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RESEARCH ARTICLE

Loop-Mediated Isothermal Amplification (LAMP) assay for rapid detection of Banana Bunchy Top Virus (BBTV) infecting Abaca (*Musa textilis* Nee) in the major abaca plantations in Caraga Region

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ABSTRACT

This study evaluated the diagnostic efficacy of two methods for detecting Banana Bunchy Top Virus (BBTV) in abaca: conventional Polymerase Chain Reaction (PCR) and a novel colorimetric Loop-Mediated Isothermal Amplification (LAMP) assay. The study's purpose was to determine which method is more effective for field-based surveillance in the Philippines, a country with significant economic reliance on abaca production. A total of 243 leaf samples from key abaca-producing municipalities in the Caraga Region were collected and tested. The LAMP assay exhibited a significantly higher detection rate, identifying 85.2% of samples as positive compared to only 35.8% by conventional PCR ($\chi 2 = 121.92$, $df = 1$, $p < 0.001$). This diagnostic superiority was consistent across all four provinces surveyed, with the most pronounced difference in Surigao del Norte, where LAMP detected 86.04% of infections compared to a mere 2.33% by PCR. These findings reveal a substantial, previously hidden, reservoir of asymptomatic and low-titer BBTV infections that evade detection by traditional methods. The novelty of this study lies in its demonstration that LAMP is not only more sensitive but also uniquely suited for resource-limited settings due to its simple visual readout and lack of reliance on complex laboratory equipment. The results highlight that relying solely on symptom-based surveillance severely underestimates the true disease prevalence. The study concludes that adopting LAMP as a cornerstone of national surveillance efforts could provide a critical advantage in curbing the silent spread of BBTV, ensuring the sustainability of the abaca industry in the Philippines.

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1. INTRODUCTION

The global production of bananas and abaca (*Musa textilis*) is constantly threatened by the Banana Bunchy Top Virus (BBTV). This devastating, single-stranded DNA virus is transmitted by the aphid *Pentalonia nigronervosa* and has spread across tropical and subtropical regions, including Southeast Asia, the Pacific Islands, and parts of Africa, devastating both commercial and small-scale farming operations (Lao et al., 2000; Thomas and Briton, 1999). BBTV causes severe stunting, leaf distortion, and ultimately plant death, leading to significant yield losses in susceptible crops (Anand et al., 2007; Thomas et al., 1994). For the Philippines, a nation that serves as the world's leading producer of high-quality abaca fiber, the economic and social consequences of a widespread BBTV outbreak are particularly severe (DeVries and Paje, 2012; Soguilon et al., 2018).

While abaca and banana belong to the same genus, Musa, their socio-economic roles are distinct, which fundamentally changes how BBTV impacts each industry. Bananas are a high-volume food crop, primarily consumed domestically or traded internationally as a fruit. In contrast, abaca is a fiber crop, renowned for its strong, durable fibers used in specialty papers, marine cordage, banknotes, and handicrafts. Its cultivation is a cornerstone of the livelihood for many rural communities in the Philippines, often serving as a primary cash crop in traditional agroforestry systems (Lacuna-Richman, 2002). The perennial nature of abaca plants means a single infection can lead to a long-term economic loss for a farming household, making effective disease management paramount for ensuring both financial stability and national biosecurity.

Traditional disease management strategies have long relied on the visual inspection and manual eradication of symptomatic plants, a practice known as "roguing." However, this approach is often ineffective due to the latent nature of the disease. BBTV can reside in plants for extended periods without causing visible symptoms, turning them into asymptomatic reservoirs that facilitate silent disease spread (Ibrahim et al., 2018). This underscores the critical need for

early and accurate diagnostic tools that can detect the virus in both symptomatic and asymptomatic plants.

Over the past two decades, numerous diagnostic technologies have been developed to detect plant viruses, each with its own advantages and limitations. Serological methods, such as the Enzyme-Linked Immunosorbent Assay (ELISA), are widely used for large-scale screening due to their simplicity and ability to handle numerous samples simultaneously. However, they are limited by their reliance on the presence of viral proteins, which can be inconsistent in asymptomatic plants, and their lower sensitivity compared to molecular techniques. Conversely, molecular methods based on nucleic acid detection offer high specificity and sensitivity. The Polymerase Chain Reaction (PCR) and its quantitative variant (qPCR) have become the gold standard for BBTV detection due to their precision and ability to quantify viral load. However, their widespread adoption in resource-limited settings is hindered by the need for expensive equipment (thermal cyclers) and skilled laboratory personnel (Alabi and Roubtsova, 2012; Hobbs et al., 2010; Kumari et al., 2019). More recently, cutting-edge technologies like Next-Generation Sequencing (NGS) and CRISPR-based assays offer unparalleled comprehensiveness and speed but are confined to high-tech laboratories due to their complexity and cost.

In this context, Loop-Mediated Isothermal Amplification (LAMP) has emerged as a promising alternative for field diagnostics. Developed by Notomi et al. (2000), this molecular technique amplifies DNA at a single, constant temperature, eliminating the need for a thermal cycler and enabling rapid, on-site application. The colorimetric readout allows for a simple visual result, bypassing the need for gel electrophoresis and specialized detection equipment. While LAMP has been widely validated for banana virus detection, its systematic application in abaca, particularly within resource-limited areas like the Caraga Region, remains understudied. This region is a vital hub for national abaca production, yet it is disproportionately affected by the diagnostic gap created by conventional laboratory-based methods (Albayalde, 2018; DA-FIDA, 2017).

The objective of this study was to bridge this critical gap by evaluating the utility and performance of the LAMP assay for BBTV detection in abaca plants from the high-risk Caraga Region (Galan and Obedoza, 2019). Specifically, we aimed to directly compare its diagnostic efficacy with that of conventional PCR to demonstrate its potential as a robust, accessible tool for proactive disease management in an economically crucial agricultural sector. Our findings will provide a scientific basis for the broader adoption of this technology, empowering local communities and policymakers to implement more effective surveillance strategies.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 243 leaf samples were collected from major abaca plantations across four provinces in the Caraga Region: Agusan del Norte, Agusan del Sur, Surigao del Norte, and Surigao del Sur. The sampling locations were chosen from key municipalities known for their significant abaca production. To ensure a representative sample of the disease's prevalence, the collections included both plants showing visible symptoms of BBTV (symptomatic) and those that appeared healthy (asymptomatic). The number of samples collected from each province varied based on the size and number of plantations surveyed in that area.

2.2. DNA extraction for PCR

DNA was extracted from 100 mg of fresh abaca leaf tissue using a modified CTAB (cetyltrimethylammonium bromide) method (Doyle and Doyle, 1991). Modifications were made to the standard protocol to optimize for abaca's high polysaccharide content, including the addition of 2% PVP-3000 to the CTAB buffer and a three-step ethanol wash to improve DNA purity. Purified DNA samples were stored at -20°C until use.

2.3. Loop-Mediated Isothermal Amplification

The LAMP protocol for BBTV detection was adapted from Galvez et al. (2021). The assay used four specific primers (F3, B3, FIP, and BIP) meticulously designed to target six distinct regions within the BBTV DNA-S sequence (Genbank Accession No. KM607521.1). The precise binding positions and sequences of these primers, crucial for the highly specific and efficient amplification characteristic of LAMP, are visually represented in Figure 1 and listed in Table 1. The master mix composition for each 10 µl reaction is detailed in Table 2. Reactions were incubated at 65°C for 30 minutes, followed by a three-minute cool-down period before visualization.

Table 1: BBTV LAMP primer list.

Primer	Sequence (5' to 3')	Length (bp)
BBTV-F3	CCCTGCAAGCCATCTAGA	18
BBTV-B3	TGCGACTCCTGCTTCAAG	18
BBTV-FIP	CTTGTTGCCGCCTTGCTTGCAAATGGCGAGGTTTCCG	37
BBTV-BIP	GTTGGTGTGCAAGGTGAAGCCTGGTGCTTCCAGACATG	38

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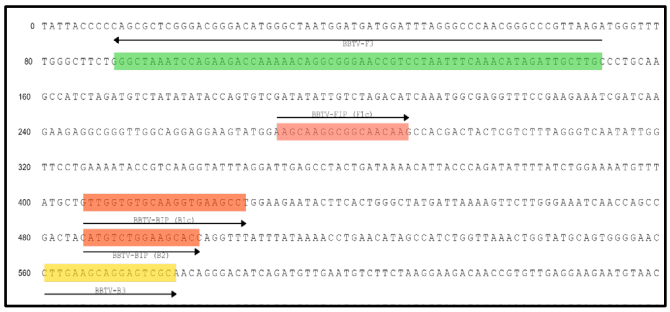

Figure 1: BBTV LAMP primers within the DNA-S sequence of BBTV (Genbank Accession No. KM607521.1). The figure indicates the binding sites and orientation of the F3, B3, FIP, and BIP primers used in the assay.

To ensure consistency and to minimize pipetting errors across all reactions, a master mix solution was prepared in a 1.5 mL microcentrifuge tube. The master mix contained specific volumes of each component, which were calculated to achieve the desired final concentrations for the assay. The composition of the master mix per reaction is outlined in Table 2.

Table 2: BBTV LAMP master mix.

Components	Stock Concentration	Final Concentration	1x (µl)
Nuclease-free Water	-	-	4
BST Buffer	10.00 X	1.000 X	1
MgSO4	100.00 mM	6.000 mM	0.6
dNTP (each A,T,C,G)	10.00 mM	1.400 mM	1.4
F3	5.00 µM	0.200 µM	0.4
B3	5.00 µM	0.200 µM	0.4
FIP	40.00 µM	1.600 µM	0.4
BIP	40.00 µM	1.600 µM	0.4
BST pol	8.00 U/µl	0.320 U/µl	0.4
Template	-	50ng/µl	1
TOTAL	-	-	10

2.4. Polymerase Chain Reaction

Conventional PCR assay was performed for comparison using the BBTV-specific primers BBTV-Rep-F (5'-GAGTTGATGCCGCAAGAG-3') and BBTV-Rep-R (5'-GAATCAGCACGTAGGCCATG-3') (Pande et al., 2012). The reaction cocktail, with a total volume of 10 µL, was prepared according to the composition outlined in Table 3.

Table 3: BBTV PCR cocktail mix.

PCR Component	Stock Concentration	Final Concentration	Volume per Reaction (µL)
Green Mastermix	2x	1x	5
BBTV-Rep (Forward)	20 µL	0.5 µM	0.25
BBTV-Rep (Reverse)	20 µL	0.5 µM	0.25
Sample total DNA	50 ng/µL	5 ng/µL	1
dH2O	—	—	3.5
Total			10

**Sterile distilled water was added to adjust the final volume to 10 µL.

2.5. Visualization of results

Results from the LAMP assay were visually detected by adding 1 µL of SYBR Green I dye. A positive result was indicated by a color change from orange to green, while a negative result remained orange. Conventional PCR products were analyzed by gel electrophoresis. A 1% agarose gel was run at 80 V for 30 minutes, and the DNA bands were visualized using a Gel Doc EZ imaging system. The presence of a specific band at the expected size confirmed a positive result.

3. RESULT AND DISCUSSION

The diagnostic efficacy of the LAMP and conventional PCR assays was assessed on 243 abaca leaf samples from the Caraga region. The overall detection rates revealed a significant difference between the two methods.

3.1. Overall assay performance

To investigate the relationship between visible symptom expression and molecular detection of BBTV, leaf samples were classified as either symptomatic or asymptomatic prior to diagnostic testing. Symptomatic abaca plants displayed typical features of Bunchy Top Disease (BTD), including severe stunting, chlorotic streaks along the leaf veins, upright and narrow leaf orientation, and the characteristic “bunchy top” arrangement (Figure 2). In contrast, asymptomatic plants appeared morphologically normal, with no visible signs of infection, but were sampled to account for the possibility of latent or low-titer infections.

LAMP assay results revealed that a substantial proportion of asymptomatic samples tested positive for BBTV, indicating the presence of infections not detectable through symptom-based assessment alone. This finding underscores the limitation of relying solely on visual field diagnosis and highlights the higher sensitivity of LAMP for early or asymptomatic infections. The difference between visible symptoms and molecular confirmation demonstrates that asymptomatic plants may serve as hidden reservoirs for BBTV, facilitating undetected viral spread within plantations.

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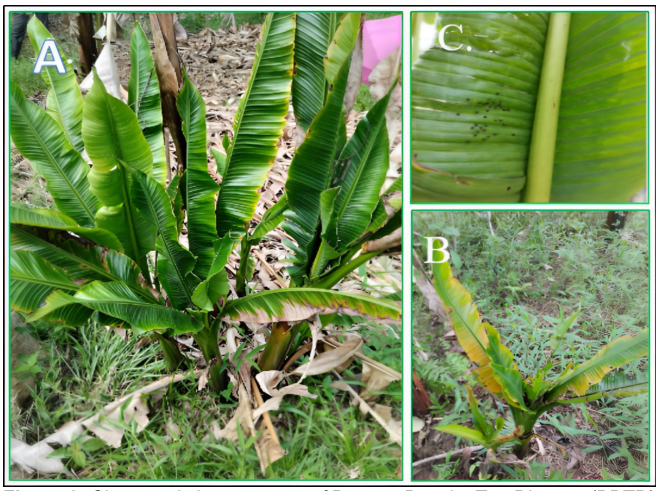

Figure 2: Characteristic symptoms of Banana Bunchy Top Disease (BBTD) observed in abaca plants from Surigao del Sur. Visible features include stunted growth, chlorotic streaks along the leaf veins, upright and narrow leaves, and the typical "bunchy top" arrangement at the crown. These symptoms were used as the basis for classifying plants as symptomatic prior to molecular validation by LAMP.

Table 4: Disease incidence of Banana Bunchy Top Virus (BBTV) in abaca plants across the Caraga Region, categorized by symptomatic and asymptomatic samples. Incidence was validated using the LAMP assay. Values represent the number of plants testing positive out of the total samples examined.

Province	Sample Tested	Symptomatic			Asymptomatic			LAMP Result (%)
		V	(+)	(-)	V	(+)	(-)	
Agusan del Sur	60	45	45		15	15		100
Agusan del Norte	80	56	39	17	24	12	12	62.50
Surigao del Sur	60	42	42		18	18		100
Surigao del Norte	43	28	22	6	15	15		86.04

*V- Visual assessment

As shown in Table 4, the number of symptomatic and asymptomatic samples varied across the surveyed municipalities, but LAMP consistently detected BBTV in both categories. In Agusan del Sur and Surigao del Sur, all symptomatic and asymptomatic samples tested positive, suggesting widespread infection regardless of visible symptoms. In contrast, results from Agusan del Norte and Surigao del Norte revealed more variability among asymptomatic plants, with only a subset testing positive. These findings confirm that visual inspection alone is insufficient for reliable diagnosis and demonstrate the utility of LAMP in identifying hidden infections that do not manifest through symptom expression.

3.1. Results visualization

Representative assay outputs are shown in Figures 3 and 4. Conventional PCR required post-amplification analysis by gel electrophoresis (Figure 3), where band visualization confirmed the presence of BBTV. In contrast, the LAMP assay produced a direct colorimetric change in the reaction tubes (Figure 4), providing rapid and straightforward interpretation without the need for specialized equipment. The clear contrast between these outputs illustrates not only the operational simplicity of LAMP but also its suitability for field diagnostics. While PCR remains dependent on laboratory infrastructure, LAMP enables immediate detection in resource-limited settings, making it an effective tool for regional surveillance.

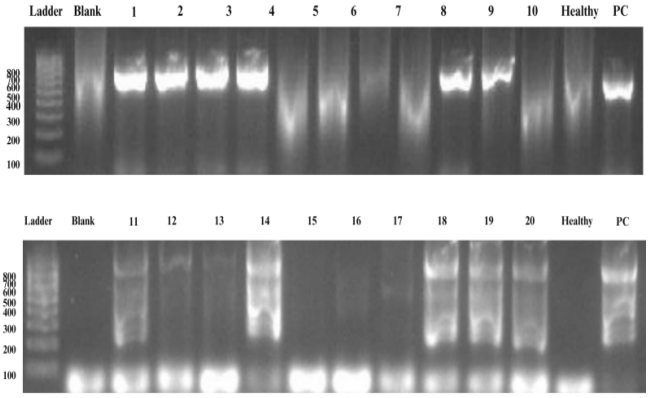

Figure 3: PCR detection of Banana Bunchy Top Virus (BBTV) in abaca leaf samples from Marihatag, Surigao del Sur. Amplicons were visualized on GelGreen-stained agarose gel. (A) Lanes 1–14: Lane 1, DNA ladder (876 bp, Vivantis); Lanes 2–11, abaca leaf samples; Lane 12, negative control; Lane 13, positive control. (B) Lanes 1–14: Lane 1, DNA ladder (876 bp, Vivantis); Lanes 2–11, abaca leaf samples; Lane 12, negative control; Lane 13, positive control. Together, gels A and B represent PCR results for 20 field-collected samples.

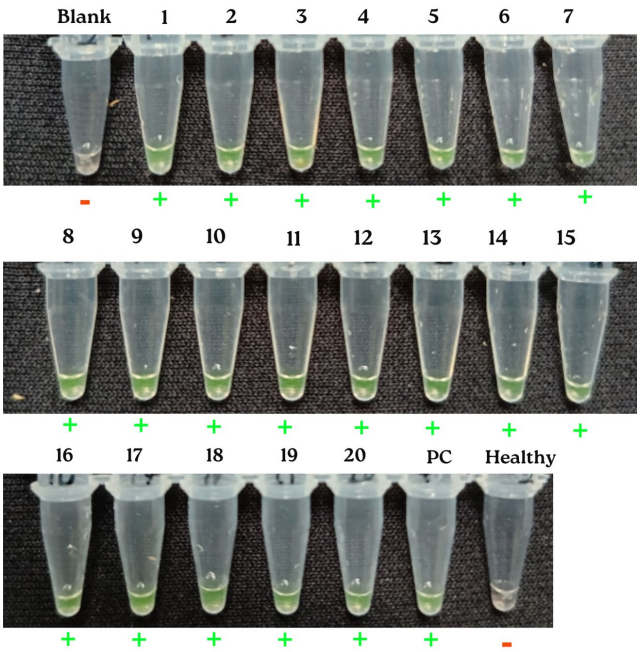

Figure 4: Representative Loop-Mediated Isothermal Amplification (LAMP) assay results for Banana Bunchy Top Virus (BBTV) detection in abaca leaf samples from Marihatag, Surigao del Sur. The same 20 samples shown in Figure 1 were tested. A positive reaction is indicated by a color change from orange to bright green. Sample numbers are shown above each tube, with (−) and (+) symbols below to denote negative and positive reactions.

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3.2. Overall assay performance

The LAMP assay consistently outperformed conventional PCR in detecting BBTV across the Caraga Region. Of the 243 abaca leaf samples tested, LAMP identified 85.2% as positive, whereas PCR detected only 35.8%. The distribution of detection rates is first summarized in Table 5, with corresponding graphical comparisons presented in Figure 5.

At the provincial level, LAMP maintained a markedly higher sensitivity than PCR. In Agusan del Sur and Surigao del Sur, all tested plants were identified as positive by LAMP, compared with only 61.67% and 43.33% by PCR, respectively. The disparity was most pronounced in Surigao del Norte, where LAMP detected 86.04% infection, while PCR identified only 2.33%. These results highlight LAMP's ability to uncover infections that PCR failed to detect, underscoring its diagnostic advantage in regional surveillance.

Table 5: Disease Incidence of BBTV in Caraga Region as validated by LAMP and PCR.

Province	No. Sample	LAMP Results (%)	PCR Results (%)
Agusan del Sur	60	100	61.67
Agusan del Norte	80	62.5	28.75
Surigao del Sur	60	100	43.33
Surigao del Norte	43	86.04	2.33
Caraga Region	243	85.2	35.8

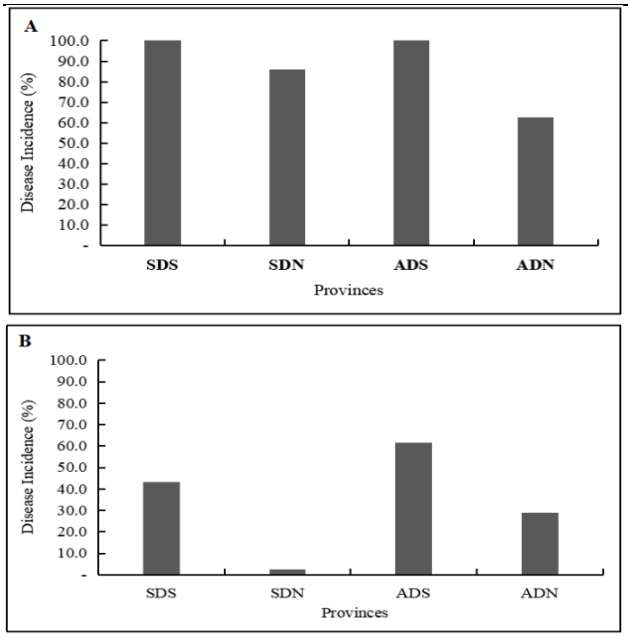

Figure 5: Comparative disease incidence of Banana Bunchy Top Virus (BBTV) in four provinces of the Caraga Region as detected by A.) Loop-Mediated Isothermal Amplification (LAMP) and B.) conventional PCR.

3.3. Statistical comparison of assay performance

To assess the statistical significance of differences in detection rates between assays, Pearson's Chi-square test was applied to the regional dataset. Results demonstrated that LAMP detected significantly more BBTV-positive samples than conventional PCR ($\chi^2 = 121.92$, df = 1, $p < 0.001$). At the provincial level, Chi-square tests likewise revealed significant differences in Agusan del Norte, Agusan del Sur, and Surigao del Sur ($p < 0.05$). In Surigao del Norte, where the low number of PCR-positive samples resulted in expected cell counts below Chi-square assumptions, Fisher's exact test confirmed the significant discrepancy ($p < 0.001$). The disparity in this province was especially pronounced, with LAMP detecting 86.04% of samples compared to only 2.33% by PCR shown in Table 6. Collectively, these findings demonstrate the statistically robust and consistent superiority of LAMP over conventional PCR for BBTV detection across all provinces surveyed.

Table 6: Statistical comparison between LAMP and PCR detection of BBTV.

Region	Test Used	χ^2 Statistic	df	p-value
Agusan del Sur	Fisher's exact	–	–	1.74×10^{-8}
Agusan del Norte	Chi-square test	17.03	1	3.68×10^{-5}
Surigao del Sur	Fisher's exact	–	–	1.49×10^{-13}
Surigao del Norte	Fisher's exact	–	–	1.41×10^{-16}
Caraga Region	Chi-square test	121.92	1	2.40×10^{-28}

The study demonstrated that the Loop-Mediated Isothermal Amplification (LAMP) assay reveals a substantially higher prevalence of BBTV infection in the Caraga Region compared to conventional PCR. This aligns with findings in other plant virus systems where LAMP's enhanced sensitivity often surpasses conventional PCR by orders of magnitude (LiJun et al., 2022). The stark discrepancy observed, particularly in Surigao del Norte, suggests that a significant number of plants harbor low viral titers or remain asymptomatic. These asymptomatic carriers are epidemiologically significant, as aphid vectors can transmit BBTV from them, thereby sustaining undetected reservoirs of inoculum and driving disease spread (Hooks and Valenzuela, 2008).

The underperformance of conventional PCR in our study can be attributed to several factors inherent to the molecular biology of both the virus and its host. Firstly, the reliance of conventional PCR on a thermal cycler with a precise temperature profile makes it vulnerable to fluctuations and inhibits its use in remote field locations. More critically, the technique's sensitivity threshold is often insufficient to detect BBTV in the early, asymptomatic stages of infection where the viral load is low (Hobbs et al., 2010). This limitation is compounded by the complex biochemistry of abaca tissue. Plants in the Musa genus are rich in polysaccharides and polyphenolic compounds, which are known inhibitors of DNA

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polymerase (Alabi and Roubtsova, 2012). These compounds, co-extracted with the viral DNA, can interfere with the enzyme's activity, leading to false negatives or reduced amplification efficiency, even when the viral template is present. Furthermore, as a DNA virus, BBTV exhibits a degree of genetic variability that can lead to primer-template mismatches, potentially reducing the efficiency of conventional PCR primers that are designed for highly conserved regions (Kumari et al., 2019). The cumulative effect of these challenges explains the significant under-detection of BBTV by conventional PCR and underscores its limitations as a primary surveillance tool in a dynamic field setting.

In contrast, the superior performance of LAMP is a direct result of its unique technical advantages. The assay operates isothermally, at a single constant temperature, which eliminates the need for a thermal cycler and makes it highly adaptable for on-site diagnostics. This method utilizes a set of four to six primers that recognize six to eight distinct regions on the target BBTV DNA, resulting in a high degree of specificity and an extremely rapid amplification process (Notomi et al., 2000). The amplification is highly efficient due to a continuous process of strand displacement and self-priming, generating a vast amount of target DNA in a short period (around 30 minutes). The true innovation for field deployment lies in the visual readout. In our study, the colorimetric dye changed from its initial color to a different color in positive samples, providing a clear, unambiguous result that can be interpreted with the naked eye, removing the need for post-amplification analysis like gel electrophoresis.

While the strengths of LAMP are considerable, its practical application is not without limitations. A key concern is the potential for non-specific amplification, which can lead to false positives if primers are not meticulously designed or if the reaction conditions are not optimized. This risk is particularly relevant in field settings where sample preparation may be less controlled. Additionally, the colorimetric interpretation, while convenient, can be subjective for individuals who are not well-trained, especially in cases where the color change is subtle. The stability of the reagents in the high-temperature and high-humidity conditions of tropical regions like Caraga presents another challenge. While lyophilized reagents can mitigate this issue, cold chain storage remains a consideration for long-term reagent viability. Lastly, a fundamental limitation of the qualitative LAMP assay is its inability to provide information on viral load. While it can confirm the presence of BBTV, it cannot quantify the amount of virus in the sample, which limits its epidemiological resolution compared to quantitative PCR (qPCR) which is a crucial tool for understanding disease progression and transmission dynamics.

The success of LAMP in our study mirrors its documented effectiveness in controlling other devastating plant viruses on an international scale. For example, LAMP assays have been successfully developed for detecting cassava mosaic virus (Oluwafemi et al., 2016) and sweet potato virus (Gowda et al., 2009), both of which are major threats to food security in tropical regions. Furthermore, its application in banana farming has been well-established, demonstrating its utility in a related crop. Our findings extend this proven track record to abaca, a distinct and equally important fibrous crop, highlighting its versatility. While similar molecular detection tools like CRISPR-based assays are gaining traction, they are often more complex and remain largely confined to advanced research settings (Zhou et al., 2021). The unique gap that our study addresses is the systematic application of a simple, robust, and validated molecular tool directly in the context of the socio-economically vital abaca crop in a resource-limited, high-risk region like Caraga.

The implications of our findings for BBTV epidemiology are profound. By demonstrating the presence of a significant reservoir of asymptomatic infections, we confirm that symptom-based surveillance alone is insufficient for effective disease management. The integration of a field-friendly diagnostic tool like LAMP into routine surveillance enables the detection of these hidden infections, which are the most critical drivers of disease spread. This allows for a proactive rather than reactive approach to roguing and vector control. Furthermore, by mapping BBTV prevalence with greater accuracy, agricultural extension workers and policymakers can better allocate resources, targeting localized outbreaks before they become widespread epidemics. The findings also underscore the need for an integrated disease management strategy that combines LAMP-based detection with continuous monitoring of the aphid vector population. A holistic approach that links the presence of the virus in both symptomatic and asymptomatic plants with the population dynamics of its vector is essential for breaking the disease cycle and achieving sustainable control. Our findings establish LAMP not only as a diagnostic tool but as a critical component of modern BBTV management. By providing a realistic and accessible foundation for designing effective disease control strategies, it is critical for safeguarding the abaca industry's long-term viability and strengthening national biosecurity against one of the most destructive viral threats to both banana and abaca production.

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4. CONCLUSION

This study established the marked superiority of the Loop-Mediated Isothermal Amplification (LAMP) assay over conventional PCR for detecting Banana Bunchy Top Virus (BBTV) in abaca within the Caraga Region. LAMP consistently identified a higher proportion of infected samples, including low-titer and asymptomatic infections that were largely missed by PCR. The pronounced discrepancy, particularly in Surigao del Norte, underscores how traditional diagnostics may grossly underestimate true infection rates, allowing silent viral reservoirs to persist and spread the disease. By combining high sensitivity with operational simplicity and rapid turnaround, the LAMP assay provides a robust, field-friendly diagnostic platform for large-scale surveillance and early detection, offering a critical advantage in curbing BBTV's silent spread.

Based on these findings, we recommend the immediate integration of the colorimetric LAMP assay into the Philippines' national BBTV surveillance program. For effective implementation, comprehensive training should be provided to agricultural extension workers and farmer cooperatives on proper sample collection and on-site testing protocols. We further propose the establishment of mobile diagnostic units equipped with these assays to conduct regular, targeted surveys in abaca plantations. This proactive, diagnostic-informed approach would enable the swift identification and targeted roguing of asymptomatic carriers, which is crucial for breaking the disease cycle and mitigating economic impact.

Future research should focus on refining the LAMP assay for broader epidemiological applications. A priority is the development of a quantitative LAMP method to measure viral load, which could better inform the severity of infection. Further studies are warranted on the transmission dynamics of BBTV from asymptomatic plants to healthy plants via the aphid vector, *Pentalonia nigronervosa*. Finally, a detailed cost-benefit analysis of widespread LAMP implementation versus current disease management practices would provide the necessary data for policymakers to justify and secure funding for this vital biosecurity initiative, ensuring the long-term sustainability of the abaca industry.

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