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ORIGINAL ARTICLE

Recent distribution and diversity analysis on banana bunchy top virus of banana and alternative host in Indonesia

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Abstract
Banana bunchy top is one of the most damaging diseases in banana cultivation. However, the distribution and molecular diversity of banana bunchy top virus (BBTV) as well as its alternative hosts in Indonesia have not been reported since 1998. A total of 257 banana leaf samples were collected from the islands of Sumatra, Java, Kalimantan, Sulawesi, Maluku, Papua, Bali, Sumbawa, Timor, and Sumba from 2016 to 2019. The BBTV was detected through PCR in samples from all islands except Kalimantan. A molecular analysis showed that all BBTV isolates belonged to the South East Asian (SEA) subgroup. Based on the DNA-S and DNA-C analysis, the isolates from Sulawesi and Halmahera islands were closely related to those from the Philippines, while the remaining isolates were highly similar to those previously reported from Sumatra, Java, and Bali. Natural infection of BBTV was also recorded on abaca (*Musa textilis*), wild banana (*M. acuminata* subsp. *sumatrana*), ornamental pink banana (*M. velutina*), galangal (*Alpinia galangal*), and turmeric (*Curcuma longa*).

Keywords Distribution · Bunchy top virus · Banana · Alternative host

Introduction

Banana (*Musa*) is an economically valuable fruit crop both in tropical and subtropical areas, where it is consumed as fresh fruit and a main or alternative staple food, and its leaves, stem, and flowers are also used for food and other purposes. Banana is grown in Indonesia from lowlands to an altitude of up to around 1000 m above sea level. As Indonesia forms part of the center of origin of *Musa*, high germplasm diversity is found in this country.

Bunchy top disease is recognized as the most devastating viral disease in banana, causing tremendous loss in many banana-producing countries (Wickramaarachchi et al. 2016; Ngatat et al. 2017; Abiola et al. 2020). Although production losses have not been quantified in Indonesia, the disease has a significant impact on farmers' livelihoods. Wirya et al. (2020) reported that the incidence of BBTD across Bali island reached 8 to 44% with the disease severity ranged from 2.6 to 30%, indicating that the plant loss was potentially higher than 40% since the systemic disease usually destroys the whole cluster of the infected plant.

The initial symptom on infected plants comprises chlorosis on the margin of new leaves and dark green dots and streaks on the petiole and minor veins of the leaf lamina. There can be whitish streaks on the veins of newly emerging leaves which will become dark green when the leaves unfurl. Ultimately, the young leaves become shorter and narrower with the chlorotic or necrotic upturned margins, and an erect habit (Elayabalan et al. 2015; Thomas 2015). BBTV-infected plants, especially when infected early in the cropping cycle,

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usually fail to produce fruit; therefore, severe yield loss occurs (Ngatat et al. 2017; Qazi 2016). With later infections, smaller fruits which are not marketable may be produced. Symptoms can appear on the flower sheaths (bracts), characterized with dark green lines on the sheath tip (Thomas 2015).

The genome of BBTV consists of six single-stranded, circular DNA components of about 1.1 kb. Each genome component is named for the one open reading frame (ORF) it encodes: DNA-R (replication initiation protein), -U3 (unkown function), -S (coat protein), -M (movement protein), -C (cell-cycle-link protein) and –N (nuclear shuttle protein) (Baldodiya et al. 2019; Thomas 2019). DNA-R uniquely has a second small ORF of unknown function embedded within the major ORF. Yu et al. (2019) reported that the DNA-N, DNA-S, and DNA-U3 were highly expressed followed by the DNA-M with moderate expression, DNA-R at the lower expression, and DNA-C at the lowest one.

According to genome sequence analyses, there are two different groups of BBTV: (i) the South Pacific group (alternatively named Pacific-Indian Oceans) comprising isolates from Africa, Australia, Pacific islands, South Asia, Myanmar, and (ii) the Asian group (alternatively South-East Asian) (Stainton et al. 2015; Das and Banerjee 2018). In Indonesia, BBTV was first reported in 1978 in West Java Province (Cimahi and Padalarang, Bandung). Then, it was also documented in Sumatra (Riau, West Sumatra, and Lampung provinces), Java (West Java, Central Java, the Special Region of Yogyakarta and Bali (Nurhadi and Setyobudi 2000) and Kutai Kartanegara, East Kalimantan (Irwansyah and Akhsan 2019)). BBTV was initially perceived to only infect species in the Musaceae family (*Musa* and *Ensete*) (Selvarajan and Balasubramanian 2013). However, recent studies have identified several alternative hosts; *Alpinia zerumbet* (shell ginger), *Canna indica*, and *Colocasia esculenta* with isolates from Japan and the Philippines (Pinili et al. 2013) and *Heliconia aurantiaca* in Hawaii (Hamim et al. 2017). BBTV can be transmitted by two species of the aphid genus of *Pentalonia*: *P. nigronervosa* and *P. caladii* (Watanabe et al. 2013). The former is predominantly found on *Musa* sp. and occasionally on members of the Zingiberales, and the latter is largely spotted on the members of the Zingiberales and Araceae, but occasionally on *Musa* sp. (Bagariang et al. 2019; Duay et al. 2014; Watanabe et al. 2013; Suparman et al. 2017).

The molecular characteristics of Indonesian BBTV have been previously reported for isolates from Central Java (Furuya et al. 2004), Bali (Pinili et al. 2011), and Sumatra (Chiaki et al. 2015). This paper reports the distribution of BBTV from these areas and additional islands in Indonesia, the molecular diversity of the isolates, and some new host records.

Material and methods

Sample collection and BBTV detection

The surveys and random samplings of BBTV were targeted in the islands of Sumatra, Java, Kalimantan, Sulawesi, Seram, Halmahera, Papua (New Guinea), Bali, Sumbawa, Sumba, and Timor (Fig. 1). Young banana leaves with the symptom of bunchy top were collected from the visited fields. Each sample was kept in a paper bag or envelope and stored in the cool box during the survey. All samples were cut into small pieces and preserved by drying over silica gel and storing in the refrigerator before extraction. An observation and sampling were carried out on the potential alternative host from the Zingiberales growing around the infected banana.

DNA extraction was conducted in the Laboratory of Plant Protection at Universitas Gadjah Mada, Yogyakarta, Indonesia and in the Plant Pathology Laboratory of Queensland Alliance for Agriculture and Food Innovation (QAAFI), the University of Queensland, Australia. Total DNA was extracted using ISOLATE II Genomic DNA Kit (Bioline Reagents Limited, London, UK) according to the manufacturer's instructions. The virus was detected by PCR using back-to-back primer pairs specific for DNA-R, DNA-S, DNA-C and DNA-N (Stainton et al. 2012).

PCR amplification and sequence analysis

The DNA was amplified using MyTaq™ HS Red Mix (Bioline) PCR kit. A total reaction volume of 12.5 μl consisted of 2 μl of DNA template, 1 μl of each primer stock, 5 μl of My Taq Red Mix, and 3.5 μl of RNAse-free water. The initial denaturation was performed at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min, and a final extension at 72 °C for 3 min. The amplicons were electrophoresed in a 1% agarose gel in 1× TBE buffer and visualized by staining using ethidium bromide. The PCR products were sent to First Base Laboratory in Selangor, Malaysia for DNA sequencing.

Genetic diversity and phylogenetic analysis

The sequences were aligned using MAFFT and continued with sequence cleaning by BMGE on the NGPhylogeny.fr web service (Criscuolo and Gribaldo 2010; Katoh and Standley 2013; Lemoine et al. 2019). The phylogenetic trees were constructed by MEGA X software using maximum likelihood with Tamura-Nei (TN93) parameter model, and 1000 bootstrap replicates were used to verify the significance of the trees (Tamura and Nei 1993; Kumar et al. 2018). The complete nucleotide (nt) sequences from DNA-R, DNA-S, DNA-C, and DNA-N were compared to the available nucleotide

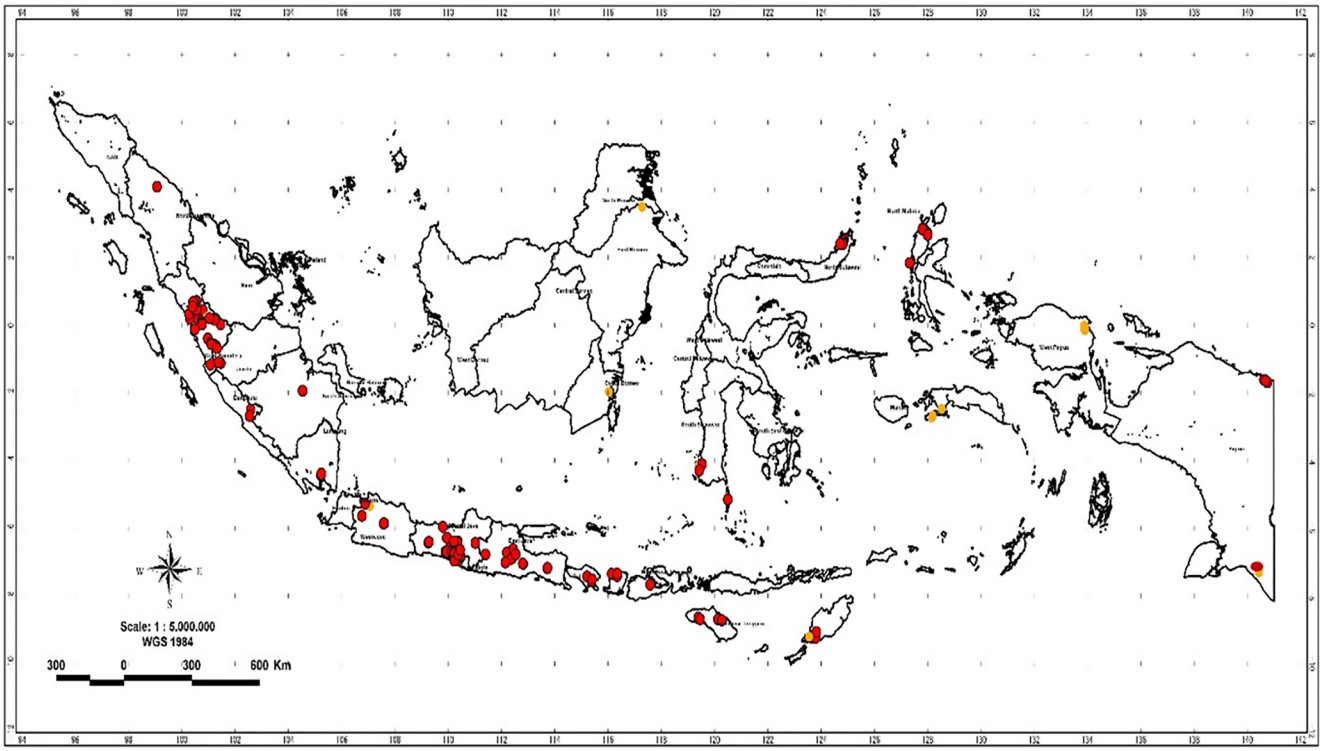


Fig. 1 Distribution of BBTV in Indonesia. Red indicates BBTV-positive samples; yellow dots indicate BBTV-negative samples

sequences of BBTV in GenBank representing the global distribution and population of BBTV. Two abaca bunchy top virus (ABTV) accessions were used as the outgroups.

Alternative host

The observation and sampling for alternative host of BBTV were conducted simultaneously with the sampling of banana plants with bunchy top symptoms, focusing on plants from the Zingiberaceae family. The samples were selected according to the presence of symptoms or aphid vectors on the plants. BBTV detection and greenhouse assay for artificial inoculation were performed to provide evidence of the information.

Artificial inoculations were done in the greenhouse using banana aphids *P. nigronervosa* that were reared on healthy *Caladium bicolor*. Different species outside the genus *Musa* were tested as potential virus reservoir. These include ginger (*Zingiber officinale* L.), canna (*Canna edulis* L.), canna lily (*Canna indica* L.), and false bird of paradise (*Heliconia rostrata* Ruiz&Pav.). Unequal number of plants per species was used in the tests.

The inoculation technique was done according to Jebakumar et al. (2018), with a slight modification. Healthy, wingless adults (instar 3) of *P. nigronervosa* were transferred from healthy Caladium to the BBTV-infected banana plant cv. Mas. After 48 h of acquisition period, 20 virulivirous aphids were transferred individually using a wet paint brush to the healthy plants. The inoculated plants were placed in a separate room (screen house) with a temperature of 25–30 °C.

Results

Distribution map of BBTV in Indonesia

The current surveys and sampling on symptomatic banana plants revealed the extended distribution areas of bunchy top disease in Indonesia, including Timor and Sumba islands in East Nusa Tenggara and Ternate island of North Maluku Province.

A total of 257 samples included 9 asymptomatic samples were collected from 23 provinces in Indonesia, consisted of 237 symptomatic banana leaf samples from various cultivars, 11 leaves from other plants including pink banana (*Musa velutina* H. Wendl. & Drude, n = 1) from Yogyakarta, canna lily (*C. indica* L., n = 1), bird of paradise flower (*Strelitzia* sp., n = 1), abaca (*M. textilis* Née, n = 2) from Yogyakarta and North Sulawesi, wild banana (*M. acuminata* ssp. *sumatrana*, n = 2) from Bengkulu and West Sumatra, and turmeric (*C. longa*, n = 1) from West Sumatra (see Supplementary Table 1). The highest incidence was found in Sumatra (cultivars Muli and Ambon Kuning) and in Java (cultivars Mas, Ambon Kuning, and Awak). The distinct symptoms of bunchy top disease were found on cultivars Mas (AA), Muli (AA), Raja (AAB), Ambon Kuning (AAA), Maraseba

(AAA), Awak (AAB), Goroho (ABB), Mulu Bebek (AAB), and Kepok/Sepatu (ABB). Banana plants with typical symptom of bunchy top disease were not found in the provinces of the Special Capital Region of Jakarta, North Kalimantan, South Kalimantan, Central Sulawesi, Maluku, and West Papua. PCR tests of these symptomless banana samples were also negative.

Molecular characteristics

A total of 28 BBTV isolates were selected to represent positive samples for molecular analysis. These isolates were obtained from different islands and cultivars and characterized according to the sequence of DNA-R. The additional characterization on the sequencing of DNA-S was conducted on 13 samples while DNA-C and DNA-N on 12 samples by selecting 1 isolate each from each island except 3 isolates each from Sumatra and Java (Table 1). This study analyzed the sequences on four components of BBTV amplified with the related specific primers. All amplicons were about 1.0–1.1kb.

Total length of the DNA-R was 1104 nucleotides consisting of one open reading frame (ORF) with the length of 861 nucleotides from the nucleotide number 103–963th coding for 287 amino acids (33.6 kDa). DNA-S had 1058 nucleotides with one of ORF at the length of 513 nucleotides, coding for 171 amino acids (19.4 kDa). The size of DNA-N is 1083 nucleotides with one ORF of 483 nucleotides from the nucleotide number 246–728th that forming 161 amino acids. DNA-C has 1014 nucleotides with an ORF of 561 nucleotides at the nucleotide numbers of 149–709th and forming 187 amino acids. There were no gaps, deletions, nor insertions in all sequences.

These components of BBTV isolates from Indonesia possessed CR-SL and CR-M, functioning in the regulation of their replications and primer synthesis, respectively. In DNA-R, the stem loop common region (CR-SL) contained the conserved nonanucleotide sequence of TATTATTAC at the nucleotide position of 13–21. The intron sequence GGGAC (F1, F2) and R (GTCCC) were located at the position of 33–42, 204–208, and 368–372, respectively. The stem loop common region was also found in the DNA-S, DNA-C, and DNA-N (Fig. 2). The DNA sequences from this study have been submitted to NCBI with the assession numbers listed in Table 1.

Relationship among isolates

Worldwide BBTV isolates were clustered into two groups, the Asia and the South Pacific/Pacific Indian Ocean groups which were separated according to the sequences of DNA-R, DNA-N, and DNA-S. They were considered as more stable components than the other DNAs (King et al. 2011). A previous study described that BBTV isolates of Indonesia were the members of Asia/South East Asia (SEA) group (Chiaki et al. 2015). All isolates indicated above 90% similarity with the SEA isolates based on the DNA-R that encoded replication protein (Fig. 3).

In this paper, BBTV isolates were also clustered by the DNA-S that encodes coat protein (Wanitchakorn et al. 1997), DNA-N which encodes nuclear shuttle protein (Wanitchakorn et al. 2000) and DNA-C encodes a cell-cycle link protein (Clink) that associates with the cell cycle and organize viral DNA synthesis in the host cells (Yu et al. 2019). Figure 4 configures the cluster of Indonesia's BBTV according to the DNA-S, and the group was build based on the coat protein DNA sequence. BBTV from Bali MT433370 showed a high similarity to the previous sample studied Bali5 (Pinili et al. 2011); therefore, they properly stand in the same group. Interestingly, Bali's isolate also shares common group to the previous isolate from Central Java IG3 (Furuya et al. 2004). The samples from Sumba island MT433373 and Timor island MT433372 in the East Nusa Tenggara province proved the similarity, and it put them together in one cluster. The sample originated from Bengkulu MT433366 in Sumatra island showed a high similarity to the previous research on BBTV from Sumatra KM607538.1, the isolate Q568. The isolates obtained from North Sulawesi province of Sulawesi island and North Maluku province in Halmahera island indicated a closer relationship to the KM607448.1 isolate 522B from the Philippines. Meanwhile, the isolate obtained from Papua MT433375 indicated a relationship to the BBTV isolate MT433371 from West Nusa Tenggara province (Fig. 4).

Comparison of phylogenetic tree showed a small variation cluster composition according to DNA-R, DNA-S, DNA-N, and DNA-C (Figs. 3, 4, 5, and 6)

The isolate from Papua MT433362 and Bali's isolate (MT433358) were relatively close to the Indonesian isolate of Q568 (KM607389). The isolate of North Sulawesi MT433353 and the Philippines isolate 768 KM607323.1 built one cluster and showed a close relationship. The BBTV of East Nusa Tenggara province in Sumba island (MT433361) and another isolate from Bengkulu province in Sumatra island (MT 433354) were in the same cluster. However, another isolate from the same province but different island of Timor (MT 433360) built one cluster. Meanwhile, BBTV from East Java province of Java island (MT 433356) and the isolate from Sumbawa island in West Nusa Tenggara province (MT 433359) were in one cluster and showed a relationship to the two isolates mentioned previously, MT433361 and MT 433360.

Figure 6 represents the relationship and grouping of Indonesia's BBTV isolates according to the DNA-C. Timor's isolate (MT433348) remains separated from the other isolate from the same province East Nusa Tenggara but different island, Sumba (MT433349). However, the isolate from

Table 1 Characteristics of BBTV isolates based on DNA-R, DNA-S, DNA-N and DNA-C

Island	Sample	Location		Cultivar	GenBank Accession Number			
		Latitude	Longitude		DNA-R	DNA-S	DNA-N	DNA-C
Sumatra	GM_109001	3.24'21,64"LS	102.38'31,69"	Ambon Kuning	MK940788	MT433366	MT433354	MT433341
	GM_109003	3.29'32,98"LS	102.34'29,13"	subsp, sumatrana	MK940789	MT433367	MT433355	MT433342
	GM_103020	0.31'18,00"LS	100.33'43,00"	pisang lidi/lilin	MN073184			
	GM_103047	1.40'33,00"LS	101.19'57,00"	subsp, sumatrana_2	MN073185			
	GM_110010	5.22'27,83"LS	105.14'15,37"	Muli	MN055481			
	GM_110008	5.23'0,17"LS	105.13'35,74"	Muli	MN055482			
	GM_110004	5.25'29,79"LS	105.13'23,51"	Raja bulu	MN073183			
	GM_110007	5.22'14,78"LS	105.14"17,41"	Raja	MN067518			
Java	GM_214020	7.21'29,45"LS	110.12'31,428"	Ambon	MK805529			
	GM_216023	7.45'15,36"LS	112.31'28,13"	Abaca	MN017711	MT433369	MT433357	MT433345
	GM_212004	6.50'13,428"LS	107.35'2,844"	Awak	MN037878			
	GM_216016	8.1'31,5"LS	112.48'0,24"	Mas	MN037879	MT433368	MT433356	MT433343
	GM_214019	7.27'35,47"LS	110.13'10,64"	Raja	MN055476			
	GM_216024	7.24'54.72"LS	109.15'14.50"	Kepok		MT433376	MT433363	MT433351
Bali	GM_317006	8.29'33,71"LS	115.23'30,91"	Awak	MN067519	MT433370	MT433358	MT433346
Lombok	GM_418004	8.19'3,69"LS	116.20'26,28"	Ketip	MN037876			
	GM_418002	8.19'28,13"LS	116.8'17,27064"	Ketip	MN037877	MT433371	MT433359	MT433347
Timor	GM_519006	9.37'23.00"S	119.23'48.00"E	Mas	MN037874			
	GM_519005	10.11'35"LS	123.35'40"	Raja	MN037875	MT433372	MT433360	MT433348
Sumba	GM_619001	9.37'23"LS	119.23'48"	Mas	MN037873	MT433373	MT433361	MT433349
Sulawesi	GM_72008	1.26'10,69"LS	124.50'42,42"	Abaca	MN017712	MT433365	MT433352	MT433340
	GM_72007	1.26'10,70"LS	124.50'42,42"	Gapi	MN017713			
	GM_72006	1.26'10,71"LS	124.50'42,42"	Goroho	MN017714	MT433364	MT433353	MT433339
	GM_72005	1.26'10,72"LS	124.50'42,42"	Mas	MN017715			
Halmahera	GM_827017	1.50'31,628"LS	127.48'12,163"	Sangate	MN055478			
	GM_827009	1.48'0,6"LS	127.54'7,2"	Mulu bebek	MN055479	MT433374		
	GM_827008	1.46'31,04"LS	127.56'34,663"	Goroho	MN055480			
	GM_827016	1.50'31,628"LS	127.48'12,163"	Sepatu putih/Gohu	MN089582			
Papua	GM_1129006	2.37'15,606"LS	140.41'23,66"	Musa sp.	MN037872	MT433375	MT433362	MT433350

Sumba was in one cluster with the isolate from Sumbawa in province West Nusa Tenggara. The isolate from North Sulawesi MT433339 was in the same cluster with that from the Philippines isolate MS 15 (KM607083).

Alternative host

The wild banana sub species sumatrana (*M. acuminata* subsp. *sumatrana*) found in Bengkulu and West Sumatra generated the symptoms of bunchy top disease, such as bunchy and yellowing leaves, as well as *M. velutina* in Yogyakarta expressed the typical symptoms of bunchy top disease (Fig. 7). In West Sumatra, it was noted that aphid colonized turmeric which was co-planted with banana (Fig. 8). PCR detection showed that the turmeric plants were infected by BBTV (Fig. 9). The search for alternate hosts was done using plant species that are frequently co-planted with banana such as ginger, turmeric, and aromatic ginger. These plants exhibited chlorosis and confirmed positive to BBTV through PCR (Fig. 9) with the interval between incubation periods to the first detection was 10, 14, and 30 days after inoculation, respectively. Also, the infected plants showed the symptoms of chlorosis (Fig. 10). Canna, canna lily, and false bird of paradise plants did not show any symptoms and no virus was detected up to 30 days after inoculation. It is suggested that zingiberaceaus plants (ginger, turmeric, and aromatic ginger)

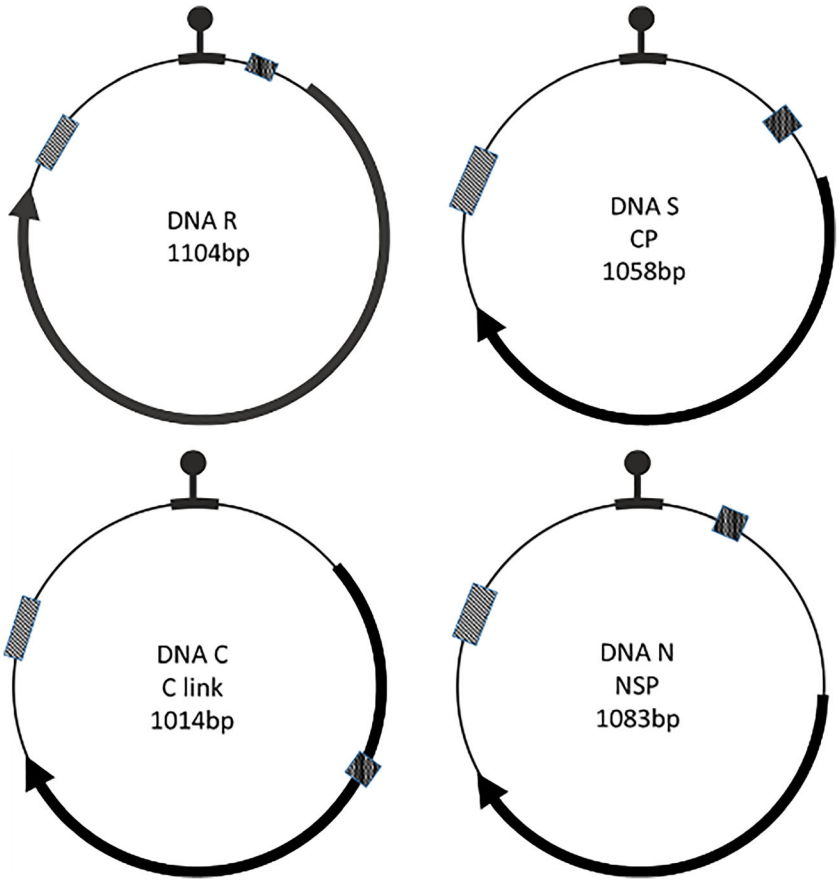


Fig. 2 Genomic organization of four DNA components of BBTV Mas cultivar isolate showing CR-SL, CR-M, TATA Box, and ORF

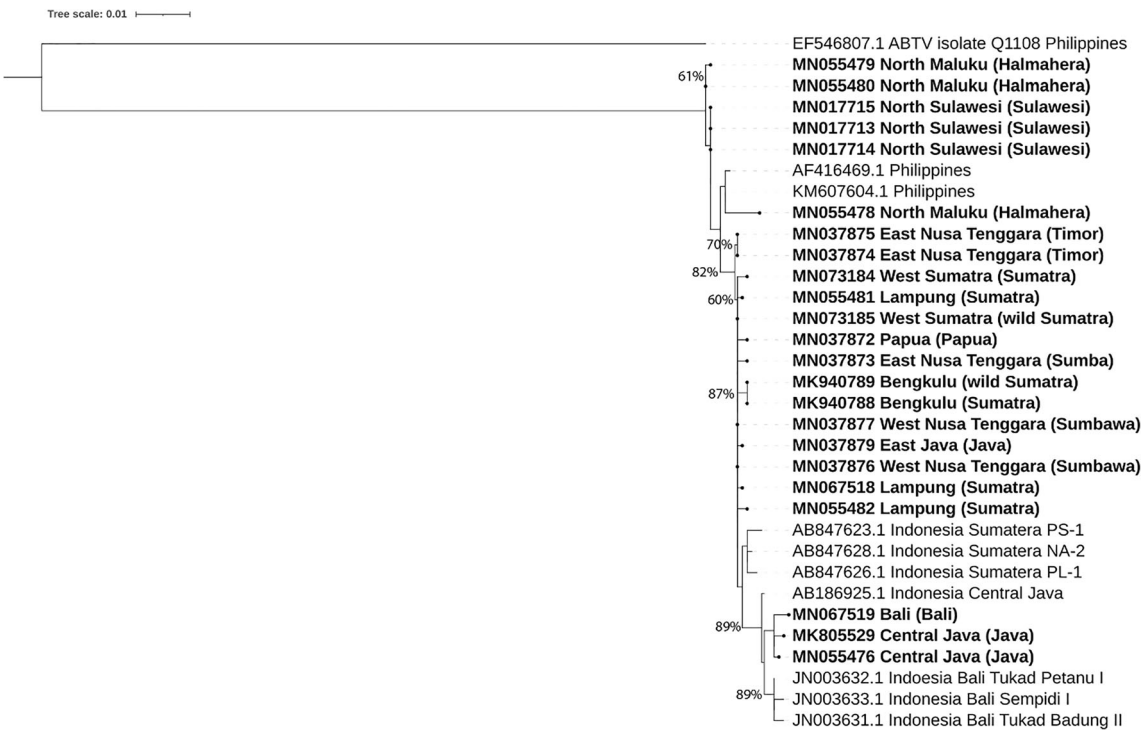


Fig. 3 Phylogenetic analysis based on nucleotide sequence of DNA-R component. The evolutionary history was inferred by using the maximum likelihood method and TN93-parameter model with 1000 bootstrap replications

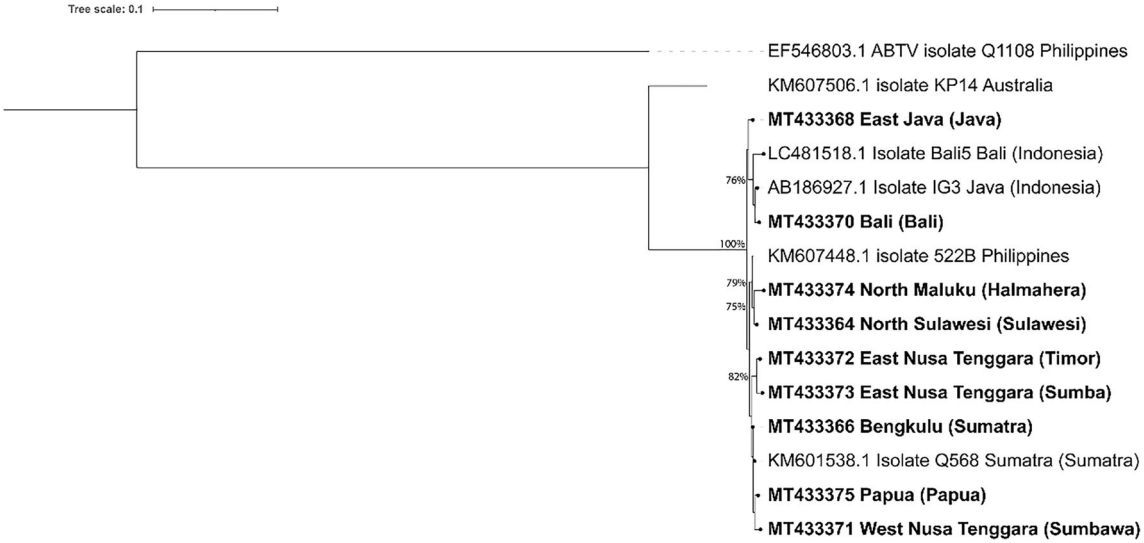


Fig. 4 Phylogenetic analysis based on nucleotide sequence of DNA-S component. The trees were constructed using the maximum likelihood method and TN93-parameter model with 1000 bootstrap replication

were more susceptible than Canaceae (canna and canna lily) and Heliconiaceae (false bird paradise). The genetic relationship based on the DNR-R sequences among BBTV isolates obtained from non-cultivated bananas, including those from *M. acuminata* subsp. sumatrana, *M. velutina*, abaca, and tumeric is presented in Fig. 11.

BBTV is transmitted and dispersed by *P. nigronervosa*. In addition to staying on banana plant, the aphid also completed a life cycle and transmitted the virus onto non-banana plants. In Sumba, colocasia plant was found to be an alternative host for *P. nigronervosa*. The artificial inoculation of BBTV banana cv Mas isolate on *Zingiber officinale*, *C. longa*, and *Kaempferia galanga* resulted in the chlorosis symptom which was confirmed by the positive results of PCR analysis.

Figure 11 suggests that the BBTV of wild banana *M. acuminata* subsp. *sumatrana* MK940789 obtained from Bengkulu is highly similar to that of cultivated banana from Bengkulu MK940788 and stands in the same cluster. Furthermore, the other BBTV isolate of *M. acuminata* subsp. *sumatrana*, MN073185, from West Sumatra province has a farther distance relationship to those isolates from Bengkulu; however, they are in one group. The isolate of BBTV obtained from Tumeric GM 103053 showed similarity to that of the cultivated banana in the same area in West Sumatra MN073184.

On the other hand, the BBTV abaca from Yogyakarta MN017711 is closely related to that of the cultivated banana isolate from Central Java MK805529 and similar to that of *M. velutina* from Yogyakarta. Furthermore, the BBTV isolate of abaca from North Sulawesi MN017712 is closely related to that of cultivated banana from the same area in North Sulawesi province, MN017713, and has a farther distance relationship with that of abaca isolate from Yogyakarta in Java.

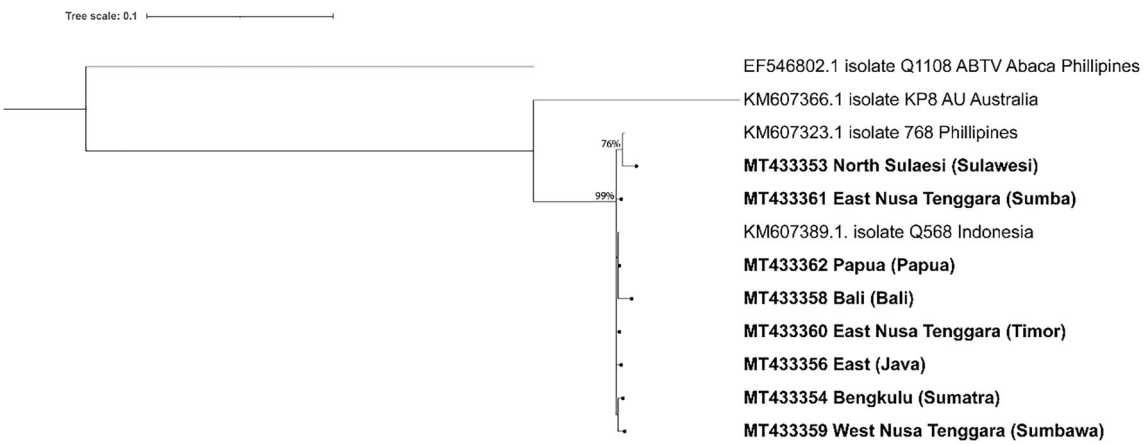


Fig. 5 Phylogenetic analysis based on nucleotide sequence of DNA-N component. The evolutionary history was inferred by using the maximum likelihood method and TN93-parameter model. With 1000 bootstrap replications

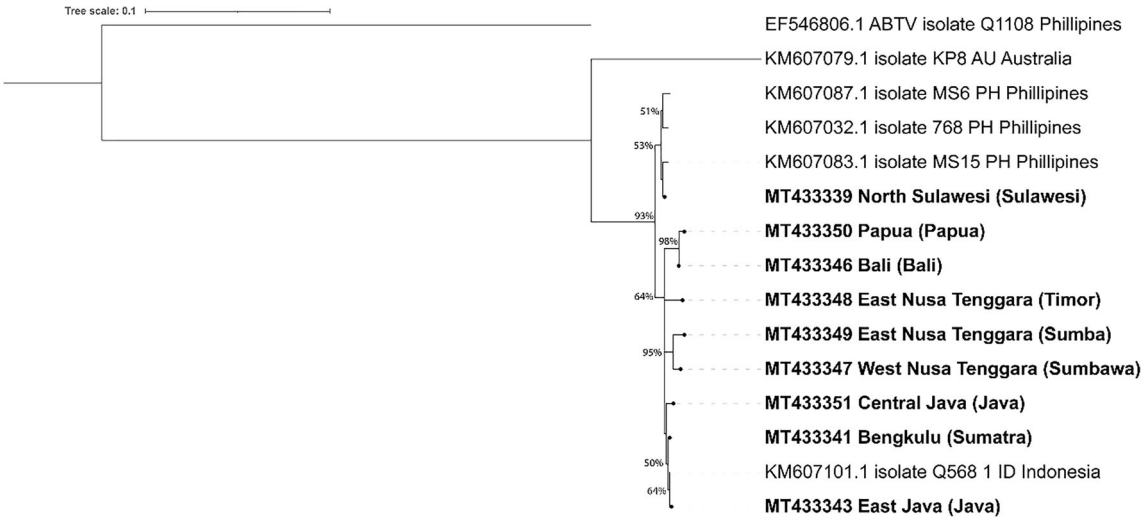


Fig. 6 Phylogenetic analysis based on nucleotide sequence of DNA-C component. The evolutionary history was inferred by using the maximum likelihood method and TN93-parameter model. With 1000 bootstrap replication

Discussion

BBTV is a major limiting factor for banana production in Indonesia. The virus is persistently transmitted by the aphid vector *P. nigronervosa*, and from the infected mother plants to their suckers or through tissue culture. Even if virus-free bananas are produced through tissue culture (Tchatchambe et al. 2020), reinfection in the nurseries can occur. In Indonesia, propagation from suckers is a common practice, and these suckers can be infected with BBTV especially when the mother plants are planted in an open area or in the field.

The movement of banana planting materials without any pest or disease monitoring into the new area, furthermore when suckers or plantlets are transported long distance through ground transportation, aphid infestation may occur during the transportation period leading to aphid propagation and virus transmission on the planting materials.

BBTV distribution in Sumatra, Java, Kalimantan, Sulawesi, Bali, and West Nusa Tenggara was reported (Nurhadi and Setyobudi 2000; Hermanto 2011). However, in 2012 it was reported that Kalimantan was not included in the BBTV distribution data in the previous year (Hermanto et al. 2012). Meanwhile, Chiaki et al. (2015) confirmed the BBTV was spread in Sumatra island and they identified the genetic structure and its diversity based on DNA-R, DNA-S, and DNA-U3. Irwansyah and Akhsan (2019) reported on the identification and severity of BBTV in Kutai, East Kalimantan. The present research provides new information on the distribution of bunchy top disease in Indonesia with the confirmation through molecular detection. It is recognized that bunchy top disease has been distributed through almost all islands and provinces in Indonesia. The symptomatic plants are found more frequently in the islands of Sumatra and Java. Plantlets from the nurseries in Java or Sumatra

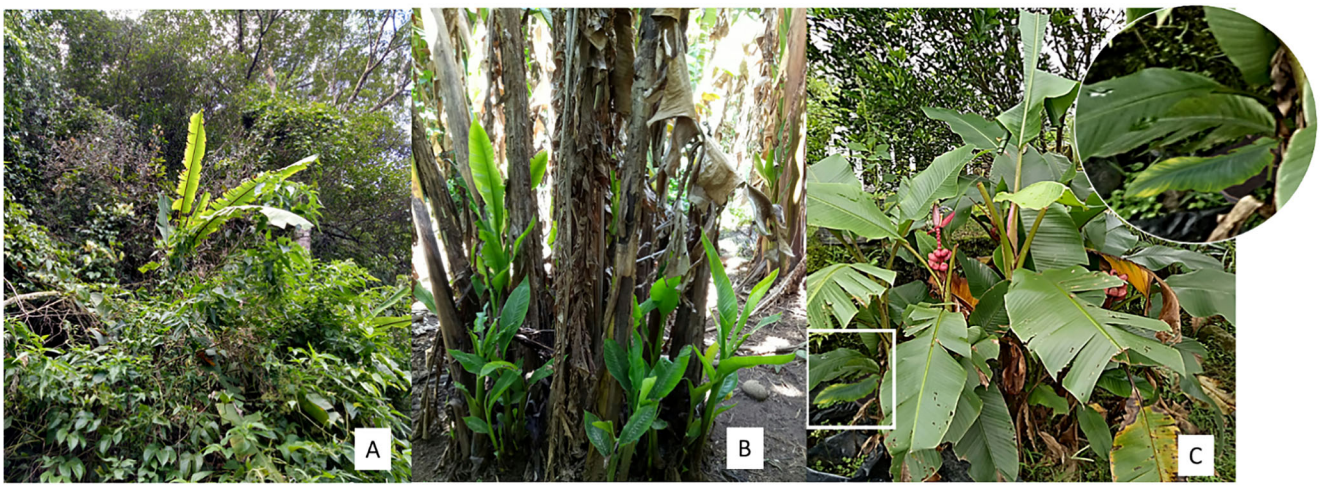


Fig. 7 Natural infection of BBTV on Musa group. **A** Wild banana, **B** abaca, **C** pink banana

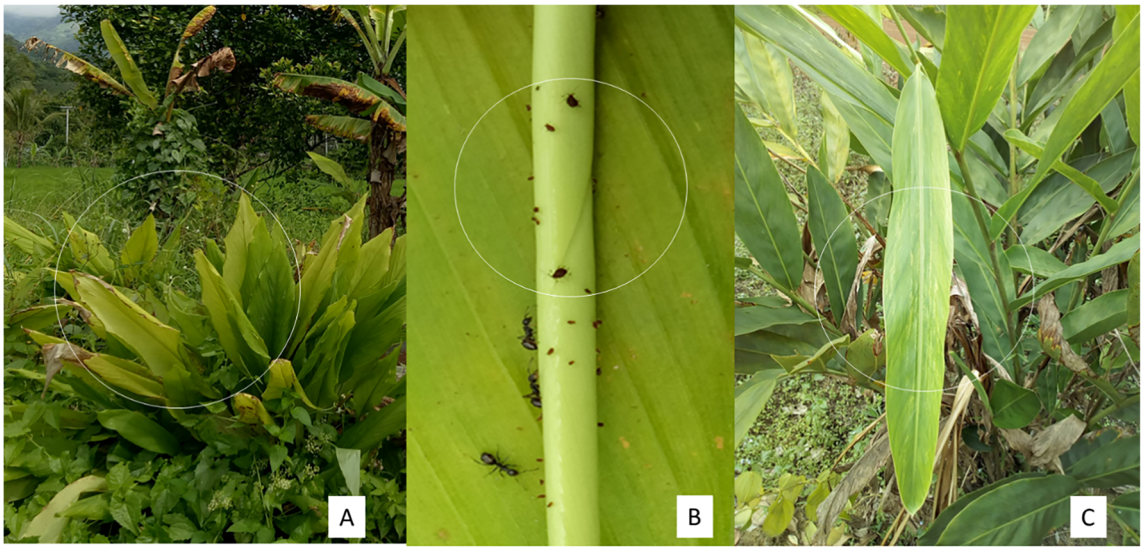


Fig. 8 Natural infection of BBTV on Zingiberaceae. **A** Turmeric in the same farm of banana plant with BBTV symptom. **B** Aphids on turmeric leaves. **C** BBTV symptom on galangal

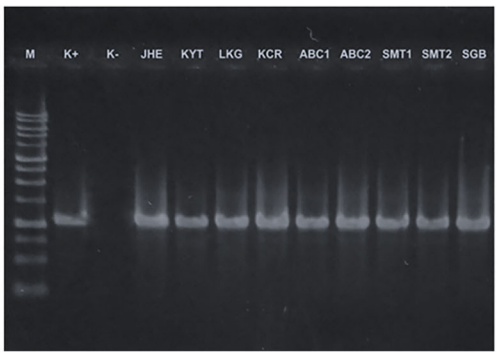
may cross the provinces and islands in Indonesia through trading or transmigration. The BBTV infection of *M. acuminata* subsp. *sumatrana* in the provinces of Bengkulu and West Sumatra was in the plants growing along the roads that connected to the cultivated banana farms, suggesting that BBTV could have been transmitted from wild banana to the cultivated ones or *vice versa*.

The surveys detected BBTV in all banana cultivars containing an A genome (AA, AAA, AAB, ABB), but not in cv. Klutuk (BB). Wild bananas of *M. acuminata* subsp. *sumatrana* (AA), *M. textilis* (abaca), and *M. velutina* (pink banana/sanggabuana) were naturally infected by similar BBTV isolates as those in nearby cultivated banana plants. Similar distinct and typical symptoms of bunchy top disease were present on all banana cultivars, including bunching leaves on the top of the plant, except the cv. Kepok (ABB) where significant changes in color or leaf dimension were non-existent.

The sequences from all isolates in this study were clustered in the SEA group in phylogenetic analyses. The isolates from Sumatra, Java, and Bali showed a high sequence similarity to the previously sequenced isolates from those islands (Furuya et al. 2004; Chiaki et al. 2015; Pinili et al. 2011).

The BBTV from Sumatra, Java, Bali, Lombok, Timor, Sumba, and Papua are highly similar. Meanwhile, the isolates of BBTV in the islands of Sulawesi and Halmahera are closely related to those of the Philippines BBTV. The genetic resemblance of BBTV in most of islands in Indonesia occurred due to human trans-movement, particularly from Sumatra and Java to other islands. The similar traits shared between BBTV in northern of Sulawesi, Halmahera islands, and Philippines suggest that human movement and trading activities across country borders were in place. The coastal proximity between islands and the population mobilization due to trade partly determines the distribution of the disease and the similar genetic traits on BBTV isolates.

Papua is quite far from Java, but the BBTV isolates found in both islands are highly similar, potentially due to seedling trans-movement from Java taken to Papua via transmigration. This may be due to exchange of infected banana planting materials between these places.



M = Marker 1000 bp
K+ = Positive control (infected banana cultivar Mas)
K- = Negative control (healthy banana cultivar Mas)
JHE= Ginger (*Z. officinale* artificial inoculation)
KYT= Turmeric (*C. longa* natural infection)
LKG= Galangal (*A. galanga* natural infection)
KCR= Kaemferia (*K. galanga* artificial inoculation)
ABC1= Abaca (*M textilis* natural infection) from Sleman
ABC2= Abaca (*M* textilis natural infection) from North Sulawesi
SMT1= sumatrana(*M acuminata* subsp *sumatrana* natural infection) from Bengkulu
SMT2= sumatrana (*M acuminata* subsp *sumatrana* natural infection) from West Sumatra
SGB= Pink banana (*M velutina* natural infection)

Fig. 9 Gel electrophoretic analysis on inoculated plant species showing expected band size amplified from DNA-R genome of banana bunchy top virus. Including the 1000-bp size marker, positive and negative control

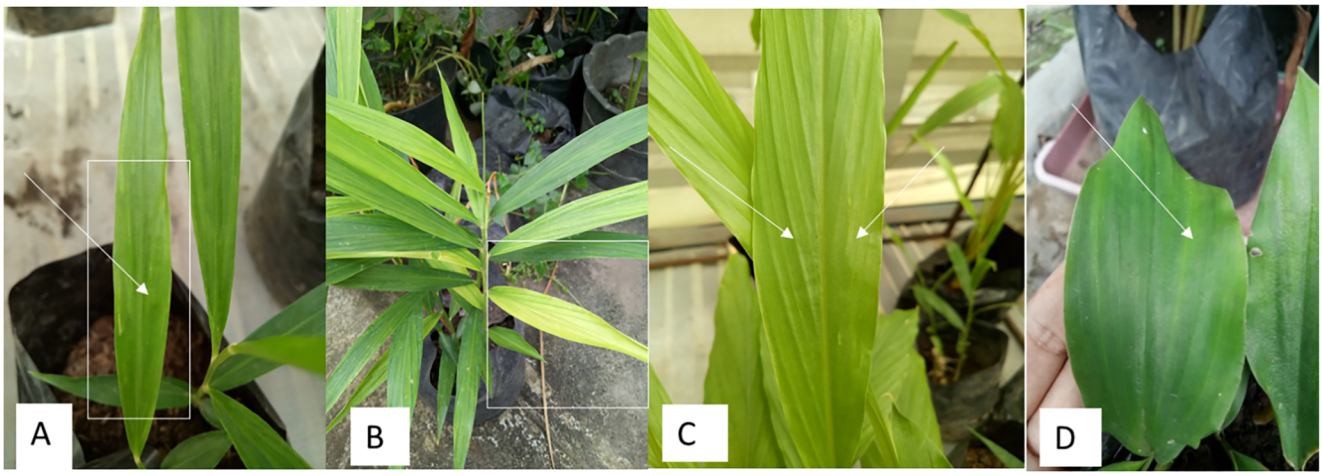


Fig. 10 Characteristic symptoms of BBTV infection: light green streak and yellowing on *Zingiber officinale* (**A**, **B**), *Curcuma longa* (**C**), and *Kaemferia galangal* (**D**)

Meanwhile, the non-existence of BBTV symptomatic banana plants in Manokwari in West Papua province or some in Merauke in Papua province suggests that the rainforest buffers the proximity from Jayapura that limits the spread of the disease through land transportation. Kalimatan region used to be free from banana bunchy top disease but recent occurrence on cultivated banana (Irwansyah and Akhsan 2019) and on wild banana (Sila et al. 2020) was reported.

Furthermore, BBTV was not found in Ambon nor Seram islands, Maluku province. Areas like Ambon where bunchy top disease had not been reported yet must be protected from disease incursion.

The prevalence of BBTV in Sumba island was a new introduction found on three infected banana plants in three different locations. Meanwhile, bunchy top disease in Timor island was documented in some locations with a higher disease incidence and intensity than that in Sumba. The distribution of BBTV in Sumba island was strongly suspected due to the trans-movement activity of human and seedlings from Timor to Sumba. It was supported by the close molecular relationship of BBTV from Timor and Sumba based on the DNA-R and DNA-S, and personal communications with the infected banana owners. Aphids as the vector of BBTV were also recorded in Sumba on both banana and non-banana plantations, either within or beyond the infected plant area. The prevalence of this vector indicated a potency for a wider distribution of bunchy top disease in Sumba.

The present results suggest that BBTV diversity in Indonesia is more related to the geographical origin of the isolates rather than that of the host plant species. The different isolates from *M. acuminata* subsp. *sumatrana* from Bengkulu and West Sumatra were not closely related, and the isolates of Abaca in Yogyakarta had some genetic distance to that of the same host plant species originated from North Sulawesi. On the other hand, a close relationship was found among isolates originated from the same locations, for example, the isolates of tumeric and banana from West Sumatra, the isolates of Abaca from Yogyakarta and banana from Central Java, and the isolates of Abaca from North Sulawesi and banana from the same province (Fig. 11).

Many studies on the alternative host plants for BBTV had been undertaken (Pinili et al. 2011; Hamim et al. 2017). The

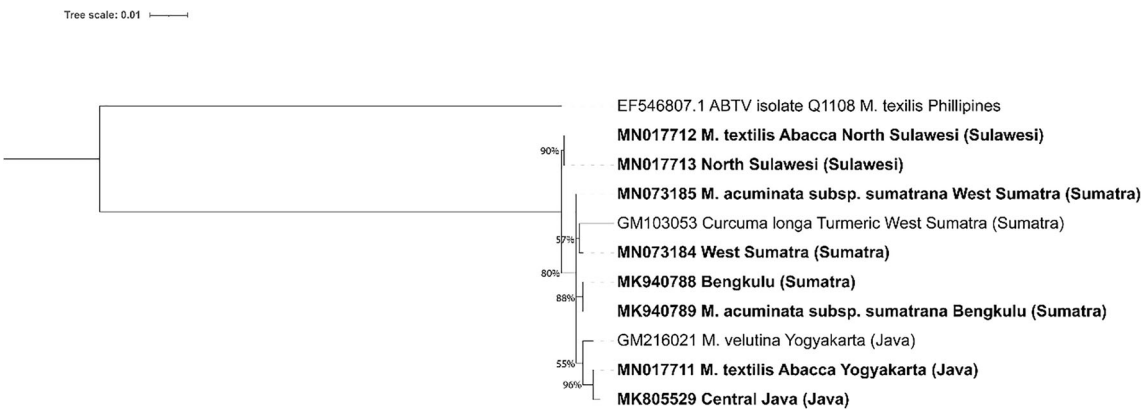


Fig. 11 Phylogenetic analysis of BBTV isolate from non-banana plants based on the nucleotides sequences of DNA-R. The evolutionary history was inferred by using the maximum likelihood method and TN93-parameter model. With 1000 bootstrap replication

present work shows that BBTV can infect and cause symptoms on ginger (*Zingiber oficinale* L.), turmeric (*Curcuma longa* L.), and aromatic ginger (*Kaempferia galanga* L.) that are intercropped with banana plants. The success of artificial transmission of BBTV on ginger, turmeric, and galangal plants showed that the aphids can feed sufficiently to transmit BBTV to the plants in Zingiberaceae.

In Sumba, *P nigronervosa* with morphological identification was found colonizing Colocasia plants as an alternative host. Banana aphid *P nigronervosa* could reproduce well on the Colocasia plants, *Caladium bicolor*, that were used for aphid mass rearing in the glass house as the vector for BBTV transmission studies. A previous study mentioned that ginger and canna were also good alternative hosts for the aphid (Watanabe et al. 2013). Alternative hosts play an important role in spreading bunchy top disease (Pinili et al. 2013). The ability of the banana aphid to live and propagate on alternative hosts needs to get more attention for better management of the disease. Plants in the Zingiberaceae family and Colocasia may have a vital role as the alternate hosts for both banana aphids and BBTV. A high vegetative propagation capability may include the risk factor in the dissemination of aphids and the virus. Further research on the expression genes related to viruliferous and non viruliferous of *P. nigronervosa* (Subandiyah et al. 2020) could be conducted on the interaction of the aphid BBTV vector related to different species of host plants. The capability of viruliferous aphids from zingiberaceae plants to infect the banana plant should be investigated further.

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Susamto Somowiyarjo: supervisor, conceptual
Siti Subandiyah : supervisor, conceptual, project leader at UGM, survey and sampling, data analysis, manuscript writing and reviewing
Ruly Kusuma: GIS analysis and distribution map
Ady Bayu Prakoso: sampling and bioinformatic analysis
Kathy Crew: laboratory advice and assistance
Megan Vance: laboratory advice and assistance
Jane Ray: sample collection assistance
John E. Thomas: conceptual, data analysis and manuscript writing and reviewing

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

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