapla* from infected root galls using loop-mediated isothermal amplification combined with FTA technology. *Scientific Reports*, *7*, 44853. <https://doi.org/10.1038/srep44853>.

Peng, H., Peng, D., Hu, X., He, X., Wang, Q., Huang, W.K. & He, W. (2012). Loop-mediated isothermal amplification for rapid and precise detection of the burrowing nematode, *Radopholus similis*, directly from diseased plant tissues. *Nematology*, *14*, 977-986. <https://doi.org/10.1163/156854112X638415>.

Song, Z.Q., Cheng, J.E., Cheng, F.X., Zhang, D.Y. & Liu, Y. (2017). Development and evaluation of loop-mediated isothermal amplification assay for rapid detection of *Tylenchulus semipenetrans* using DNA extracted from soil. *Plant Pathology Journal*, *33*, 184-192. <https://doi.org/10.5423/PPJ.OA.10.2016.0224>.

Stone, A. (1973). *Heterodera pallida* n.sp. (Nematoda: Heteroderidae), a second species of potato cyst nematode. *Nematologica* *18*:591. doi: 10.1163/187529272X00179.

Talavera, M., Andreu, M., Valor, H. & Tobar, A. (1998). Nematodos fitoparasiticos enareas productoras de patata de Motril y Salobreña. *Invest Agric Prod Prot Veg*, *13*, 87-95.

Tomita, N., Mori, Y., Kanda, H. & Notomi, T. (2008). Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nature Protocols*, *3*, 877-882. <https://doi.org/10.1038/nprot.2008.57>.

Urwin, P. E., Troth, K. M., Zubko, E. I. & Atkinson, H. J. (2001). Effective transgenic resistance to *Globodera pallida* in potato field trials. *Molecular Breeding*, *8*, 95–101. doi:10.1023/a:1011942003994.

Verma, G., Sharma, S. Raigond, B. Pathania, S. Naga K. & Chakrabarti, S. K. (2019). Development and application of fluorescent loop mediated isothermal amplification technique to detect *Phytophthora infestans* from potato tubers targeting ITS-1 region. *3 Biotech*, *9*, 345. <https://doi.org/10.1007/s13205-019-1878-2>.

Wollenweber, H. (1923). Krankheiten und Beschädigungen der Kartoffel. Berlin: Arb. Forsch. Inst. Kartof, 56.

Yu, L.Z., Song, S.Y., Yu, C., Qi, L.J., Yu, Z.X., Jiao, B.B. & Yang, J. (2018). A loop mediated isothermal amplification (LAMP) assay for rapid and reliable detection of *Anguina wevelli*, a grass parasitic nematode. *European Journal of Plant Pathology*, *150*, 725-734.

Zhang, L. & Gleason, C. (2019). Loop-mediated isothermal amplification for the diagnostic detection of *Meloidogyne chitwoodi* and *M. fallax*. *Plant Disease*, 103, 12-18.

Zhou, Q. J., Cai, Y., Gu, J.F., Wang, X. & Chen, J. (2017). Rapid and sensitive detection of *Meloidogyne mali* by loop-mediated isothermal amplification combined with a lateral flow dipstick. *European Journal of Plant Pathology*, 148,755-769.

Zasada, I. & Dandurand, L.M. (2018). PCN by the Numbers." *Globodera Alliance Newsletter*, December 2018. [https://www.globodera.org/sites/default/files/newsletters/Issue 7 GloboderaAlliance Newsletter Dec 2018_0.pdf](https://www.globodera.org/sites/default/files/newsletters/Issue%207%20GloboderaAlliance%20Newsletter%20Dec%202018_0.pdf).

Table 1

Table 1 List of PCR and LAMP primers used in the present study

Code	Sequence (5'-3')	Length (bp)	Target
F3	ACAGGGGCTGGTGCTTTA	18	<i>G. pallida</i> -specific LAMP
B3	GCAAAATTTGGGGCGGGA	18	
FIP	CCGTAGGCGCATACTGTGGATC- ATCCTACCTCTACTGACCCG	42	
BIP	AACTCGGCAGTTTGGTGTTCGA- AATTCGATACCGCGCTCG	40	
FP	ACAGGGGCTGGTGCTTTA	18	<i>G. pallida</i> specific PCR (225 bp) for characterization of mt-COI region.
BP	GCAAAATTTGGGGCGGGA	18	

Figures

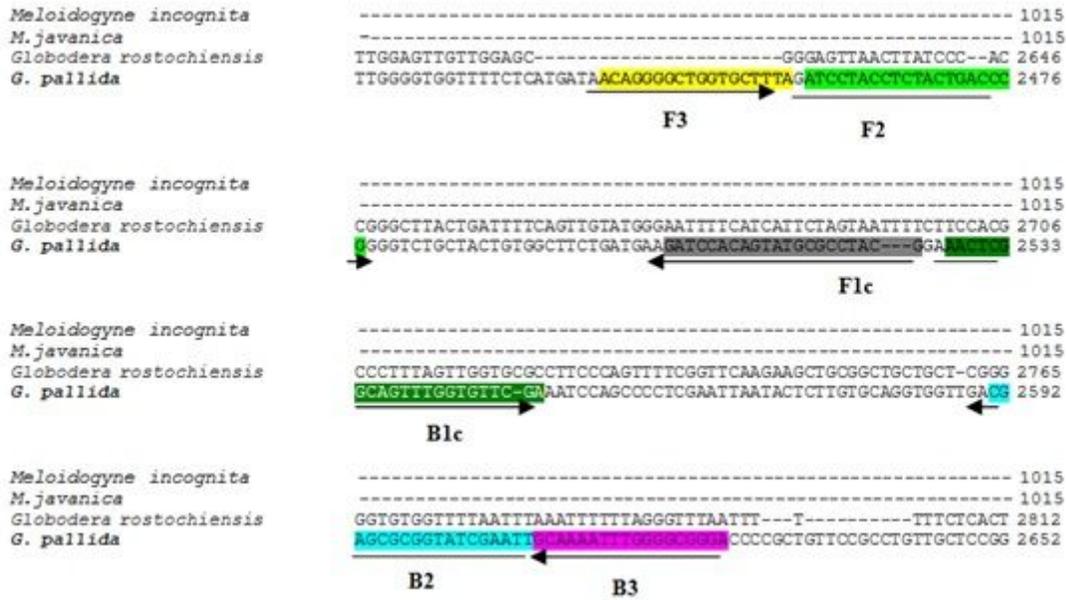


Figure 1

Sequence alignment of mt-COI gene showing position of LAMP primers. G. pallida LAMP targeted mt-COI region sequence is highlighted.

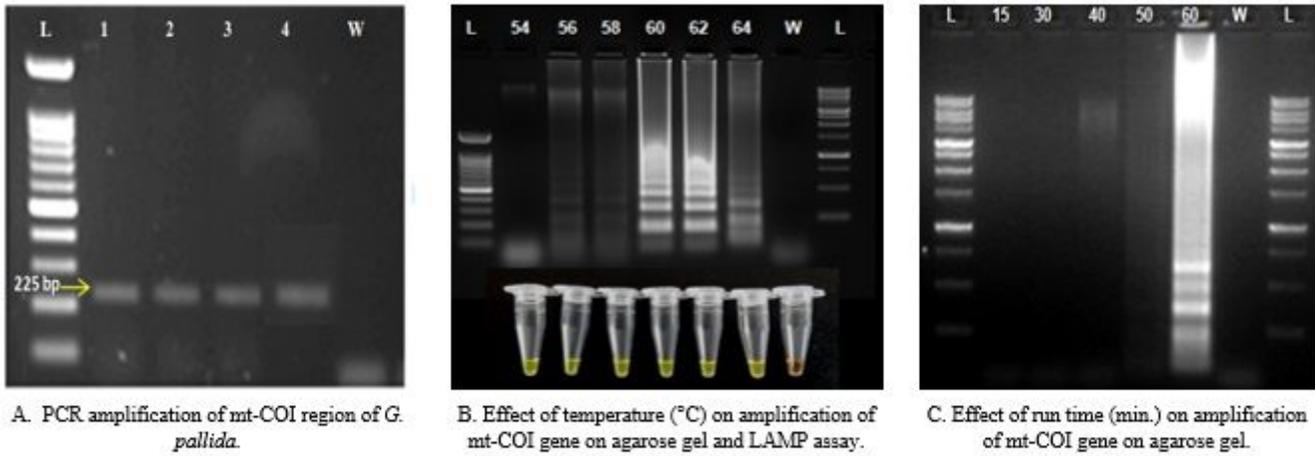


Figure 2

Standardization of LAMP assay for the detection of *G. pallida*. W: water; L: Ladder (100 bp).

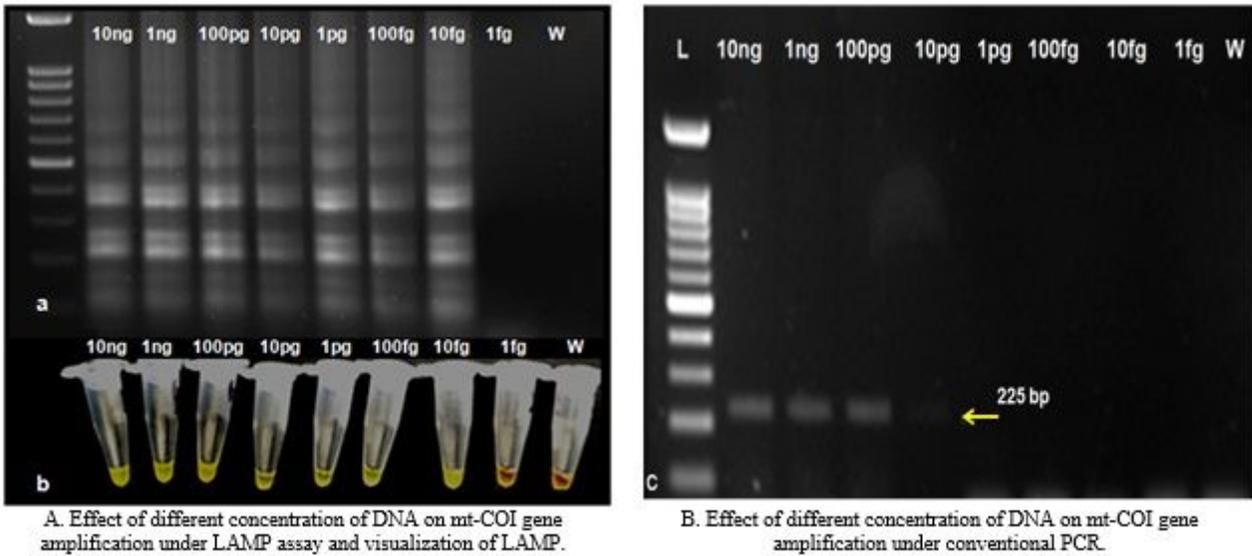


Figure 3

Comparison of sensitivity of LAMP assay with conventional PCR assay. W: water; L: Ladder (100 bp).

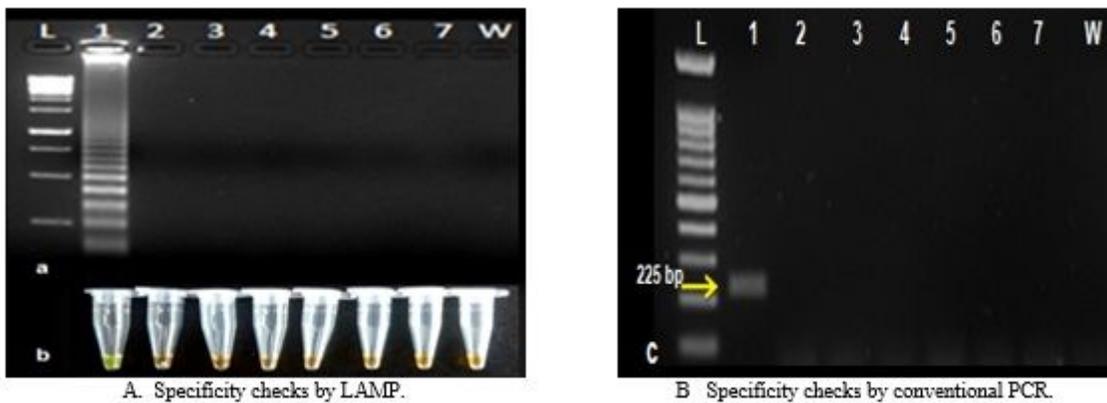
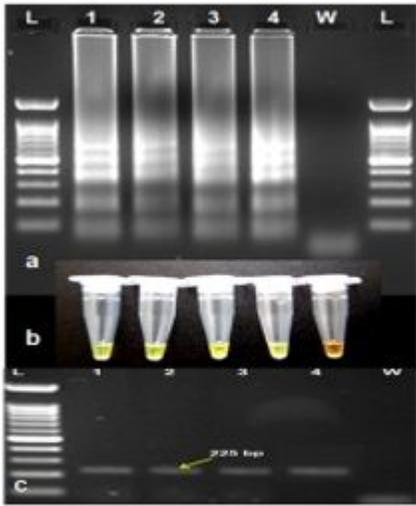


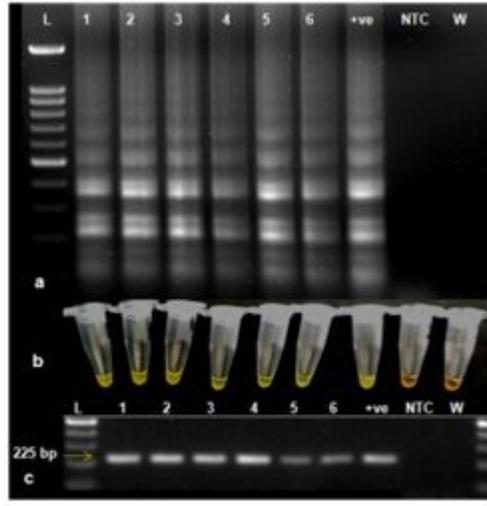
Figure 4

Specificity assay of LAMP and conventional PCR primers. 1. *G. pallida*, 2. *G. rostochinensis*, 3. *M. incognita*, 4. *M. javanica*, 5. *H. avenae*, 6. *H. carotae* 7. *Cactodera*. W: water; L: Ladder (100 bp).



A. Detection of *G. pallida* directly from artificially inoculated soil.

1. Single cyst; 2. Three cysts; 3. Five cysts; 4. Ten cysts; W: Water; L: Ladder (100bp).



B. Detection of *G. pallida* directly from PCN sick soil. 1-3 soil samples collected from Tamilnadu; 4-6 from Himachal Pradesh ; +ve: *G. pallida*; NTC: Healthy soil; W: Water; L: Ladder (100bp).

Figure 5

Detection of *G. pallida* directly from soil samples through LAMP assay and conventional PCR.