Jurnal Perlindungan Tanaman Indonesia, Vol. 24, No. 1, 2020: 11–16
DOI: 10.22146/jpti.54882
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Disease Severity and Molecular Identification of *Banana bunchy top virus*, Infecting Local Banana in Bali Island

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Received March 20, 2020; revised May 15, 2020; accepted May 22, 2020

ABSTRACT

Bunchy top symptoms on banana has been reported in Bali Island since early 2011. Symptoms variation were observed in the field similar to infection of *Banana bunchy top virus* (BBTV). The identity of the BBTV in Bali on the basis of DNA-S nucleotide sequence has not been studied, therefore research was conducted to identify the species of BBTV infecting local banana in Bali based on sequence analysis. Research activities were initiated by collecting field samples from several local banana growing areas in Bali Island. Incidence of bunchy top disease in all locations reached 8% to 44% with disease severity ranged from 2.6% to 30%. Identification of BBTV from field samples were done by polymerase chain reaction using specific primers for BBTV (CPF/CPR) followed by sequence analysis of amplified DNA target. Specific BBTV DNA fragment was successfully amplified from 10 field samples; sequence analysis of DNA fragments showed their highest homology with BBTV. In addition the phylogenetic analysis confirmed the close relationship of BBTV isolates from Bali with various BBTV isolates from Indonesia and other isolates from the Asian group in GeneBank.

Keywords: *Banana bunchy top virus*; disease severity; homology sequence analysis; phylogenetic analysis; polymerase chain reaction

INTRODUCTION

Banana bunchy top disease is considered as the most important disease on banana in Bali, Indonesia due to its effect on yield loss. Dale (1987) reported up to 100% yield loss due to bunchy top disease. The disease is now spreading very fast across the areas in Indonesia especially in the production region of banana such as in Bogor, Bali, and Yogyakarta (Leiwakabessy *et al.,* 2017; Pinili *et al.,* 2011; Furuya *et al.,* 2004).

The symptom of bunchy top disease on banana was first observed in Bali in 2011. Disease incidence and severity in Bali has not been studied. The disease was rapidly spreading in Bali because banana is planted continuously in some areas, such as in Karangasem, Bangli, Tabanan, Gianyar, Buleleng, Badung, Klungkung and Jembrana (Statistics of Bali Province, 2016). Along with the increasing area of bunchy top disease, the production of banana in those areas declined significantly, from 190,235 tons in 2015 to 183,210 tons in 2016 (Statistics of Bali Province, 2016).

Banana bunchy top virus (BBTV) belongs to the family *Nanoviridae* and genus *Babuvirus*, a small family of plant-infecting, circular ssDNA viruses (Vetten *et al*., 2005). Natural transmission of BBTV is occurred only by its insect vector aphid (*Pentalonia nigronervosa*) (Furuya *et al.,* 2006). Hooks *et al.* (2008) reported, incubation study of BBTV showed that the incubation period ranged between 25 and 85 days after inoculation (DAI). The causal agent of bunchy top disease on banana in Bali has been identified to amplify the complete DNA-R component as *Banana bunchy top virus*, a member of *Babuvirus.* That studies on detection of BBTV in Bali only amplified the complete DNA-R component for identification, using pair primers D11 forward (5'-GGAAGAAGCCTCTCATCTGCTTCAGACARC -3') and D12 reverse (5'-TTCCCAGGCGCAC ACCTTGAGA-AACGAAAG-3') (Pinili *et al*., 2011), however identification to amplify the complete DNA-S component has not been studied. This research was conducted in order to analyze and compare the incidence and severity of banana bunchy top disease

in Bali and detection of BBTV from field samples, as well as to analyzed the sequence homology and phylogeny.

MATERIALS AND METHODS

Field Survey and Samples Collection

Survey was conducted in February 2019 in several banana growing areas in Bali Island. Disease symptoms, incidence and disease severity was observed during survey as well as collecting leaf samples for DNA isolation. Disease incidence (DI) was measured as the proportion of diseased plants in a population, regardless of the weight or severity, to the total of plant samples. Disease severity (DS) was estimated based on disease score, where DS (%) = [sum (class frequency x score of rating class)]/[total number of plants) x (maximal disease index)] × 100. Assessment on disease scores was based on the variations of visual symptoms (Leiwakabessy *et al.,* 2017) (Table 1).

Leaves showing symptoms from each survey location were collected and preserved in plastic bags containing silica gel for virus confirmation in the laboratory.

Detection and Identification of BBTV

Field samples were subjected for BBTV detection by polymerase chain reaction-based detection method using specific primers CP1/F (5'-CCCGGGAGA ATACTTCACTGGGCTATGATT-3') and CP1/R (5'-CCCGGGCTTCACCTTGCACACCAACAG CAT-3') (Mansoor *et al.,* 2005). Total DNA isolation from leaf samples was performed using CTAB (Doyle & Doyle, 1987). Preserved leaf tissue (0.1 g) was freezed up in liquid nitrogen and the tissue was finely ground using mortar and pistil and transferred to micro tube (2.1 ml). Five hundred microliter of buffer extracting solution (EDTA-20 mM, Tris-HCl, pH 8-100 mM, NaCl-1.4 M, CTAB-2%, and Mercaptoethanol-0.2%) was added on to the micro tube followed by incubation at 65°C for 60 minutes with occasional mixing by gently inverting the tube. After incubation, equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and the tube was inverted several times followed by centrifugation at 12,000 rpm for 15 minutes.The upper phase was transferred to a new micro tube followed by adding sodium acetate (1/10x volume) and cold isopropanol (2/3xvolume), then the mixture was incubated overnight at -20^{o}C to precipitate DNA. After the incubation, the tube was centrifuged at 12,000 rpm for 10 minutes and the supernatant was removed. The pellet containing total DNA was washed with 70% ethanol and centrifuged at 8,000 rpm for 5 minutes. Pellet DNA was air-dried and resuspended in TE buffer solution (1x) and stored at -80°C for further use.

Amplification reaction was prepared using ready to go PCR bead (Amersham Pharmacia Biotech. Inc.). For each reaction, 2 µl of DNA, 1 µM of each CP1/F/CP1/R primer, 0.5 µl of $MgCl_2$, 12.5 µl of 2x MyTaqTM HS Red Mix Bioline and distilled water to final reaction volume of 25 µl was added to a PCR bead. DNA amplification was conducted in thermal cycler (Gene Amp, PCR System 9700 PE Applied Bio-system) started with pre-heating cycle for 5 minute at 94^{o}C, followed by 35 cycles of denaturation (30 seconds at 94^{o}C), annealing (45 seconds at 55^{o}C), and extension (30 seconds at 72^{o}C). The last cycle ended at 72^{o}C for 7 minute and cooled down to 4^{o}C. The amplicon was then visualized by electrophoresis using 1% agarose gel in 0.5x TBE (Tris-Boric acid-EDTA) buffer. The electrophoresis was performed at 50 V for 50 minute, then the gel was soaked on to 0.1% EtBr for 10 minute, washed with H_2O for 20–30 minute, and visualized under UV transilluminator.

Table 1. Disease score based on symptoms variation of BBTV infection on banana (Leiwakabessy *et al.*, 2017)

Disease score	Symptom type
0	Symptomless
1	Limited vein clearing and dark green streaks on the lower part of lamina and on petiole. No significant reduction of lamina width.
2	Vein clearing, upturned leaf, chlorotic, and ragged margins. Significant reduction in petiole length, distance, and lamina width.
3	Brittle lamina with upturned, chlorotic, and ragged margins, sometimes with necrotic symptom. Leaves failed to emerged, giving a clear bunched appearance and dwarf.

Sequence Analysis

PCR product was subjected to direct sequencing and the sequence data was analyzed using BioEdit V.7.0.5 software program, CLC Sequence Viewer 8, and MEGA 6.06.

RESULTS AND DISCUSSIONS

Incidence of bunchy top disease in all locations reached 8% to 44% with disease severity ranged from 2.6% to 30% (Table 2). Infected plants were easily recognized in the field due to their unique symptoms, the appearance of dark green streak, slightly chlorotic margins along the new developing leaves, and dwarf (Figure 1). These types of symptoms had also been reported as typical of bunchy top disease of banana in Sumatra, Indonesia (Chiaki *et al.,* 2015).

The survey conducted in 10 locations in Bali Island indicated that the appearance of dark green streak of leaf were the early symptom banana infecting viruses, followed by slightly chlorotic margins along the new developing leaves, plants will become dwarfs when infected with the virus as the vegetative period. The type of symptoms associated with the disease in Bali Island is same from those reported by Thomas & Iskra-Caruana (2000).

Further detection using specific primers confirmed the infection of BBTV. Specific DNA fragment of 1,083 bp was successfully amplified from all field samples (Figure 2). Two out of 10 field samples (Sesetan 2 and Tegallalang 1) representing ten different survey location in Bali were then subjected to direct sequencing.

Sequence and Phylogenetic Analysis

Two sequences was obtained and further analysis showed their highest homology (98.9%) with several isolates of *Banana bunchy top virus* (BBTV) in Indonesia (KM607538) and other isolates in Asia. Interestingly, their sequence homology is low (43.2% to 43.4%) to out group, *Abaca bunchy top virus* (ABTVr) from Malaysia (EF546813) (Table 3).

Phylogenetic analysis showed that isolates from Bali can be differentiated into three groups, whereas the isolates of BBTV from Pakistan, Congo and Australia formed a separate group (Figure 3). Most of the isolates Bali 2 (LC481517), Bali 5 (LC481518), Java (AB186928), Sumatra (AB848108), Taiwan (KM607541), Indonesia (KM607541), Philippines (KM607521, KM607523), India (KM607450), China (KX779467), Thailand (MF039880) and Viet Nam (AB113661) were clustered in the first group, however isolates from Bali separated from other Indonesian isolates, this indicates that isolates from Bali have different strains than other Indonesian isolates. Isolates Pakistan (AM418565) and Congo (KM607492) were in the second group together with Australia (KM607442); and one isolate *Abaca bunchy top virus* (ABTVr) Malaysia (EF546813) were clustered in the out group.

Rahim *et al.* (2015) reported that symptoms of viral infections found in the field showed similarities or variations in symptoms caused by viruses, plant

Table 2. Disease incidence, severity and field symptoms of BBTV infecting banana in Bali Island, Indonesia

Location (Village, regency)	Cultivar/Genome	Field symptoms	Disease incidence (%)	Disease severity (%)	PCR detection
Sanur 1, Denpasar	Raja/AAB	green streak	12.0	6.0	+
Sanur 2, Denpasar	Emas/AA	green streak	14.0	8.0	+
Sesetan 1, Denpasar	Uli/AA	green streak, dwarf	34.0	24.6	+
Sesetan 2, Denpasar	Andong/unidentified	green streak	22.0	13.3	+
Tampaksiring 1, Gianyar	Uli/AA	green streak	18.0	10.0	+
Tampaksiring 2, Gianyar	Emas/AA	green streak	8.0	2.6	+
Payangan 1, Gianyar	Sari/AAB	green streak	10.0	4.6	+
Payangan 2, Gianyar	Uli/AA	green streak	16.0	8.0	+
Tegallalang 1, Gianyar	Temaga/unidentified	green streak, chlorotic, and dwarf	44.0	30.0	+
Tegallalang 2, Gianyar	Raja/AAB	green streak	22.0	10.6	+

Figure 1. BBTV infection in several cultivation area at Denpasar City and Gianyar Regency (A); slightly chlorotic margins along the new developing leaves (B); the appearance of dark green streak (C), dwarf (D), and a healthy banana plant (E)

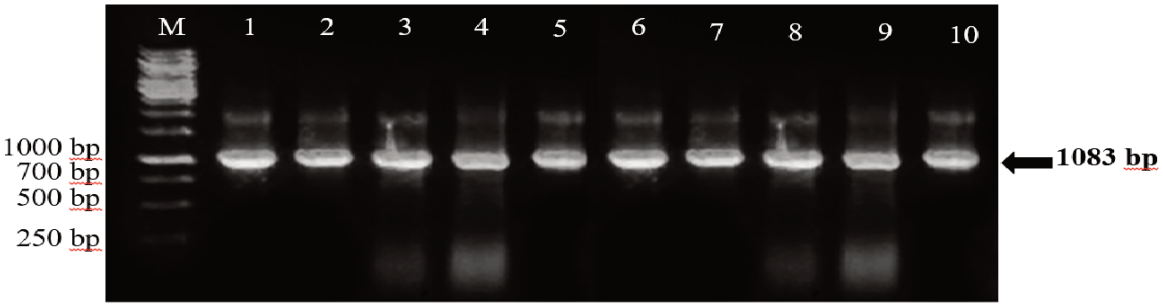

Figure 2. Visualization of DNA amplification of BBTV from leaf samples using primers for BBTV (CPF/CPR) on 1% gel agarose; M: DNA marker (1kb ladder); line 1 to 10, field samples from Denpasar city (Sanur and Sesetan) and Gianyar Regency (Tampaksiring, Payangan, and Tegallalang), respectively

Table 3. Nucleotide sequence homology (%) of BBTV infecting banana in Bali Island with BBTV reported earlier in GeneBank

BBTV in GeneBank		BBTV infecting banana in Bali/ Accession Number			
Accession Number	Isolate	BBTV_Bali2 (LC481517)		BBTV_Bali5 (LC481518)	
		nt	aa	nt	aa
KM607541	Taiwan (TAI)	99.2	98.4	98.3	96.5
KM607538	Indonesia (IDN)	98.9	97.5	98.3	96.5
KM607523	Philippines (PHI)	98.1	96.5	97.8	95.9
KM607521	Philippines (PHI)	98.2	96.8	97.9	96.2
KM607450	India (IND)	98.5	81.7	98.2	81.1
AB186928	Indonesia (Java)	98.8	98.4	98.7	97.8
AB848108	Indonesia (SMT)	98.7	98.1	98.3	97.1
KX779467	China (CHN)	97.9	95.9	97.7	95.3
AM418565	Pakistan (PAK)	86.5	59.7	86.8	59.4
KM607492	Congo (CON)	86.9	59.5	86.8	59.2
MF039880	Thailand (THA)	90.7	70.0	90.8	70.0
AB113661	Viet Nam (VNM)	91.7	76.4	91.8	76.4
KM607442	Australia (AUS)	86.5	62.8	86.4	62.5
EF546813	ABTVr Malaysia (MLY)	43.2	11.6	43.4	11.6

Remarks: nt = nucleotide, aa = amino acid

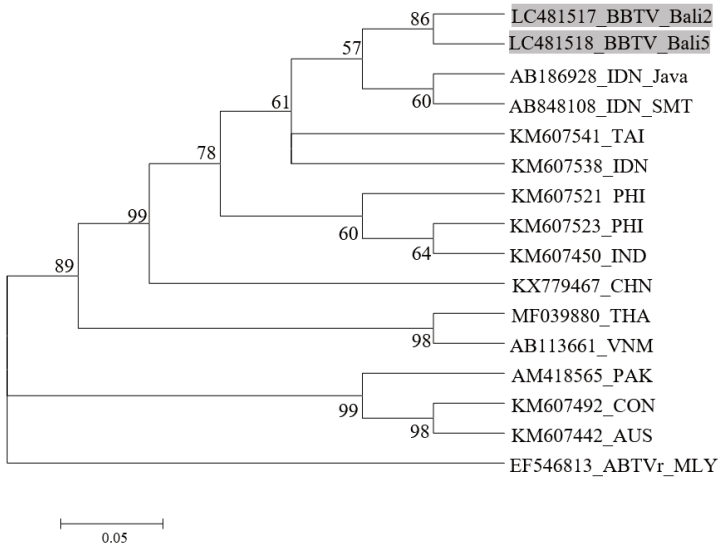

Figure 3. Phylogenetic trees of nucleotide sequences of full-length CP BBTV of the DNA-S using Mega 6.06 (Algorithm Neighbor Joining with 1,000 bootstraps replicates), gene isolates from Bali against 12 BBTV isolates at Genebank; Abaca bunchy top virus (ABTV) is used as a comparison out group; isolates given highlight marks were isolates from Bali (Bali 2 and Bali 5); (Indonesia–IDN, Taiwan–TAI, China–CHN, Philippines–PHI, India–IND, Pakistan–PAK, Congo-CON, Thailand-THA, Vietnam-VNM, Australia–AUS, Malaysia-MLY, Sumatra–SMT)

varieties and environmental conditions that influence the expression of symptoms. Niyongere *et al.* (2012) reported, the incidence of banana bunchy top disease (BBTD) significantly varied according to trial banana cultivar, planting materials, and location. However, it is suggested that the higher incidence of BBTD can be attributed to the presence of large number of winged aphid (*P. nigronervosa*).

Pinili *et al.* (2011) reported, that studies on detection of BBTV in Bali only amplified the complete DNA-R component for identification, however the identity of the BBTV in Bali on the

basis of DNA-S nucleotide sequence has not been studied. The DNA-R BBTV genome size is 1,111 nt with parts of the mRep gene measuring 860 nt which are in number nucleotides 102 to 962. The amplification target of the mRepF / mRepR primary pair is part of the mRep gene which is in nucleotides number 435 to 674 (Mansoor *et al.,* 2005). The DNA–S BBTV genome is smaller than DNA-R, which is 1,075 nt. The amplification target of the primary pair CP1F/CP1R is the entire DNA-S genome, including the protein envelope gene (coat protein/CP) which is at number 213 to 740 nucleotides (Amin *et al.,* 2008).

CONCLUSIONS

These results showed that BBTV on banana crops were distributed evenly in Bali. The infections of BBTV on local banana in Bali shows the potential of BBTV to spread geographically. Therefore, efforts to control and prevent the spread of BBTV that is more widespread in Indonesian need to be done immediately.

LITERATURE CITED

Amin, I., J. Qazi, S. Mansoor, M. Ilyas, & R.W. Briddon. 2008. Molecular Characterization of *Banana bunchy top virus* (BBTV) from Pakistan. *Virus Genes* 13: 191–198.

Chiaki, Y., N. Nasir, H. Herwina, Jumjunidang, A. Sonoda, T. Fukutomo, M. Nakamura, & H. Iwai. 2015. Genetic Structure and Diversity of the *Banana bunchy top virus* Population on Sumatra Island, Indonesia. *European Journal of Plant Pathology* 143: 113–122.

Dale, J.L. 1987. Banana Bunchy Top: An Economic Important Tropical Plant Virus Disease. *Advances in Virus Research* 33: 301–325.

Doyle, J.J. & J.J. Doyle. 1987. A Rapid DNA Isolation of Procedure for Small Quantities of Fresh Leaf Tissue. *Phytochemical Bulletin* 19: 11–19.

Furuya, N., S. Somowiyarjo, & K.T. Natsuaki . 2004. Virus Detection from Local Banana Cultivars and the First Molecular Characterization of *Banana bunchy top virus* in Indonesia. *Journal of Agricultural Science* 49: 75–81.

Furuya, N., T.O. Dizon, & K.T. Natsuaki. 2006. Molecular Characterization of *Banana bunchy top virus* and *Cucumber mosaic virus* from Abaca (*Musa textilis* Nee). *Journal of Agricultural Science* 51: 92–101.

Hooks, C.R.R., M.G. Wright, D.S. Kabasawa, R. Manandhar, & R.P.P. Almeida. 2008. Effect of *Banana bunchy top virus* Infection on Morphology and Growth Characteristics of Banana. *Annals of Applied Biology* 153: 1–9.

Leiwakabessy, M., S. Nurulita, & S.H. Hidayat. 2017. Disease Incidence and Molecular Analysis of *Banana bunchy top virus* in Bogor, West Java. *In* D. Efendi & A. Maharijaya (eds), *The Future of Tropical Horticulture. International Proceeding on Tropical Horticulture 2016: The Future of Tropical Horticulture*. Bogor, Indonesia, November 28–29, 2016.

Mansoor, S., J. Qazi, I. Amin, A. Khatri, I.A. Khan, S. Raza, Y. Zafar, & R.W. Bridon. 2005. A PCR-based Method with Internal Control for the Detection of *Banana bunchy top virus* in Banana. *Molecular Biotechnology* 30: 127–129.

Niyongere, C., T. Losenge, E.M. Ateka, D. Nkezabahizi, G. Blomme, & P. Lepoint. 2011. Occurrence and Distribution of Banana Bunchy Top Disease in the Great Lakes Region of Africa. *Tree and Forestry Science and Biotechnology* 6: 102–107.

Pinili, M.S., D.N. Nyana, G. Suastika, & K.T. Natsuaki. 2011. Molecular Analysis of *Banana bunchy top virus* First Isolated in Bali, Indonesia. *Journal of Agricultural Science* 56: 125–134.

Rahim, Y.F., A.D. Tri, & G. Munif. 2015. Detection of Viruses that Infect Soybeans in West Java. *Jurnal Fitopatologi Indonesia* 11: 59–67.

Statistics of Bali Province. 2019. *Banana Fruit Production Specified by Regency/City in Bali, 2000–2017*. Available via DIALOG. https://bali.bps.go.id/dynamictable/2017/05/18/129/produksi-buah-pisang-dirinci-menurut-kabupaten-kota-di-bali-2000-2017.html, modified 28/05/ 2019.

Thomas, J.E. & M.L. Iskra-Caruana. 2000. Bunchy Top, p. 241–253. *In* R.D. Jones (ed), *Diseases of Banana, Abaca and Ensete*. CAB International, Wallingford.

Vetten, H.J., P.W. Chu, J.L. Dale, R.M. Harding, J. Hu, L. Katul, M. Kojima, J.W. Randles, Y. Sano, & J.E. Thomas. 2005. Nanoviridae, p. 343–352. *In* C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball (eds.), *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego.