Survey and Detection of Banana Bunchy Top Virus (BBTV) in Abaca (Musa textilis Nee) in Major Abaca Farms in Caraga Region, Philippines

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Survey and Detection of Banana Bunchy Top Virus (BBTV) in Abaca (Musa textilis Nee) in Major Abaca Farms in Caraga Region, Philippines

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Abstract: *Banana Bunchy Top Virus (BBTV) is a major constraint in abaca (Musa textilis) production, causing substantial yield losses. This study aimed to detect and assess BBTV incidence in selected abaca farms across Surigao del Sur and Surigao del Norte using Polymerase Chain Reaction (PCR) with BBTV-Rep primers. A total of 103 leaf samples—both symptomatic and asymptomatic—were collected from five municipalities. Results revealed a significantly higher infection rate in Surigao del Sur (65%) compared to Surigao del Norte (2.3%), with Tago showing the highest incidence (75%) and Gigaquit showing no infection. Notably, BBTV was also detected in asymptomatic plants, highlighting the virus's silent spread and the need for proactive surveillance. The study underscores the value of PCR-based diagnostics for early BBTV detection, which is essential for effective disease management and the long-term sustainability of abaca cultivation in the Caraga Region.*

Keywords: Banana bunchy top virus; Abaca; PCR; Disease incidence; Asymptomatic infection

1. INTRODUCTION

Abaca (Musa textilis), commonly recognized as Manila hemp, stands as an exceptionally vital economic crop in the Philippines, profoundly impacting the national economy and the livelihoods of its rural population. This resilient fiber sustains an estimated 1.5 million people through direct and indirect employment across its value chain and contributes significantly to the country's export earnings, yielding over P2 billion in annual revenue [1, 2]. Its unique properties, including superior strength, durability, and salt-water resistance, make abaca fiber indispensable for a diverse array of global industries, ranging from specialized paper products (such as banknotes and tea bags) and marine cordage to high-performance textiles and advanced composite materials for automotive and aerospace sectors [3]. The Philippines consistently holds the prestigious position as the world's leading producer of abaca, commanding a significant share of the global market [4, 5]. Within this national context, regions such as Surigao, with their favorable climatic conditions and rich agricultural heritage, play a crucial role in extensive abaca cultivation, fulfilling both national and international demands for this prized natural fiber [6]. Despite its immense economic significance, abaca cultivation in the Philippines faces persistent and escalating threats from various plant pathogens, with viral infections being among the most destructive. Foremost among these is the Banana Bunchy Top Virus (BBTV), a highly virulent and globally distributed Babuvirus that has devastated banana and abaca plantations worldwide. BBTV is primarily transmitted by the aphid Pentalonia nigronervosa, commonly known as the banana aphid [7, 8]. This aphid vector facilitates the rapid and efficient spread of the virus within and between plantations, making containment exceptionally challenging. BBTV is widely regarded as the most destructive viral disease affecting abaca, frequently leading to severe yield reductions, substantial fiber quality degradation, and, in severe cases, complete plantation abandonment due to its capacity to induce severe stunting, chlorosis, and the characteristic "bunchy top" phenotype. These symptoms significantly impair photosynthetic efficiency and hinder plant growth, leading to irreversible damage. Further complicating accurate field diagnosis and effective disease management are instances of mixed viral infections [9, 10]. Abaca plants can be simultaneously infected by BBTV alongside other economically important viruses such as the Banana Bract Mosaic Virus (BBrMV). Such co-infections can lead to atypical or exacerbated symptoms, making visual identification unreliable and prone to misdiagnosis. This diagnostic ambiguity often results in delayed intervention, allowing the virus to spread undetected and unchecked, thus exacerbating economic losses for farmers [11]. Given the destructive nature of BBTV and the limitations of symptom-based identification, molecular diagnostic tools are indispensable for precise and timely disease intervention. Techniques such as Polymerase Chain Reaction (PCR) provide a highly sensitive and specific method for detecting viral genetic material, enabling early and accurate identification of infected plants, including those that may be asymptomatic carriers [12]. Early detection is critical for implementing effective control measures, such as immediate rogueing (removal of infected plants) and vector management, thereby breaking the disease cycle and preventing further dissemination. This study was designed to address the urgent need for robust diagnostic capabilities in abaca-growing regions of the Caraga Administrative Region. Specifically, it aimed to apply PCR-based molecular detection to accurately identify the presence and assess the incidence of Banana Bunchy Top Virus (BBTV) in abaca plantations within selected municipalities of Surigao del Sur (Tago, Marihatag, and San Agustin) and Surigao del Norte (Gigaquit and Mainit). By combining systematic field surveys with precise molecular confirmation, this research seeks to provide foundational data for developing more effective surveillance programs and targeted management strategies to safeguard the abaca industry in the Philippines.

2. Materials and Methods

2.1 Time and Place of Study

This comprehensive study on the incidence of abaca bunchy top disease was meticulously conducted over an extended period, from August 2023 to May 2024. All laboratory analyses, including DNA extraction, quantification, and Polymerase Chain Reaction (PCR) amplification, were performed at the Molecular Biology and Agricultural Microbiology (MolBam) Laboratory, situated within the College of Agriculture and Agri-Industries at Caraga State University in Butuan City, Philippines. The selection of this laboratory provided access to state-of-the-art equipment and specialized expertise essential for molecular diagnostics. The geographical focus of the study encompassed key abaca-producing areas within the Caraga Region, specifically targeting three municipalities in Surigao del Sur (Tago, Marihatag, and San Agustin) and two municipalities in Surigao del Norte (Gigaquit and Mainit). These sites were chosen based on their significance to abaca production and anecdotal reports of past viral disease incidence, ensuring that the study addressed areas of high relevance for disease management.

2.2 Field Survey and Sample Collection

Field surveys and subsequent sample collection were systematically executed across selected abaca farms within the identified municipalities of Surigao del Sur and Surigao del Norte. A total of 103 abaca leaf samples were collected. The sampling strategy primarily focused on plants exhibiting characteristic symptoms suggestive of bunchy top disease, such as severe stunted growth, noticeably distorted and brittle leaves, a distinctive bunchy appearance (apical clustering of leaves), and marked leaf narrowing. However, a limited number of apparently healthy or asymptomatic plants were also sampled to assess the presence of latent infections. The precise geographical coordinates of each sampling location were meticulously recorded using a handheld Global Positioning System (GPS) device. This georeferencing was crucial for potential future spatial analysis and mapping of disease incidence. Detailed documentation of observed symptoms, including their severity and specific manifestations, was carried out for each sampled plant. For molecular analysis, three representative leaf samples were carefully collected from each plant, typically comprising one young, symptomatic or fully expanded leaf, one older symptomatic leaf (if present), and one healthy-looking leaf (if the plant was asymptomatic). The collected leaf samples were promptly cut into small pieces (approximately 1-2 cm^2) using sterile razor blades to facilitate subsequent DNA extraction. These pieces were immediately placed into pre-labeled 2 ml sterile microcentrifuge tubes. To maintain sample integrity and prevent nucleic acid degradation, the tubes were placed in a portable cool box containing ice packs immediately after collection. Within 72 hours of collection, the samples were transported to the MOLBAM Laboratories at Caraga State University and immediately preserved at $-80\,^\circ C$ until further processing. This cold chain management was critical to ensure the quality and stability of nucleic acids for downstream molecular detection, DNA extraction, and PCR amplification.

2.3 DNA Extraction, Quantification, and PCR Amplification

DNA extraction was performed at the Molecular Biology and Agricultural Microbiology Laboratory of Caraga State University, employing a modified Cetyl Trimethylammonium Bromide (CTAB) method, originally described by Doyle and Doyle [13], known for its effectiveness in extracting high-quality plant DNA, even from samples rich in polysaccharides and phenolic compounds. Approximately 100-200 mg of fresh or frozen abaca leaf tissue was ground into a fine powder using a sterile, pre-chilled mortar and pestle with liquid nitrogen to prevent DNA degradation and facilitate cell lysis. The finely ground tissue was then transferred to a 2 ml microcentrifuge tube. A preheated (65 $^\circ$C) CTAB extraction buffer, comprising 2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 0.2% β-mercaptoethanol (added just prior to use for antioxidant properties), and 2% Polyvinylpyrrolidone (PVP-3000) (to bind phenolic compounds), was added to the homogenized samples. The mixture was then thoroughly vortexed and incubated at 65 $^\circ$C for 45 minutes, with gentle inversion every 10-15 minutes to ensure proper cell lysis and DNA release. Following incubation, the mixture was centrifuged at 12,000 rpm for 5 minutes at room temperature to pellet cellular debris. The aqueous supernatant, containing the liberated DNA, was carefully transferred to a new sterile tube. An equal volume of chloroform:isoamyl alcohol (24:1 v/v) was added to the supernatant, gently mixed by inversion for 5 minutes, and then centrifuged again at 12,000 rpm for 5 minutes. This step effectively separates proteins, lipids, and other contaminants from the nucleic acids. The resulting upper aqueous phase was carefully transferred to a fresh tube, and DNA was precipitated by adding 0.7 volumes of ice-cold isopropanol. The mixture was gently inverted several times and stored at $-80\,^\circ$C for 1 hour to maximize DNA precipitation. The precipitated DNA pellet was subsequently obtained by centrifugation at 12,000 rpm for 10 minutes at 4 $^\circ$C. The pellet was then washed twice with 500 µl of cold 75% ethanol to remove residual salts and impurities. After the final wash, the ethanol was carefully decanted, and the DNA pellet was air-dried at room temperature for 10-15 minutes until no visible ethanol remained. Finally, the purified DNA pellet was resuspended in 50-100 µl of nuclease-free water to prevent degradation and stored at $-20\,^\circ$C for long-term preservation until further use. Quantification of the extracted DNA was performed using a K5500 plus spectrophotometer. The absorbance of each DNA sample was measured at 260 nm (A260) to determine nucleic acid concentration (ng/µl) and at 280 nm (A280) and 230 nm (A230) to assess DNA purity. A critical quality control parameter was the A260 /A280 ratio, where a value between 1.8 and 2.0 typically indicated pure DNA, largely free from significant protein contamination. Similarly, the A 260/A230 ratio, ideally between 2.0 and 2.2, was monitored to confirm the absence of contaminating organic compounds (e.g., carbohydrates, phenols, guanidine salts) that could interfere with downstream PCR reactions. Samples not meeting these purity criteria were re-extracted or further purified. PCR

Amplification: PCR amplification was carried out using a BIO-RAD T100 Thermal Cycler. The cycling conditions were meticulously optimized following protocols established by Galvez et al. [14] and Piamonte [12], which have been successfully applied for BBTV detection in Musa species. Each 25 µl PCR reaction mixture typically contained 1x PCR buffer, 1.5 mM MgCl$_2$, 0.2 mM of each dNTP, 0.4 µM of each forward and reverse primer, 1 unit of Taq DNA polymerase, and 50-100 ng of template DNA. Specific primers, BBTV-rep F (5'-GCATCCCGACCATTTGAA-3') and BBTV-rep R (5'-AATGTTAAGGAACCATCTAA-3'), were utilized. These primers are designed to amplify an 876 bp fragment of the BBTV replicase (rep) gene, a conserved region ideal for BBTV detection. The thermal cycling program was as follows: an initial denaturation step at 95 ◦C for 5 minutes, followed by 35 cycles of denaturation at 95 ◦C for 30 seconds, primer annealing at 61 ◦C for 30 seconds, and DNA elongation at 72◦ C for 1 minute. A final extension step at 72 ◦C for 7 minutes was performed to ensure complete synthesis of amplified products. Positive control samples, consisting of DNA extracted from abaca plants previously confirmed to be infected with BBTV by PhilFIDA, were included in each PCR run to validate reaction efficacy. Conversely, DNA extracted from healthy, disease-free abaca plants, confirmed negative by prior molecular testing, served as negative controls to check for contamination or non-specific amplification.

2.4 Gel Electrophoresis

Following PCR amplification, the resulting DNA products were meticulously visualized and analyzed through agarose gel electrophoresis. This technique separates nucleic acid fragments based on their size and charge. Since DNA is negatively charged, it migrates towards the positive electrode through the porous matrix of the agarose gel. Smaller DNA fragments navigate the gel matrix more easily and thus migrate faster and further than larger fragments. To prepare the agarose gel, 0.65 g of molecular biology grade agarose was accurately weighed and mixed with 50 ml of 1x TAE (Tris-Acetate-EDTA) buffer in an Erlenmeyer flask. The mixture was then microwaved for approximately 1-2 minutes until the agarose was completely dissolved and the solution became clear, ensuring thorough mixing. Factors such as gel pore size (determined by agarose concentration), the size of the DNA fragments, and the applied voltage significantly influence nucleic acid migration and fragment separation, as noted by Lee et al. (2012). For optimal separation of the 876 bp target fragment, a 1.3% agarose gel was prepared. The hot agarose solution was allowed to cool slightly before adding 2 µl of SYBR Safe DNA Gel Stain (or equivalent), a fluorescent dye that intercalates with DNA. The prepared gel mixture was then poured into a casting tray equipped with a comb inserted to create wells for sample loading. Once the gel solidified (approximately 30-45 minutes at room temperature), the comb was carefully removed.

Prior to loading, 5 µl of each PCR product was mixed with 1 µl of 6x DNA loading dye, which contains tracking dyes to monitor the progress of electrophoresis and glycerol to help the DNA sample sink into the wells. A 1 kb DNA ladder (e.g., Vivantis DNA Ladder) was loaded into designated wells to serve as a molecular weight marker for estimating the size of the amplified DNA fragments. Electrophoresis was performed in a horizontal electrophoresis unit submerged in 1x TAE buffer, running at a constant voltage of 85 volts for approximately 25 minutes. After the run, the agarose gel was carefully transferred to a Gel Doc EZ system for visualization under UV light. The presence of an 876 bp band, corresponding to the expected size of the amplified BBTV replicase gene fragment, was indicative of a positive result for BBTV infection. Images of the gels were captured and analyzed using the accompanying software.

3. Results and Discussion

3.1 Sample Distribution

A total of 103 abaca leaf samples, predominantly exhibiting symptoms indicative of bunchy top disease, were collected from five different municipalities across the Surigao provinces. The highest proportion of samples originated from abaca plantations in Surigao del Sur, contributing 60 samples (approximately 62% of the total collected samples), reflecting the significant abaca cultivation in this province and potentially a higher incidence of reported disease. Surigao del Norte accounted for the remaining 43 samples (approximately 38%). The sampling strategy aimed to include both symptomatic and a limited number of asymptomatic plants, providing a more comprehensive understanding of the disease's prevalence, including potential latent infections.

3.2 Symptom-Based Incidence

Field-based symptom observation provided initial insights into the apparent incidence of Banana Bunchy Top Virus (BBTV) within the surveyed abaca plantations. A high incidence of BBTV-like symptoms was visually estimated in two key municipalities: Tago (85% of observed plants showing symptoms) and Marihatag (75% incidence). These high percentages suggest a significant and widespread problem of BBTV in these areas, likely contributing to substantial yield losses. Other surveyed locations reported moderate to low symptom expression, with estimated incidence rates ranging from 20% to 60%, indicating varying levels of disease pressure across the region. Symptomatic plants commonly exhibited classical diagnostic features unequivocally associated with BBTV infection (Figure 1). These included characteristic marginal chlorosis (yellowing along the leaf edges), distinct dark green streaks or dots along the midrib and petioles (often referred to as "dot-dash" symptoms), progressive narrowing and upright orientation of young leaves, and a characteristic apical clustering of stunted foliage, which gives rise to the descriptive "bunchy top" phenotype. In more advanced stages of the disease, infected plants also showed significant pseudostem shortening and severely inhibited sucker development, leading to overall plant stunting and reduced productivity. These visual indicators were critically utilized for preliminary screening and careful selection of representative samples for subsequent molecular confirmation via PCR, as they serve as the first line of suspicion for field diagnosis.

While symptom-based diagnosis offers a rapid, cost-effective, and practical method for estimating field-level disease incidence, it inherently remains prone to misidentification. This is primarily due to the potential for overlapping symptoms with nutrient deficiencies (e.g., potassium deficiency causing leaf yellowing) or co-infecting viruses (e.g., Banana Bract Mosaic Virus causing mosaic patterns). Such ambiguities can lead to false positives (misidentifying non-viral problems as BBTV) or false negatives (missing actual BBTV infections due to atypical or mild symptoms). Consequently, molecular assays, such as PCR, are indispensable. They provide the necessary specificity and sensitivity to verify BBTV presence accurately, differentiate it from other stressors, and precisely inform disease mapping and management efforts, thereby guiding more effective and targeted interventions.

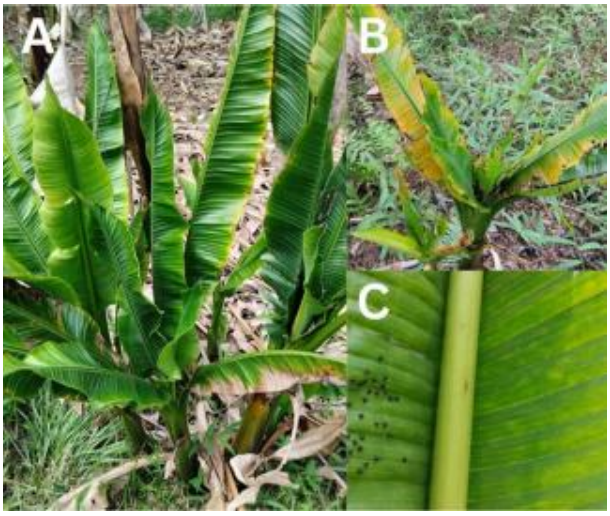

Fig. 1. Abaca plants showing typical Banana Bunchy Top Virus (BBTV) symptoms observed in Tago and Marihatag. Notable signs include upright narrow leaves, chlorotic margins, and leaf bunching at the apex.

3.3 PCR-Based Confirmation

To confirm the presence of Banana Bunchy Top Virus (BBTV) in the abaca plants sampled, PCR testing was done on leaf tissues from plants both showing symptoms and those appearing healthy without symptoms. The results, summarized in Table 3, show how many samples tested positive for BBTV in different municipalities of Surigao del Sur and Surigao del Norte, along with whether the plants had symptoms or not. Most of the samples taken from plants with visible symptoms tested positive for the virus, confirming that the typical symptoms were caused by BBTV infection. However, some samples from plants without any visible signs of disease also tested positive. This means that the virus can be present in plants that do not yet show symptoms, or in cases where symptoms are very mild and hard to detect by visual inspection alone. Because of this, relying only on visible symptoms to identify infected plants may not be enough. Molecular techniques such as PCR are necessary to detect the virus accurately, even in plants that look healthy. This information is important for managing the disease effectively, as plants that seem healthy could still spread the virus to other plants.

Table 1. Number of Samples Positive to BBTV by PCR Detection from Symptomatic and Asymptomatic Samples Collected from Surigao Provinces

Sites	No of Sample	Symp. PCR+	Asymp. PCR+	Total PCR +
Marihatag	20	8	3	11
Tago	20	15	0	15
San Agustin	20	0	0	0
Gigaquit	23	0	0	0
Mainit	20	1	0	1
Total	103	24	3	26

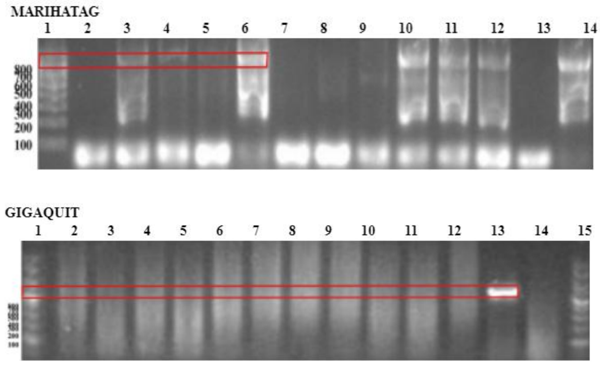

Fig. 2. Detection of Banana Bunchy Top Virus (BBTV) in abaca samples from (Top) Marihatag, Surigao del Sur and (Bottom) Gigaquit, Surigao del Norte using PCR with BBTV-rep F/R primers. Top gel: Lane 1 – DNA ladder (876 bp, Vivantis), Lane 2 – Blank, Lanes 3–12 – Field samples, Lane 13 – Healthy abaca (negative control), Lane 14 – BBTV-infected abaca (positive control). Bottom gel: Lane 1 – DNA ladder, Lane 2 – Blank, Lanes 3–12 – Field samples, Lane 13 – Positive control, Lane 14 – Negative control, Lane 15 – DNA ladder.

This study examined the presence and spread of Banana Bunchy Top Virus (BBTV) in abaca plants from selected areas in Surigao del Sur and Surigao del Norte. A total of 103 samples were collected, mostly from Surigao del Sur. Field observations showed a high rate of visible BBTV symptoms in Tago and Marihatag, with 85% and 75% of plants affected, respectively. Common symptoms included yellowing at the leaf edges (marginal chlorosis), dark green streaks along the central leaf veins, leaves that became narrow and stood upright, and the classic "bunchy top" appearance where new leaves are stunted and clustered together. In more advanced cases, plants also showed shorter stems and poor growth of new shoots. While looking for symptoms in the field is a quick and low-cost way to estimate BBTV infection, it can sometimes be misleading because other problems, like nutrient deficiencies or different diseases, can cause similar symptoms. To confirm the presence of BBTV, PCR testing was done on both plants with and without symptoms.

The PCR results showed that most symptomatic plants were indeed infected with BBTV. Importantly, three plants without any visible symptoms also tested positive, which means the virus can be present in plants before symptoms appear or in plants with very mild symptoms. This highlights that relying only on visual inspection may miss some infected plants, allowing the virus to spread unnoticed. Some samples from plants with symptoms tested negative for BBTV, which could mean that other diseases or conditions caused the symptoms or that the virus level was too low for detection. These results show the importance of combining both symptom observation and molecular testing to accurately detect BBTV. Overall, the study confirms that PCR testing is necessary for reliable BBTV detection, especially to find infections in plants that look healthy. Accurate detection helps in managing the disease better and protecting abaca crops from further damage.

4. Conclusion and Recommendation

This study confirmed the widespread presence of Banana Bunchy Top Virus (BBTV) in abaca plantations across Surigao del Sur and Surigao del Norte, with significantly higher infection rates observed in Surigao del Sur. The use of PCR-based molecular diagnostics proved highly effective in accurately detecting BBTV, including in asymptomatic plants that might not be identified through visual inspection alone. These findings highlight the limitations of relying solely on symptom-based diagnosis due to the similarity of BBTV symptoms with other viral infections or abiotic stresses, which can lead to misdiagnosis and underestimation of disease prevalence. Therefore, integrating molecular diagnostic tools into routine surveillance is essential to enable precise and early detection of BBTV, allowing timely interventions that can reduce virus spread and minimize yield losses in abaca production.

To enhance disease management, intensified monitoring efforts should be prioritized in high-incidence areas such as Surigao del Sur, with systematic sampling from both symptomatic and asymptomatic plants. Building the capacity of agricultural extension workers and farmers in symptom recognition, proper sample collection, and the significance of molecular confirmation is crucial for early detection and effective control. Moreover, molecular surveillance data should be incorporated into comprehensive disease management strategies, including rogueing infected plants, vector control, and quarantine measures, to improve the efficiency of interventions. Further research focusing on the epidemiological role of asymptomatic carriers is recommended to better understand their contribution to virus dissemination and refine management approaches accordingly. Collectively, these scientifically grounded measures provide a practical framework to strengthen BBTV control and support the sustainability of abaca production in the Caraga region.

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