PCR and LAMP based diagnostic kit for detection of *Fusarium* dieback in tea

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Abstract: Detection of plant pathogens plays a vital role to implement proper management strategies. PCR have played crucial role in detection of several plant pathogen and also helps in to identify the putative pathogen at molecular level. Beside this, implementation of LAMP based detection methods also have helped to overcome the problems associated with PCR based methods. Implementation of PCR and LAMP based detection kit for diagnosis of dieback causing pathogen in tea ecosystem will provide better and proper management strategies to manage this biotic stress at the tea growing regions.

Keywords: database, genome, monitoring, primer, validation

Introduction

Dieback disease caused by *Fusarium* spp. in tea is one of the major challenges among the biotic stresses as this pathogen can alone causes 20-60% yield losses. The pathogen initially attacks the plucking shoots and later it progresses downwards and causes complete crop loss under favourable conditions (Das *et al.*, 2025). Based on detection and identification of the causal agent for dieback pathogen, recent studies revealed that this disease is favoured by *Fusarium* species complex. Molecular based identification and analysis have revealed the association of four species of *Fusarium i.e. F. concentricum*, *F. fujikuroi*, *F. solani* and *F. oxysporum*. Among these, *F. concentricum* was found to be dominant and virulent species causing dieback disease in the tea ecosystem of Assam and North Bengal (Pandey *et al.*, 2024). Therefore, to enable the precise identification of the pathogen at the molecular level, use of Polymerase Chain Reaction (PCR) and Loop-mediated isothermal amplification (LAMP) based detection will play a crucial role in identification of the pathogen over traditional morphological methods (Vázquez-Rosas-Landa *et al.*, 2021). This article highlights the different steps involves for the development of diagnostic kit for *Fusarium* pathogen causing dieback disease in tea by PCR and LAMP techniques.

PCR based detection of *Fusarium* spp.

For proper identification of the pathogen causing dieback in tea ecosystem, PCR based technique is rapid and more reliable. In PCR based detection of this pathogen, primers like ITS1, ITS4, ITS-Fu-f, ITS-Fu-r etc. are mainly used (Sarkar, 2025). Tang *et al.* (2024) identified four species of *Fusarium* viz. *F.concentricum*, *F. solani*, *F. fujikuroi*, *F. oxysporum* where they have mainly used ITS, *tef*-1 α, *tub2*, and *rpb2* sequences in PCR amplification. In another study conducted by Liu *et al.* (2026) studied the molecular characteristics of *Fusarium solani* species complex which causes dieback disease in tea. For this, ITS, *tef-1alpha*, *beta-tubulin*, *RPB1*, *RPB2* and *CAL5* gene regions were amplified and PCR products were then amplified for its identification. For the development of PCR based diagnostic kit for the detection of dieback pathogen involves several sequential and laboratory experiments for its final validation. Initially, the diseases infested samples showing prominent symptom of dieback will be collected from the tea growing areas and then isolation of the pathogen will be conducted by performing standard protocol (Das *et al.*, 2025). After the isolation of pathogens, the cultural plates will be incubated at 25-27 °C until the full hyphal emergence occurs. Fresh culture (7 days old culture) of the isolates will be used for the DNA extraction. The genomic DNA can be extracted by using 2% cetyltri-methylammonium bromide (CTAB) methods. Then the PCR amplification can be performed by using aforementioned primers. After that, all the primer products will be subjected for electrophoresis in 2% agarose gel and then it can be purified by using gel extraction kit (GenElute™ Gel extraction kit, HiPurA® Quick Gel Purification Kit). The DNA sequences of primers (ITS, tub2, tef-1α, tub2, and rpb2) then used in the phylogenetic analysis. The closest matches then identified by BLAST (Basic Local Alignment Search Tool) searches in NCBI (National Centre for Biotechnology Information) databases. For the detection and identification of the pathogen causing dieback disease in team selection of targeted gene region and primer designs plays a crucial role. Proper primer designing and target region selection will further help in amplification of the targeted DNA without amplifying the non-targeted DNA from the other microbes and plant sources (Costa *et al.*, 2017).

Steps involves in development of PCR based detection kit

1. **Collection, isolation and identification of the putative pathogen causing dieback in tea:** Dieback infested tea samples will be collected from the tea ecosystem and then isolation of the pathogen will be done from the infested leaf, stem, root sections. The pathogen can also isolate from the soil samples collected from the tea plantation areas showing prominent symptom of dieback. After the isolation of the putative pathogen, characterisation can be done based on cultural and microscopic

characteristics. The data obtained from these characterisations can be correlated with the published literatures for its identification up to genus level.

2. **Extraction of genomic DNA:** The extraction of genomic DNA can be performed by using CTAB method or by using commercial fungal DNA extraction kits. The quality and quantity assessment of the extracted DNA can be done by using gel electrophoresis, nanodrop or by spectrophotometric analysis (Brandfass *et al.*, 2008; Jiménez-Fernández *et al.*, 2010).
3. **Selection of target gene region:** The unique region specific to *Fusarium* spp. causing dieback in tea is mainly selected for its PCR amplification. For this, some selected primers are commonly used such as Internal Transcribed Spacer (ITS), β-tubulin, elongation factor-1 alpha etc. These regions are manly used for species specific identification of *Fusarium* spp. (Xu *et al.*, 2022; Teng *et al.*, 2024)
4. **Primer designing:** The primers that are species specific to *Fusarium* sp. are designed by using the sequence from databases like NCBI. Several bioinformatic tools are used for the design of a particular primer that specifically amplify the targeted *Fusarium* spp. causing dieback disease in tea. To develop molecular diagnostic tool for *Fusarium*, Suga *et al.* (2013) developed PCR primers by identifying the portions of transposable elements (TEs) in the *Fusarium* spp. Specialized methods like Transposon Display (TD), inter-retrotransposon Amplified Polymorphism (IRAP) and Sequence-Specific Amplification Polymorphism (SSAP) are used for designing primers to match the ends of TEs to amplify the regions. For the identification of primers, bioinformatic tools like RJ primers are used for genome scanning for its sequences related to TE insertion junction by using BLASTN searches databases (Castanera *et al.*, 2016; Dufresne *et al.*, 2011).
5. **Optimization and validation of PCR components:** To obtain clear and reproducible amplification of the PCR product parameters such as primer concentration, MgCl2 concentration, temperature for annealing and numbers of cycle required are adjusted. This ensures high sensitivity and specificity of the pathogen detection. During validation of the primer, the selected primer should amplify the targeted DNA from the *Fusarium* spp. causing dieback in tea. Mishra *et al.* (2003) have studied the PCR based assay for rapid and reliable identification of pathogenic *Fusaria*. They have highlighted that, use of fluorescent dyes in place of ethidium bromide (EtBr) can reduce the chance of exposure to carcinogenic substances. Lacmanová *et al.* (2009) also have highlighted the primers and other PCR products required for identification of toxicogenic *Fusarium* spp.
6. **Sensitivity testing for detection of PCR limit:** To determine the minimum concentration required for detection of *Fusarium* spp. under PCR, serial dilution method is followed. This method helps to know at what minimum concentration of DNA, detection of *Fusarium* spp. can be performed. Jurado *et al.* (2005) have conducted an experiment where different dilution of DNA (90 ng to 170 fg) were prepared and tested for its sensitivity in PCR.
7. **Detection from infected plant tissues:** The DNA extracted from the infected plant tissue or tea twigs can be used for detection of the pathogen. PCR is mainly performed by using the developed primers that can confirm the pathogen's presence in the dieback infested tea samples. Moricca *et al.* (1998) conducted the detection of *Fusarium* spp. in cotton plant by employing PCR. They have highlighted that, detection based on PCR found to be accurate and more sensitive and which not only helps in diagnosis but also helps in disease monitoring and forecasting.
8. **Development of diagnostic kit and its field validation and standardization:** After the validation of the PCR components and its concentration required for detection of *Fusarium*, a diagnostic kit can be assembled. Then the kit can be used for its evaluation at the filed level. For further validation, the detection ability of this kit can be standardised at different tea growing ecosystem situated at different agro-ecological conditions. Later on, the kit protocol can be documented for the purpose of rapid detection of *Fusarium* infestation at the tea gardens.

LAMP mediated detection of *Fusarium*

Loop Mediated Isothermal Amplification (LAMP) has emerges one of the most efficient tools for the detection of plant pathogens which has overcome the limitations of traditional PCR methods. This works on constant temperature unlike PCR which relies on the varying temperature inside the thermocycler for DNA amplification. Beside this, LAMP is typically more robust, specific and

sensitive than PCR. In recent days commercially, available LAMP based diagnostic kits are there and some of them are already has been officially recommended for diagnosis of certain plant diseases (Németh and Kovács *et al*., 2025). The PCR mainly detects the DNA with in 2-3 hours sometimes a day at femtogram level which is ideal for lab confirmation of detection of the pathogen. Whereas, LAMP can amplify DNA in 30-60 minutes isothermally (60-65°C) and also provides visual readouts via colour change detection methods (Dong *et al*., 2025). Ghosh *et al*. (2015) has developed LAMP assay for rapid detection of *F. oxysporum* f.sp. *ciceris* which causes wilt in chickpea. By using this, they have targeted the elongation factor 1 alpha gene sequence for the visualization of the pathogen for its detection. In another study by Gupta *et al*. (2020) has developed LAMP based detection of *F. graminearum* which causes head blight of wheat. During this investigation they have standardised the concentration of outer and inner primers, Mg^{2+} and the reaction temperature required for detection of this pathogen via LAMP assay. This assay also highlighted the sensitivity speed and specificity where it can detect less than 100 fg of DNA per reaction within 60 min. Jiang *et al*. (2021) have initially designed the primers for LAMP by using Primer Explorer V5 software afterwards they have standardised the optimized the conditions and reactions required for LAMP assay for the detection of *F. fujikuroi* causing bakanae disease in rice seedlings. Beside the detection abilities, LAMP also plays a vital role in distinguishing pathogens at the species level. Deng *et al*. (2022) have conducted LAMP assay for distinguishing *F. oxysporum* and *F. commune* in Lotus rhizomes. This helps in rapid identification of pathogens causing disease at different species level and thus facilitate efficient pesticide use for the management of the disease.

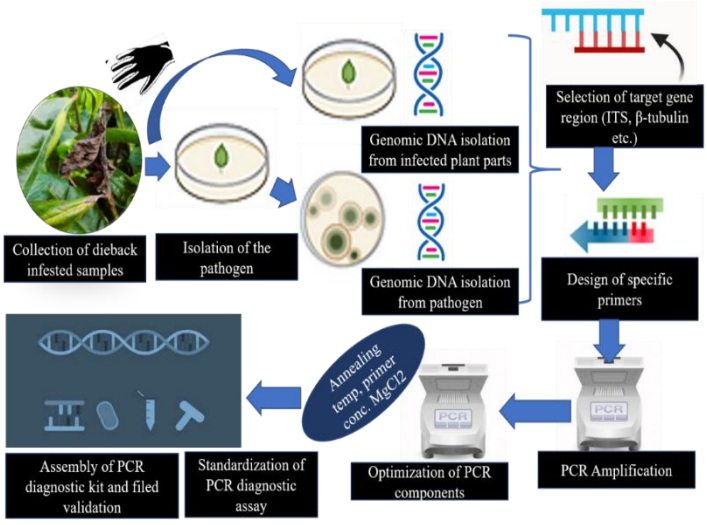

Fig 1: Steps involved in detection of *Fusarium* causing dieback disease in tea by PCR detection kit

The LAMP assay generally comprises components like templet DNA which is obtained from the fungal pathogens and requires four primers viz. forward inner primers (FIP), backward inner primer (BIP), forward outer primer (F3) and backward outer primer (B3), *Bst* DNA polymerase, dNTPs and reaction buffer and ions like Mg^{2+}. The LAMP generally works based on the auto-cycling strand displacement of DNA synthesis which is carried out by using *Bst* polymerase enzyme which has been obtained from *Geobacillus stearothermophilus*. The LAMP primers initiate the amplification process and then creates a loop like structure that allows continuous DNA amplification. During this process, the inner primers (FIP and BIP) initiate the synthesis of DNA with the help of the enzyme *Bst* polymerase which can produce the complementary DNA strand. Then the outer primers (F3 and B3) displaces the newly synthesized DNA strand which results in formation of loop like structure at both ends of the DNA. This loop structures then serve as templet for continuous amplification. This amplification results in exponential yield of DNA within 30-60 minutes at constant temperature. Then the amplified DNA can be detected by using dyes like hydroxynapthol blue or SYBR green dye (by colorimetric method), agarose gel electrophoresis methods or by measuring the turbidity by using magnesium polyphosphate methods (Silva *et al*., 2020).

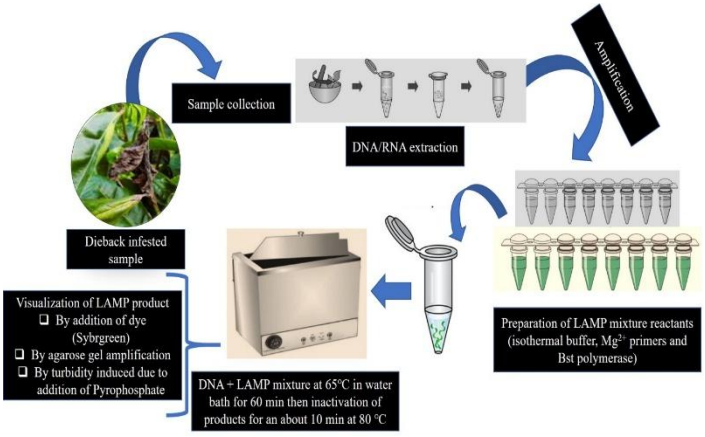

Fig 2: Steps involved in detection of *Fusarium* causing dieback disease in tea by LAMP assay

Conclusion: By implementing proper standardisation and validation technique for the development of PCR and LAMP based detection kit will helps to identify and monitor the presence of pathogen causing dieback disease in tea ecosystem. This detection kit will help to implement better management strategies to curb this disease and thus it will promote sustainable agriculture.

References

Brandfass, C. and Karlovsky, P., 2008. Upscaled CTAB-based DNA extraction and real-time PCR assays for *Fusarium*

culmorum and *F. graminearum* DNA in plant material with reduced sampling error. *International journal of molecular sciences*, *9*(11), pp.2306-2321.

Castanera, R., López-Varas, L., Borgognone, A., LaButti, K., Lapidus, A., Schmutz, J., Grimwood, J., Pérez, G., Pisabarro, A.G., Grigoriev, I.V. and Stajich, J.E., 2016. Transposable elements versus the fungal genome: impact on whole-genome architecture and transcriptional profiles. *PLoS genetics*, *12*(6), p.e1006108.

Costa, S.S., Moreira, G.M. and Pfenning, L.H., 2017. Development of a PCR protocol for the identification and detection of *Fusarium solani* f. sp. *piperis* from soil and roots of black pepper (Piper nigrum). *Tropical Plant Pathology*, *42*(1), pp.55-59.

Das, P., Mahanta, M., Bhattacharyya, A. and Dutta, P., 2025. Studies on cultural and morphological variability in the isolates of *Fusarium solani* causing dieback disease in tea ecosystem of NE India. *Plant Health Arch.* 3(1): 01-07.

Deng, S., Ma, X., Chen, Y., Feng, H., Zhou, D., Wang, X., Zhang, Y., Zhao, M., Zhang, J., Daly, P. and Wei, L., 2022. LAMP assay for distinguishing *Fusarium oxysporum* and *Fusarium* commune in lotus (*Nelumbo nucifera*) rhizomes. *Plant disease*, *106*(1), pp.231-246.

Dong, Y., Song, J., Li, S., Zhan, J. and Zhu, T., 2025. Development and comparative evaluation of LAMP, nested PCR and Real-time PCR assays for detecting *Fusarium tricinctum*, a fungal pathogen of *Zanthoxylum bungeanum*. *BMC microbiology*, *25*(1), p.568.

Dufresne, M., Lespinet, O., Daboussi, M.J. and Hua-Van, A., 2011. Genome-wide comparative analysis of pogo-like transposable elements in different *Fusarium* species. *Journal of molecular evolution*, *73*(3), pp.230-243.

Ghosh, R., Nagavardhini, A., Sengupta, A. and Sharma, M., 2015. Development of Loop-Mediated Isothermal Amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* f. sp. *ciceris*-wilt pathogen of chickpea. *BMC research notes*, *8*(1), p.40.

Gupta, S., Saharan, M.S., Gurjar, M.S., Singh, J., Bashyal, B.M. and Aggarwal, R., 2020. Molecular detection of *Fusarium graminearum* causing head blight of wheat by loop mediated isothermal amplification (LAMP) assay. *Indian Phytopathology*, *73*(4), pp.667-672.

Jiang, H., Wu, N., Jin, S., Ahmed, T., Wang, H., Li, B., Wu, X., Bao, Y., Liu, F. and Zhang, J.Z., 2020. Identification of rice seed-derived *Fusarium* spp. and development of LAMP assay against *Fusarium fujikuroi*. *Pathogens*, *10*(1), p.1.

Jiménez-Fernández, D., Montes-Borrego, M., Navas-Cortés, J.A., Jiménez-Díaz, R.M. and Landa, B.B., 2010. Identification and quantification of Fusarium oxysporum in planta and soil by means of an improved specific and quantitative PCR assay. *Applied Soil Ecology*, *46*(3), pp.372-382.

Jurado, M., Vázquez, C., Patiño, B. and González-Jaén, M.T., 2005. PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. *Systematic and Applied Microbiology*, *28*(6), pp.562-568.

Lacmanová, I., Pazlarová, J., Kostelanská, M. and Hajšlová, J., 2009. PCR-based identification of toxinogenic Fusarium species. *Czech Journal of Food Sciences*, *27*(Special Issue 2), p.90.

Liu, L., Ge, Z., Nie, Y., Ma, Y., Liu, G., Wang, W. and Yu, Y., 2026. First report of *Fusarium solani* causing collar rot and dieback on Tea plants in Shaanxi Province, China. *Plant Disease*, (ja).

Mishra, P.K., Fox, R.T. and Culham, A., 2003. Development of a PCR-based assay for rapid and reliable identification of pathogenic Fusaria. *FEMS microbiology letters*, *218*(2), pp.329-332.

Moricca, S., Ragazzi, A., Kasuga, T. and Mitchelson, K.R., 1998. Detection of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton tissue by polymerase chain reaction. *Plant Pathology*, *47*(4), pp.486-494.

Németh, M.Z. and Kovács, G.M., 2025. A comprehensive guide to loop-mediated isothermal amplification, an emerging diagnostic tool for plant pathogenic fungi. *Frontiers in Plant Science*, *16*, p.1568657.

Pandey, A.K., Hubballi, M., Sharma, H.K., Ramesh, R., Roy, S., Dinesh, K. and Babu, A., 2024. Molecular delineation and genetic diversity of Fusarium species complex causing tea dieback in India and their sensitivity to fungicides. *Crop Protection*, *181*, p.106707.

Sarkar, A. (2025). *Fusarium* dieback: An emerging issue in the tea industry. Kavaka; 61(2): 25-31. DOI: 10.36460/Kavaka/61/2/2025/25-31.

Suga, H., Hirayama, Y., Morishima, M., Suzuki, T., Kageyama, K. and Hyakumachi, M., 2013. Development of PCR primers to identify Fusarium oxysporum f. sp. fragariae. *Plant Disease*, *97*(5), pp.619-625.

Tang, Z., Zhu, J., Song, Q., Daly, P., Kong, L., He, L., Li, A., Lou, J., Wang, Z., Zhang, L. and Min, L., 2024. Identification and pathogenicity of Fusarium spp. associated with tea wilt in Zhejiang Province, China. *BMC microbiology*, *24*(1), p.38.

Vázquez-Rosas-Landa, M., Sánchez-Rangel, D., Hernández-Domínguez, E.E., Pérez-Torres, C.A., López-Buenfil, A., de Jesús García-Ávila, C., Carrillo-Hernández, E.D., Castañeda-Casasola, C.C., Rodríguez-Haas, B., Pérez-Lira, J. and Villafán, E., 2021. Design of a diagnostic system based on molecular markers derived from the ascomycetes pan-genome analysis: The case of Fusarium dieback disease. *Plos one*, *16*(1), p.e0246079.

Xu, W., Zhao, F., Deng, X., Goodwin, P.H., Xia, M., Zhang, J., Sun, R., Liang, J., Wu, C. and Yang, L., 2022. First Report of Collar Canker and Dieback of *Camellia sinensis* caused by *Fusarium solani* Species Complex in Henan, China. *Plant disease*, *106*(8), p.2263.

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