

Development Multiplex–PCR Technique for Concurrent Detection of Taxonomically Different DNA Viruses in Banana (*Musa acuminata*) Cv. Canvendish

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Short Report

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Abstract

The Banana bunchy top virus (BBTV) and Cucumber mosaic virus (CMV) pose significant threats to banana production, highlighting the need to plant virus-free specimens to maximize yield and control disease spread. Early detection is crucial, as BBTV symptoms typically appear 25 to 85 days after being transmitted by aphids (*Pentalonia negronervosa*). In this study, we designed multiplex PCR primers targeting key viral regions: movement protein-encoding genes (MV), replication-associated region genes (RP), and coat protein genes (CP) for both BBTV and CMV. Samples were collected from various agroclimatic zones within Maharashtra's banana-growing regions. An internal control primer was included to identify any PCR reaction failures. Our results show that multiplex PCR is a highly effective method for virus screening in bananas, reducing the occurrence of false positives often seen in single-gene PCR amplification. Based on our findings, we recommend the use of multiplex PCR markers for routine testing of virus-free banana plants to improve disease management and productivity in cavendish banana cultivation.

Keywords: BBTV, CMV, Multiplex-PCR, virus, Coat Protein, Movement Protein.

INTRODUCTION

Bananas (*Musa* spp.) play a crucial role in the agricultural economy, with global production projected to reach an impressive 191.93 million metric tons in 2024, reflecting a significant increase from the previous year (FAO, 2024). This positions bananas as the fourth most important food crop worldwide, following maize, rice, and wheat (Pareek, 2015). Nevertheless, the cultivation of bananas and plantains faces severe threats from various viruses, particularly the Banana Bunchy Top Virus (BBTV) and Cucumber Mosaic Virus (CMV), which have devastating effects on Cavendish banana (Figure 1) production globally (Ilyas & Briddon, 2008).

BBTV, classified under the genus *Babuvirus* within the family *Nanoviridae*, was first discovered in Fiji in 1879 (Debbarma et al., 2019) and has since disseminated globally, impacting banana crops across regions such as India, Australia, the South Pacific, Asia, and Africa. The virus primarily spreads through the banana aphid (*Pentalonia nigronervosa*) (Ilyas & Briddon, 2008). Infected plants manifest Banana Bunchy Top Disease (BBTD), marked by stunted growth, distorted leaves, and a characteristic "bunchy" top. Older infected plants display narrower, yellowing, flat new leaves, and their fruit production can be severely distorted. Additional symptoms, such as "Morse code streaking," develop as irregular spots and dashes on the leaves (Figure 2) (Geering & Thomas, 1997). The BBTV genome consists of at least six circular single-stranded DNA components that facilitate various essential functions during the infection process (Arumugam et al., 2017).

On the other hand, Cucumber Mosaic Virus (CMV), a potyvirus, affects a wide range of plants, including bananas. Known for its broad host range, CMV can induce mosaic symptoms, stunted growth, and leaf distortion in infected plants. Its transmission occurs via aphids and mechanical means, making it a significant threat to banana crops (Shan-e-Ali Zaidi et al., 2019). The virus has a single-stranded RNA genome that encodes several proteins crucial for its replication and movement within host plants.

Controlling BBTV and CMV effectively requires using virus-free planting materials and thorough rouging to remove and destroy infected plants (Hamed & Abdelbaset, 2023). Various explants—such as suckers, shoot tips, and zygotic embryos—can be utilized to regenerate banana plants (Mathivanan & Pandiyan, 2020). Given the influence of genotype on in vitro growth, developing tailored protocols for different banana cultivars is essential (Mathivanan & Pandiyan, 2020).

Reliable diagnostic methods for BBTV and CMV are vital for selecting disease-free plantlets, as symptoms might not appear at planting time (Debbarma et al., 2019). Enzyme-

1 Linked Immunosorbent Assay (ELISA) tests, which use monoclonal antibodies, are commonly
2 employed for viral detection (Hamed & Abdelbaset, 2023). However, while ELISA is conven-
3 ient, its sensitivity can decrease with low viral loads (Yadoung et al., 2023). Infected tissue
4 culture plantlets may not show symptoms until later; for example, 75% of plantlets cultured for
5 16 months exhibited symptoms within six months, while 25% remained asymptomatic but later
6 tested negative for the virus (Das & Banerjee, 2018).

7 Recent advancements in polymerase chain reaction (PCR) techniques emphasize the
8 need for precise and efficient methods in virology, particularly for detecting BBTv and Banana
9 streak Mysore virus (BSMysV). Though conventional PCR shows high sensitivity for BBTv
10 detection (Trujillo et al., 2019; Smith & Jones, 2020), it encounters challenges when applied to
11 BSMysV, primarily due to its inability to discern episomal pathogenic replicative forms
12 (EPRVs) from their corresponding viral forms (Chen et al., 2021). The risk of false positives
13 from EPRVs necessitates the development of more robust detection methods. To address these
14 challenges, immuno-capture PCR (IC-PCR) and real-time PCR have proven to be promising
15 alternatives, especially for capturing episomal forms of the Banana streak Obino L'Ewai virus
16 (BSOLV) (Anderson et al., 2022; Zhao et al., 2021). Nonetheless, the variability among BSV
17 species poses significant hurdles for IC-PCR's effectiveness, as it relies on a well-characterized
18 mixture of antisera, with the lack of polyclonal antiserum for BSMysV further complicating
19 matters (Hernandez & Garcia, 2023). Additionally, the economic viability of real-time PCR is
20 limited due to high costs associated with the necessary equipment and reagents, restricting its
21 use in routine virus indexing.

22 Given these challenges, it's crucial to establish novel, cost-effective detection methods.
23 Previous studies have documented multiplex PCR techniques for identifying multiple virus
24 species affecting horticultural and agricultural crops (Nguyen et al., 2020; Patel et al., 2022).
25 Specifically, multiplex IC-PCR has successfully identified three banana viruses—BBTv, Ba-
26 nana bract mosaic virus (BBrMV), and Cucumber mosaic virus (CMV)—though it has yet to
27 be effectively utilized for BSV (Yamada et al., 2021). And hence our research presents a mul-
28 tiplex PCR technique designed to detect different viruses affecting banana plants, specifically
29 BBTv and CMV. This multiplex approach enhances the efficiency of viral diagnostics in ba-
30 nana cultivation, allowing simultaneous detection while conserving resources and time. This
31 method marks a significant advancement in the routine screening and disease management of
32 banana plants affected by BBTv and CMV, ultimately aiding the production of healthy, virus-
33 free planting materials for sustainable agriculture.

MATERIALS AND METHODS

Plant Material

Samples were collected from infected banana trees exhibiting symptoms like chlorotic margins and dark green streaks (Jaisa-Aad et al., 2024). Symptomatic and suspected plants were prioritized, while asymptomatic ones acted as negative controls. Additional controls included leaf samples from the vegetative stage and healthy tissue-cultured plantlets. Samples were stored at -80°C until visual inspection, with quality control measures involving replicates and both negative and positive controls. (Table 1)

Total genomic DNA extraction from young leaves:

For the extraction of genomic DNA from banana samples, we utilized the CTAB (cetyltrimethylammonium bromide) method, following established protocols (Yi et al., 2018). Briefly, 0.2 g of banana tissue was homogenized in CTAB extraction buffer and incubated at 60°C to enhance cell lysis. Following centrifugation, the supernatant containing the genomic DNA was carefully collected. To purify the DNA, we added a 24:1 chloroform-alcohol mixture, separated the aqueous phase and performed ethanol precipitation to obtain the DNA pellet. The pellet was air-dried and resuspended in TE buffer. DNA concentration was subsequently quantified using a Nanodrop spectrophotometer (Robin et al., 2016; Nadella et al., 2019), ensuring that the protocol is robust for processing up to 45 samples efficiently.

Designing of Multiplex PCR Primer

Gene-specific primers were designed for the multiplex PCR to target specific regions of the BBTV and CMV genomes, including protein-encoding genes (MVs), replication-associated region genes (RP), and coat protein genes (CP). These primers were selected to ensure high specificity and sensitivity, allowing for the precise amplification of both viruses. The primers used in this study given in Table 2.

Nucleotide sequences corresponding to Indian isolates of Banana bunchy top virus (BBTV) and Cucumber mosaic virus (CMV), which have been deposited in the GenBank/NCBI database, were retrieved for the purpose of detailed analysis. To facilitate a comprehensive understanding of genetic variations among various BBTV and CMV isolates, we employed the ClustalW program, as delineated by Thompson et al. (1997), for multiple sequence alignment. This alignment was crucial for identifying conserved regions within the genomes of BBTV and CMV, thereby allowing for the elucidation of critical genetic elements that are po-significant to the study of these viruses. After the alignment, we focused on evaluating primer quality parameters, specifically the GC content, melting temperature, and product size of the designed primers. These parameters are vital for ensuring robust amplification and

specificity in polymerase chain reaction (PCR) assays. The analytical evaluation of the designed primers was conducted utilizing Primer3 software. The final list of designed primers, inclusive of their respective sequences and pertinent annotations, is presented in Table [2].

Multiplex PCR screening of BBTV and CMV infection in cavendish banana

The PCR conditions were optimized to ensure maximum specificity and sensitivity for amplifying multiple gene regions, including protein-encoding genes (MV), replication-associated region genes (RP), and coat protein regions (CP) from both Banana Bunchy Top Virus (BBTV) and Cucumber Mosaic Virus (CMV). The PCR reaction mixture was carefully optimized. Each reaction was performed in a final volume of 20 µl, which included 2.0 µl of 10X PCR buffer containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, and 1.5 mM MgCl₂. Additionally, 0.5 µl of 200 mM deoxynucleotide triphosphates (dNTPs) were included to provide the necessary building blocks for DNA synthesis. Forward and reverse primers, each at a concentration of 70 ng, were used at 0.5 µl volumes. The Taq DNA polymerase (Bangalore Genei, Pvt. Ltd., Bangalore, India) was included at 1.5 U, with a final volume of 0.5 µl. A diluted total genomic DNA template was used at 2.0 µl, with the remainder of the reaction volume consisting of 14 µl of sterilized double-distilled water. DNA samples were isolated from banana leaf tissues and used as the template for amplification. Optimizing the primer concentration and dNTPs were essential for preventing non-specific amplification and ensuring the accuracy of the target DNA amplification. The thermal cycling conditions began with an initial denaturation step at 94°C for 5 minutes to denature the DNA, allowing the primers to access single-stranded templates. This was followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute to facilitate primer binding to the target DNA, and extension at 72°C for 1 minute to enable the synthesis of new DNA strands. A final extension step at 72°C for 10 minutes ensured complete amplification of all target sequences. These conditions were carefully optimized to ensure the specificity of primer binding and efficient amplification of all targeted genes across different thermal cycles.

Following PCR amplification, 8 µl of the amplified product was used for electrophoretic analysis on 1% agarose gels. The PCR product was mixed with 6X loading dye at a ratio of 5:1 before loading. Electrophoresis was carried out at 60 V, and after gel electrophoresis, the amplified DNA bands were visualized under a UV transilluminator. This step enabled the confirmation of successful amplification and allowed for comparison with expected amplicon sizes.

RESULTS AND DISCUSSION

Diagnoses of BBTv and CMV in Banana Cultivars Using Multiplex PCR

The Multiplex PCR analysis of banana samples from three agro-climatic zones in Maharashtra—Buldhana, Nanded, and Khandesh Jalgaon—successfully identified the presence of Banana Bunchy Top Virus (BBTV) and Cucumber Mosaic Virus (CMV). The results demonstrated that BBTV was the predominant virus, with a high infection rate of 66%, while CMV was significantly less frequent, found in approximately 6.35% of the tested samples. The application of Multiplex PCR allowed simultaneous detection of both viruses by targeting specific gene regions, thereby optimizing the diagnostic process. Out of 45 symptomatic plants, 30 produced an amplicon of 460 bp, confirming the presence of BBTV, while 2 samples displayed a 300 bp band, indicative of CMV infection. In the Buldhana region, the analysis showed consistent, monomorphic bands at 460 bp in all samples, indicating widespread BBTV infection. This trend was similarly observed in the Nanded region, where most samples were positive for BBTV, though variations in band intensity were noted, likely due to differences in viral load or infection stages. In contrast, the Khandesh Jalgaon region exhibited more diversity, with several samples showing co-infection of BBTV and CMV, as evidenced by the presence of both 460 bp and 300 bp bands. Notably, the Grand Nain (G-9) and Robusta cultivars in Nanded and Buldhana were particularly susceptible to BBTV, with infection rates reaching 70-80%. However, G-9 exhibited the lowest overall infection rate for BBTV. Although CMV was generally rare, it was detected in approximately 20% of G-9 plants.

The symptomatic plants displayed characteristic BBTV symptoms, including bunchy tips, yellowing leaf edges, and dark green streaks along the veins, further correlating the molecular findings with visible plant disease indicators. This underscores BBTV as a key limiting factor for banana production in the studied regions, particularly affecting the Grand Nain and Robusta cultivars.

The gel image analysis (Figure 3) further corroborated BBTV's dominance across all three agro-climatic zones, as characterized by consistent amplification of 460 bp bands. The uniformity of these bands in Buldhana and Nanded suggested minimal genetic diversity, potentially indicating a single or closely related strain of BBTV as the main causative agent. The strong band intensities observed reflect high viral loads, while the absence of CMV bands in many samples confirm that BBTV is the primary viral threat in these regions. In Khandesh Jalgaon, the detection of co-infection by CMV and BBTV complicates disease management efforts, as samples showed both 300 bp and 460 bp bands. Differences in band intensities across regions suggest variations in viral load or the stage of infection, with stronger bands indicating

1 higher concentrations of viral DNA. One important observation was the virus-free status of all
2 plants regenerated through tissue culture. The absence of BBTV and CMV in these tissue cul-
3 ture-derived plants underscore the effectiveness of tissue culture as a method for producing
4 virus-free planting material, highlighting its potential as a critical management strategy for
5 controlling viral spread in banana cultivation. This approach could be particularly valuable for
6 minimizing the impact of BBTV, especially in regions with high infection rates. The control
7 sample, which showed both the 460 bp and 300 bp bands, validated the reliability of the Mul-
8 tiplex PCR results by confirming the accuracy and specificity of the primers used in detecting
9 both viruses. Overall, the study highlights BBTV as the most prevalent viral pathogen in the
10 Buldhana and Nanded regions, with CMV infection being comparatively rare. However, the
11 detection of co-infection in Khandesh Jalgaon calls for more targeted interventions, given the
12 additional complexity introduced by the presence of both viruses.

DECLARATIONS

Ethical Approval and Consent to Participate

This study was conducted in accordance with the ethical standards of the MGM College of Agricultural Biotechnology. Ethical approval was obtained from the Ethics Committee of the Institution.

Consent of Publication

All authors consent to the publication of this article, and consent has been obtained from all participants and contributors involved in the study.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare no competing interests.

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Authors' Contributions

RT served as the lead author, conceptualizing the study, providing critical guidance throughout the writing process and approving the final manuscript. CB and SL were instrumental in developing the research idea and drafting the initial manuscript. NM, KB, and DP contributed to manuscript preparation and revisions. ST assisted with laboratory experiments related to the research. SDT is a horticulturist and select and provided genotypes required for study.

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1 **ABBREVIATIONS**

- 2 et.al. - Italia (and associate)
- 3 mg/L - Milligram per litre
- 4 pH - Log [H] ion concentration
- 5 Viz.- Namely
- 6 %- Percentage
- 7 DAI - Days After Incubation
- 8 oC - Temperature
- 9 MS - Murashiage and Skoog
- 10 Hrs- Hour
- 11 BAP- Benzyl amino purine
- 12 N- Normal
- 13 i.e.- That is
- 14 No- Number
- 15 Cm- Centimetre
- 16 Fig.- Figure

REFERENCES

- Anderson, T., Smith, L., & Jones, R. (2022). Methods for viral diagnostics in banana. *Journal of Plant Pathology*, 104(2), 152-162.
- Arumugam, C., Kalaimughilan, K., & Kathithachalam, A. (2017). Banana Bunchy Top Viral Coat Protein (CP) Gene Expression Studies at Molecular Level in Hill Banana cv. Sirumalai (AAB). *International Journal of Current Microbiology and Applied Sciences*, 6(6), 398–411. <https://doi.org/10.20546/ijcmas.2017.606.047>
- Baldodiya, G. M., Baruah, G., Borah, B. K., Modi, M. K., & Nath, P. D. (2019). Molecular characterization and sequence analyses of Banana bunchy top virus infecting banana cultivar Jahaji (Dwarf Cavendish) in Assam, India. *3 Biotech*, 9(3), 1–10. <https://doi.org/10.1007/s13205-019-1636-5>
- Chen, Y., Tanaka, H., & Nakata, T. (2021). Challenges in the detection of viral pathogens in Musa. *Applied Microbiology and Biotechnology*, 105(4), 1501-1508.
- D’hont, A., Denoeud, F., Aury, J. M., Baurens, F. C., Carreel, F., Garsmeur, O., Noel, B., Bocs, S., Droc, G., Rouard, M., Da Silva, C., Jabbari, K., Cardi, C., Poulain, J., Souquet, M., Labadie, K., Jourda, C., Lenggellé, J., Rodier-Goud, M., ... Wincker, P. (2012). The banana (*Musa acuminata*) genome and the evolution of monocotyledonous plants. *Nature*, 488(7410), 213–217. <https://doi.org/10.1038/nature11241>
- Das, T., & Banerjee, A. (2018). Distribution, molecular characterization and diversity of banana bunchy top virus in Tripura, India. *VirusDisease*, 29(2), 157–166. <https://doi.org/10.1007/s13337-018-0451-7>
- Debbarma, R., Kumar, K. K., Sudhakar, D., & Soorianathasundaram, K. (2019). Molecular characterization of banana bunchy top virus movement protein encoding DNA-M component isolated from hill banana and Grand Naine. 10(2), 936–943. <https://doi.org/10.5958/0975-928X.2019.00120.0>
- FAO, FAOSTAT.Review, M. (2023). Market Review Preliminary results 2023-Preliminary results 2023. Rome. Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/es/#data/QC>
- Geering, A. D. W., & Thomas, J. E. (1997). Search for alternative hosts of banana bunchy top virus in Australia. 26, 250–254.

1 Guide on handling and hardening of tissue cultured bananas. (n.d.).

2 Gupta, A. K., Kaushal, A., Gupta, R., & Singh, J. (2021). Tissue Culture Techniques. Tissue
3 Culture Techniques, 2(3). <https://doi.org/10.22271/ed.book.1381>

4 Hamed, N. A., & Abdelbaset, T. E. (2023). Molecular detection of banana bunchy top virus and
5 chemotherapy for production of virus-free banana plantlets. 739–750.
6 <https://doi.org/10.21608/ejar.2023.202674.1393>

7 Hamed Taherdoost. (2016). Sampling Methods in Research Methodology: How to Choose a
8 Sampling Technique for Research Hamed Taherdoost To cite this version : HAL Id : hal-
9 02546796 Sampling Methods in Research Methodology ; How to Choose a Sampling Tech-
10 nique for. International Journal of Academic Research in Management (IJARM), 5(2), 18–27.

11 Hernandez, P., & Garcia, R. (2023). Understanding viral heterogeneity: Implications for banana
12 breeding. Plant Science Reviews , 45(3), 289-300.

13 Hernandez, P., & Garcia, R. (2023). Understanding viral heterogeneity: Implications for banana
14 breeding. Plant Science Reviews , 45(3), 289-300.

15 Ilyas, M., & Briddon, Æ. R. W. (2008). Molecular characterisation of Banana bunchy top virus
16 (BBTV) from Pakistan. 191–198. <https://doi.org/10.1007/s11262-007-0168-y>

17 Jaisa-aad, M., Muñoz-Castro, C., Healey, M. A., Hyman, B. T., & Serrano-Pozo, A. (2024).
18 Characterization of monoamine oxidase-B (MAO-B) as a biomarker of reactive astrogliosis in
19 Alzheimer’s disease and related dementias. Acta Neuropathologica, 147(1).
20 <https://doi.org/10.1007/s00401-024-02712-2>

21 Kumar, S., Singh, R., & Vashisth, S. (2017). Multiplex IC-PCR: A tool to detect multiple vi-
22 ruses in banana. Journal of Plant Pathology , 99(3), 651-657.

23 Mathivanan, S., & Pandiyan, M. (2020). Micropropagation -Tissue Culture Banana. June 2022.

24 Nadella, R. K., Vaiyapuri, M., Kusunur, A. B., Joseph, T. C., Velayudhan, L. K., & Mothadaka,
25 M. P. (2019). Isolation and characterization of sulphur oxidizin bacteria (Halothiobacillus sp.)
26 from aquaculture farm soil. Journal of Environmental Biology, 40(3), 363–369.
27 <https://doi.org/10.22438/jeb/40/3/MRN-914>

28 Nguyen, B., Patel, A., & Wang, Q. (2020). Innovations in multiplex PCR for plant virus detec-
29 tion. Virology Journal , 17(1), 28.

- 1 Pareek, S. (2015). Nutritional and Biochemical Composition of Banana (*Musa* spp.) Cultivars.
2 In Nutritional Composition of Fruit Cultivars. Elsevier Inc. [https://doi.org/10.1016/B978-0-12-](https://doi.org/10.1016/B978-0-12-408117-8.00003-9)
3 [408117-8.00003-9](https://doi.org/10.1016/B978-0-12-408117-8.00003-9)
- 4 Patel, A., Thakkar, K., & Singh, R. (2022). Review on multiplex PCR methods for virus detec-
5 tion in crops. *Curent Trends in Genetics* , 9(1), 45-53.
- 6 Robin, J. D., Ludlow, A. T., La Ranger, R., Wright, W. E., & Shay, J. W. (2016). Comparison
7 of DNA quantification methods for next generation sequencing. *Scientific Reports*, 6, 1–10.
8 <https://doi.org/10.1038/srep24067>
- 9 Shan-e-Ali Zaidi, S., Vanderschuren, H., Qaim, M., Mahfouz, M. M., Kohli, A., Mansoor, S.,
10 & Tester, M. (2019). New plant breeding technologies for food security. *Science*, 363(6434),
11 1390–1391. <https://doi.org/10.1126/science.aav6316>
- 12 Smith, L., & Jones, R. (2020). The efficacy of PCR in detecting BBTV. *International Journal*
13 *of Virology* , 15(2), 234-240.
- 14 Thakur, A., Dutta, S., & Roy, J. (2020). Application of multiplex PCR in diagnosing viral dis-
15 eases in horticultural crops. *Plant Molecular Biology Reporter* , 38, 503-510.
- 16 Trujillo, W., Diaz, D., & Ochoa, Y. (2019). Advances in PCR technology for plant viruses.
17 *Frontiers in Microbiology* , 10, 871.
- 18 Xiang, D., Yang, X., Liu, B., Chu, Y., Liu, S., & Li, C. (2023). Bio-priming of banana tissue
19 culture plantlets with endophytic *Bacillus velezensis* EB1 to improve *Fusarium* wilt resistance.
20 *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1146331>
- 21 Yadoung, S., Shimizu, S., Hongsibsong, S., Nakano, K., & Ishimatsu, R. (2023). Dopamine as
22 a polymerizable reagent for enzyme-linked immunosorbent assay using horseradish peroxi-
23 dase. *Heliyon*, 9(11), e21722. <https://doi.org/10.1016/j.heliyon.2023.e21722>
- 24 Yamada, H., Nishikawa, Y., & Tanaka, T. (2021). Detection of multiple banana viruses using
25 multiplex PCR techniques. *Journal of Horticultural Science* , 76(4), 377-384.
- 26 Yi, S., Jin, W., Yuan, Y., & Fang, Y. (2018). An Optimized CTAB Method for Genomic DNA
27 Extraction from Freshly-picked Pinnae of Fern, *Adiantum capillus-veneris* L. *Bio-Protocol*,
28 8(13), 8–13. <https://doi.org/10.21769/bioprotoc.2906>
- 29 Zhao, L., Zhang, S., & Yang, H. (2021). Real-time PCR for detection of plant viruses: Pros and
30 cons. *Plant Pathology Journal* , 37(3), 287-295.



Figure 1: Cavendish Banana (*Musa acuminata*) AAA

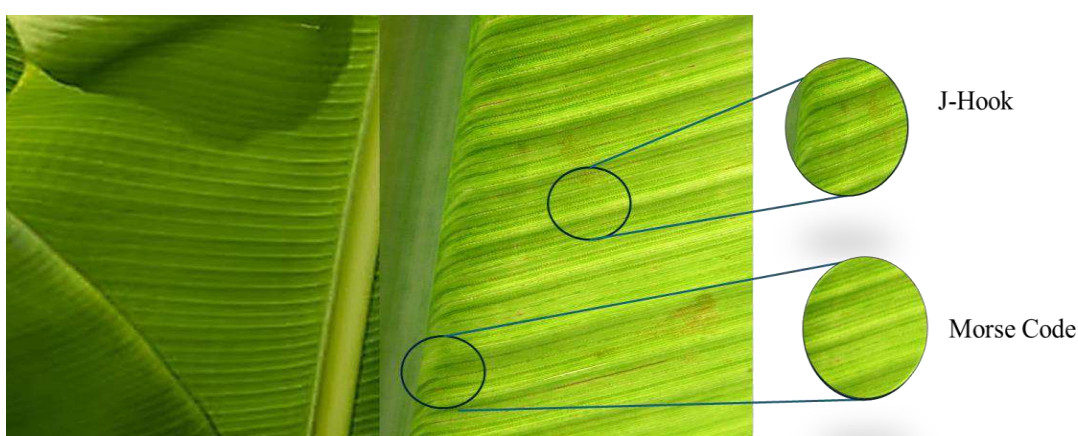


Figure 2: Banana plant exhibiting typical symptoms of viral infection, characterized by J-shaped leaf hooks and dark streaking patterns on the leaves, resembling Morse code. These visual symptoms are commonly associated with viral pathogens affecting banana crops

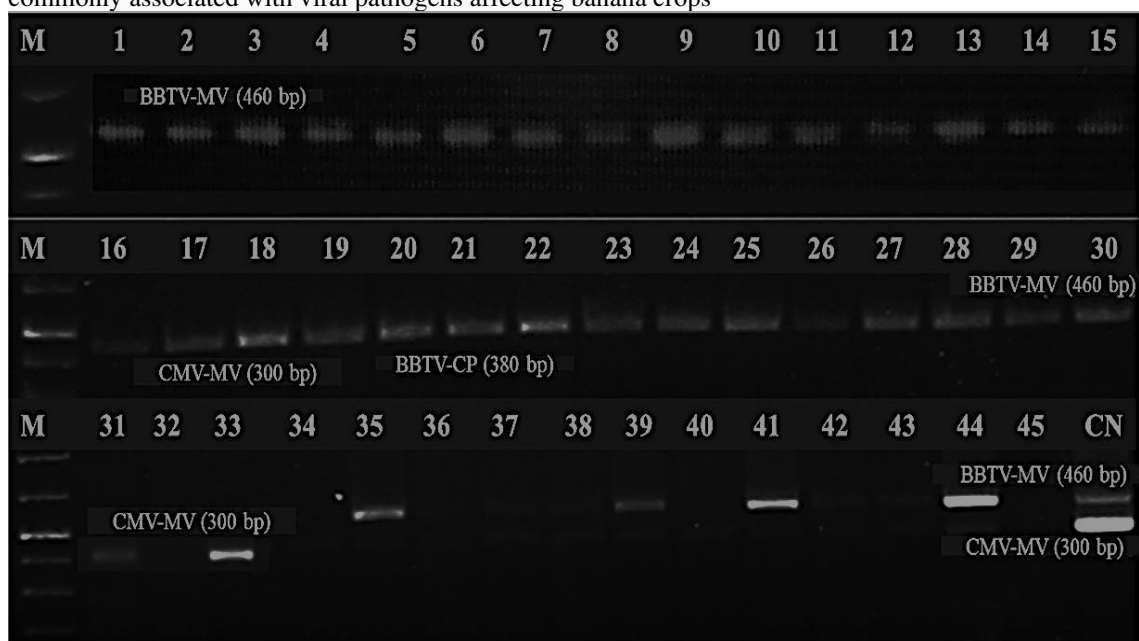


Figure 3: Validation of m-PCR for detecting BBTV and CMV in 45 leaf samples collected from field-grown plants across various agro-climatic zones. Lanes 1–15: Buldhana; Lanes 16–30: Nanded; Lanes 31–45: Jalgaon. Lane CN: Positive control (leaf sample with dual virus infection); Lane M: Molecular marker (1 kb ladder). Bands corresponding to the detected viral pathogens are indicated along with their size in bp. No bands were observed in tissue culture samples, indicating the absence of viral infection.

Table 1: Details of Samples used for Validation						
Sr No	Location	Type of Sample	No. of Positive Samples			
			No of Samples	BBTV	CMV	Dual Infection
1	Jalgaon, Maharashtra	Field grown Samples, Symptomatic/Non- Symptomatic (Random)	15	15	0	0
2	Nanded, Maharashtra	Field grown Samples, Symptomatic/Non- Symptomatic (Random)	15	14	0	0
3	Aurangabad, Maharashtra	Tissue Culture Raised Plants	15	0	0	0
4	Nanded, Maharashtra	Field grown Samples, Symptomatic/Non- Symptomatic (Random)	15	3	2	0
4	Control	Positive Control	1	1	1	1

Table 2: List of primer					
Sr. No	Target virus	Primer sequence (F/R)	Amplified gene	Product size (bp)	PCR cycling condition
1	BBTV	(F)TTTGAATGGTTTCTGTTCTTTGGAG	MV	460	94°C, 3 min 94°C, 2 min 55°C, 1 min 72°C, 2 min 72°C, 10 min
		(R)GACAGCCGGTTCTTGATCTCG			
		(F)ATGGCTAGGTATCCGAAG	CP	380	94°C, 4 min 94°C, 2 min 52°C, 1 min 72°C, 2 min 72°C, 10 min
		(R)CCAGAACTACAATAGAATGCC			
2	CMV	(F) TATGATAAGAAGCTTGTTCGCG	MV	300	94°C, 3 min 94°C, 2 min 53°C, 1 min 72°C, 2 min 72°C, 10 min
		(R) GCCGTAAGCTGGATGGACAA			