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RESEARCH PAPER

First report of *Banana bunchy top virus* on heliconia (*Heliconia* spp.) in Bali, Indonesia

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ABSTRACT

Banana bunchy top virus (BBTV) mostly infects *Musa* spp. which causes banana bunchy top disease (BBTD), the most devastating viral disease in banana cultivation all over the world. During field survey in Sekar Bumi Tropical Farm located in Kerta Village, 15 heliconia plant samples showed green streak on lamina leaves that similar to common BBTD symptoms were collected. Further research was then conducted to identify molecular characteristic of BBTD in heliconia showing green streak. Molecular identification was conducted using polymerase chain reaction (PCR). Specific primers to amplified DNA target were used in this study: mRep/F (5'-GCGTGAAACGCACAAAAGGCC-3') and mRep/R (5'-GCATACGTTGT-CAAACCTTCTCCTC-3'). The expected 240 bp fragment target of partial master replication (mRep) gene was successfully amplified from ten out of 15 samples. Sequence analysis confirmed that the symptomatic heliconia samples were infected with BBTV and falls into the same clade with BBTV from the Asian Group.

Key words: Banana Bunchy Top Diseases (BBTD), banana production, molecular detection, mRep

INTRODUCTION

Banana Bunchy Top Disease (BBTD) is one of the most devastating viral diseases in banana plants worldwide. Typical symptoms of bunchy top disease are relatively easy to be recognized based on morphological appearance including vein clearing on lower part lamina and petiole, chlorosis of the leaf margins, reduction in petiole length, and Morse code dashed "J-hooking" along the midrib of leaves, and dark green streak of petioles with bunched appearance (Leiwakabessy et al., 2017). Since the first report in Java Island, Indonesia in 1978, BBTD caused by *Banana bunchy top virus* (BBTV) has been reported in some areas of Indonesia including Sumatera, Bali, and Special Territory of Yogyakarta (Furuya et al., 2004; Pinili et al., 2011; Chiaki et al., 2015). The disease might spread out from symptomatic plant to healthy plant by its sucker, corms, and plantlet of tissue culture during the trade among islands or countries (Wickramaarachchi et al., 2016).

Almost all cases of BBTV are in banana, however some cases were reported in other plants known as alternative hosts. Alternative hosts may play important roles as the source of virus inoculums. Alternative hosts for BBTV have been investigated since the aphid vector colonies numerous plant families including Araceae, Commelinaceae, Musaceae, and Zingiberaceae (Blackman & Eastop, 1984). Other plant species such as *Canna indica* (Canna; Cannaceae) and *Hedychium coronarium* (white ginger or garland flower; Zingiberaceae) were reported to be BBTV hosts in Taiwan (Geering & Thomas, 1997; Yasmin et al., 2001). Since Watanabe et al. (2013) confirmed that *Pentalonia* spp., the vector of BBTV, can also feed on heliconia, and a recent study by Hamim et al. (2017) was successful to molecularly detected BBTV from several symptomatic heliconia in Hawaii, the viral disease was shown to have a wide bioecological range. The virus is transferred in a persistent circulative manner and can be retained in the aphid system during its life cycle of 15–20 days (Hu et al., 1996).

Heliconia plants showing dark green streak and chlorotic on the leaf, similar to common BBTV symptoms but without stunting appearance, were found during a field survey in Sekar Bumi Tropical Farm located in Payangan, Gianyar, Bali. Heliconia fall within the

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family Heliconiaceae which consists of at least 200 species. In Indonesia, this plant is popularly known as pisang hias (ornamental banana) due to its morphological appearance which is similar to banana (pisang) and utilized mostly for ornamental (hiasan) purpose. Although Heliconia was formerly included in the family Musaceae, and has been variously associated with the Strelitziaceae, it is now the only genus under Heliconiaceae of the order Zingiberales (Kress, 1990).

Heliconia as a host of BBTV could be destructive to banana production in Bali and Indonesia. Therefore, further study is needed to determine the causal agent of symptomatic heliconia and whether banana aphids can transmit BBTV from infected heliconia to healthy banana plants.

MATERIALS AND METHODS

Research Site. A field survey in Sekar Bumi Tropical Farm located in Kerta Village, Gianyar Regency (8° 20′ 11.09″ S, 115° 17′ 2.2″ E) (Figure 1) was done in early 2020. Plant samples were taken using purposive sampling method based on bunchy top common symptoms classified by Leiwakabessy et al. (2017). The collected samples were brought to Laboratory of Plant Diseases, Faculty of Agriculture, Universitas Udayana.

Detection and Identification of Virus Isolates. PCR methods were performed to molecularly identify BBTV as the causal agent of bunchy top common symptoms on heliconia samples. The molecular identification in this research includes isolation of total DNA viruses, DNA amplification, and electrophoresis.

Isolation of Total DNA. Total DNA was extracted from 25–50 mg of fresh leaf samples using EZ-10 Spin Column Plant RNA Mini-Preps Kit (Bio Basic Inc, Canada). The total DNA was stored at -80 ºC for further use.

DNA Amplification. BBTV genome was amplified by PCR technique using mRep/F (5'-GCGTGAAACGCACAAAAGGCC-3') and mRep/R (5'-GCATACGTTGTCAAACCTTCTCCTC-3') specific primer (Amin et al., 2008). A 240 bp fragment of partial master replication (mRep) gene was expected (Amin et al., 2008). The PCR mix: 1 µL total DNA, 12.5 µL NZY Taq 2x Master Mix (Nzytech, Portugal), 1 µL forward primer, 1 µL reverse primer and distilled water (ddH_2O) of 9.5 µL to final reaction volume of 25 µL. DNA amplification for BBTV was conducted in a machine Thermal Cycler (INFINIGEN Biotech Inc., USA) with program as follow 5 min at 94 ºC for pre-heating, followed by 35 cycles of denaturation (30 s at 94 ºC), annealing (45 s at 55 ºC), and extension (30 s at 72 ºC), with final extension of 7 min at 72 ºC, the cycles ends with a temperature of 4 ºC.

Visualization of PCR Product. Visualization of PCR product was done by electrophoresis gel agarose in a concentration of 1% [0.25 g of agarose was put into a 100 mL baker glass then 25 mL of buffer TBE 0.5× (0.045 M Tris-Borate; 0.01 M EDTA)] was put into a 100 mL baker glass then 25 mL of buffer TBE 0.5× (0.045 M Tris-Borate; 0.01 M EDTA) was added and then heated for 3 min until it mixed well. After the gel warms, 1.5 µL dye FlouroVue TM (Smobio, Taiwan) was added, then agarose poured into a gel tray and allowed to stand for ± 30 min until hardened. Electrophoresis was carried out at 50 volts for 50 min.

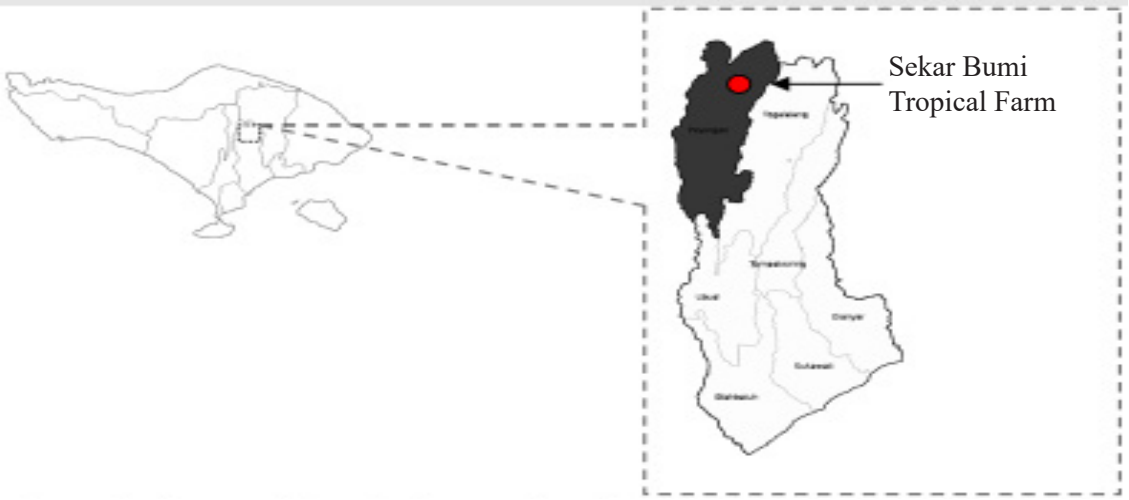

Figure 1. Location of Sekar Bumi Tropical Farm in Bali Island, indicating the sampling site.

Electrophoresis results in the form of DNA bands were visualized with an ultraviolet transilluminator and documented by digital camera.

Nucleotide Sequence and Phylogenetic Analysis. Gene master replication of one of the virus isolates was sent for sequencing at Macrogen, Singapore. Nucleotide sequence identity of the obtained virus isolates were compared to corresponding isolates from other countries available in the GenBank. The phylogenetic tree was constructed from the sequences aligned using Clustal W and MEGA 7.0 software with the neighbor-joining algorithm and bootstrap support, estimated using 1000 replicates (Kumar et al., 2016).

RESULTS AND DISCUSSION

Identification of the Virus Isolates. A total of 15 heliconia samples were applied for molecular detection of BBTV by PCR assay using a pair of specific primer mRep/F and mRep/R by Amin et al. (2008). Ten out of 15 heliconia samples were confirmed positive to amplify approximately 240 bp partial fragment of master replication of BBTV genome (Figure 2). BBTV is a circular single-stranded (ss) DNA plant virus whose genome contains at least six circular components and is associated with all geographical isolates of BBTV. The six components included DNA-R, DNA-U3, DNA-S, DNA-M, DNA-C, and DNA-N (Stainton et al., 2015; Wickramaarachchi et al., 2016; Kumar et al., 2017; Kakathi & Nath, 2018).

The length of BBTV DNA-R is 1111 nucleotides while the mRep gene is 860 nucleotides, start from a nucleotide number of 102 to 962. Amplification targets of mRepF/mRepR primers are nucleotide numbers of 435 to 674 (Mansoor et al., 2005). DNA-R encodes the master replication initiation protein (Rep) which is essential for trans-replication of the BBTV genomic components (Horser et al., 2001) through its nicking and joining activity (Hafner et al., 1997).

BBTV DNA-R is also known as BBTV DNA component 1. This component has two conserved regions: CR-SL and CR-M (Burns et al., 1995; Beetham et al., 1997). DNA-R also differs from other five components by having two ORF: major ORF and small ORF internal. The region was known to encode replication initiator protein, a component which was transferred by insect vector, *P. nigronervosa*, to initiate disease in hosts.

Symptom of BBTV. Symptoms associated with infected heliconia in Kerta village were similar with those in Hawaii (Hamim et al., 2017): dark green streak and chlorotic on the leaf (Figure 3). Similarities or variation of symptoms could be due to plant cultivars and environmental conditions (Rahim et al., 2015). In the

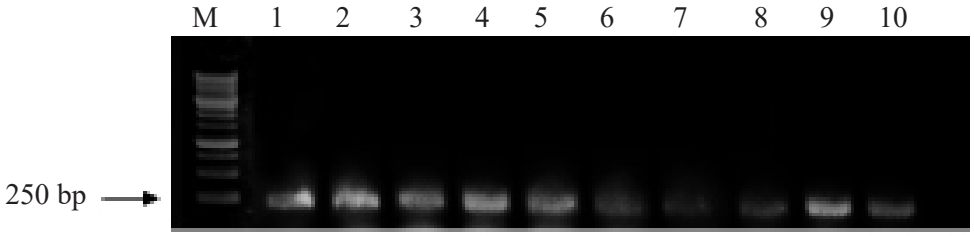

Figure 2. Visualization of DNA amplification of BBTV using mRep/F and mRep/R primers (samples 1–10) on 1% agarose gel. M, 1 kb DNA marker (Smobio, Taiwan).

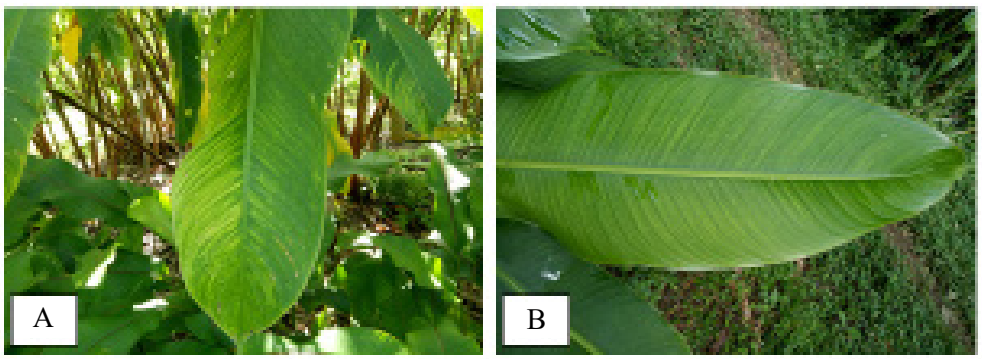

Figure 3. Symptoms of Banana Bunchy Top Diseases on heliconia. (A) Chlorosis on leaf; (B) Dark green streak on leaf.

case of BBTV in banana plants, Thomas (2008) reported that the symptom severity of BBTV infection was determined by plant response and time of infection. However further study is needed to determine symptom severity of BBTV infection in heliconia plants.

Phylogenetic Analysis. BLASTn analysis and comparison of the sequence with others available in the GenBank database (www.ncbi.nlm.nih.gov) showed that the isolates obtained in this study have the highest nucleotide sequence identity (> 98%) with BBTV Asian Group (Accession Number: JN003633, JN003632, AB847628, MK940789, MN037873, MN037876, MN037879, MN037872, JN003631, and KM607608). Moreover, phylogenetic analysis also showed that BBTV isolated heliconia in Bali fall into the same clade with isolates from Asian Group (Figure 4).

This research confirmed the association between BBTV and heliconia showed a dark green streak on the lamina. However, further research is still needed on identifying the complete genome sequence of the isolate, host range, virus transmission pathway by the insect vector, and which DNA components are needed to cause disease symptoms. Research on identifying and characterizing the complete genome of BBTV isolate on heliconia is currently ongoing.

CONCLUSION

Heliconia showing dark green streak on lamina leaf that was found in Sekar Bumi Farm of Kerta village was confirmed positive to BBTV by PCR using mRep/F and mRep/R specific primers. This work is the first report of BBTV infecting Heliconia in Bali, Indonesia. The research related to that discovery needs to be continued, moreover the complete genome, biological and genetic variabilities, and the role of insect vector transmission is not clearly studied.

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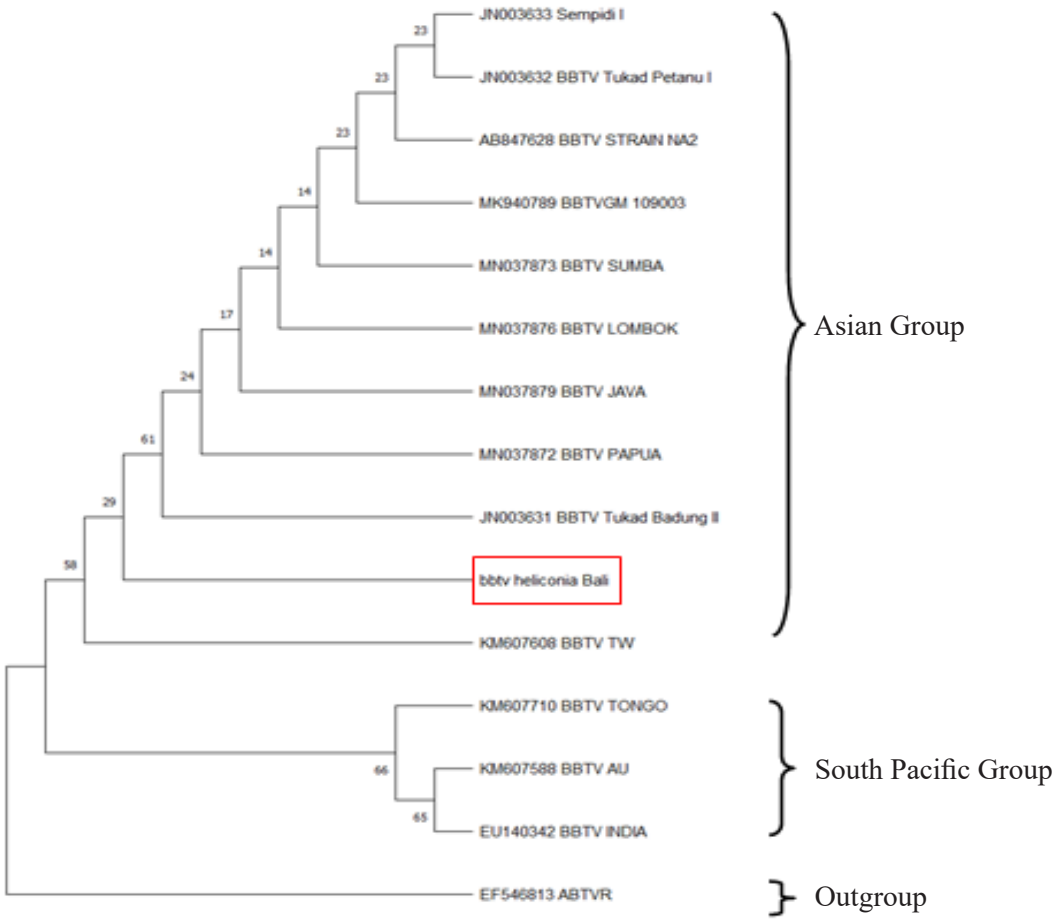

Figure 4. Phylogenetic tree of nucleotide sequence of BBTV heliconia Bali using MEGA 7.0 (Neighbor Joining method with bootstrap 1000×). *Abaca bunchy top virus* (EF546813) is used as outgroup.

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AUTHORS' CONTRIBUTIONS

GNASW and PS outlined and review the theme. MA conceptualized and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

COMPETING INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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