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Banana Bunchy Top Virus Molecular Confirmation and DNA-S Phylogenetic of some Banana Isolates from Indonesia

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ABSTRACT

This study was aimed to confirms the Banana Bunchy Top Virus (BBTV) infection of ten banana accessions from Indonesia through PCR assay using primer of BBTV coat protein (CP) gene, also the sequences characteristics and phylogeny. Preliminary morphological results showed five acessions were positively infected with slight to severe intensity symptoms i.e. Pisang Berlin, Candi, Billa, Morosebo, and Mas Kripik; and five accessions were symptomless i.e. *M. acuminata* var. *rutilifes* and *M. balbisiana*, Pisang Ebung, Madu and Moseng. However, PCR results qualitatively confirms that all accessions were positively infected. Two sizes of amplicons were identified. Pisang Candi isolate was showing ±500 bp amplicons, whereas the others were ±1000-1100 bp. The total aligned and selected BBTV CP sequences of 36 accessions (bananas and other host species) was 450 nt. It was considered highly conserved (85.55%), low G+C content (41%), and high genetic similarities (92.47 to 100%). Phylogenetic analysis delineates isolates into two large groups, i.e. the Asian group (East Asia and South-east Asia) and the South Pacific group (South Asia, Africa and the Pacific). The findings of this study are usefull for broader applicability of further banana breeding program particularly for mitigation, selection and evaluation of banana with BBTV resistance.

1. Introduction

Bananas (*Musa* spp.) are tropical fruit which considered of globally important food and cash crops as they play roles in socio-economic, cultural and food security of millions people worldwide (Heslop-Harrison and Schwarzacher 2007; Hapsari and Lestari 2016; Hapsari *et al.* 2017). The total production of bananas reached not less than 100 million tons annually, with Cavendish (AAA) as the dominant commercial cultivar (FAO 2010). Nevertheless, banana bunchy top disease caused by banana bunchy top virus (BBTV) has become the most serious and destructive viral disease to threat the world's banana production. The BBTV infections resulted in stunted growth and infected plants rarely produce a fruit bunch with almost total in yield loss (Hooks *et al.* 2008). The virus is transmitted locally by black banana aphid (*Pentalonia nigronervosa* Coq.); it is not considered as soil-borne, thus unlikely to be spread via cutting tools. Hence, uncontrolled movement and use of infected planting material is responsible for much of the spread (Thomas 2008). Some alternate hosts need to be concerns as inoculum sources also identified include taro, gingers, *Canna, Anthurium,* arrowroot, etc. (Pinili *et al.* 2013).

Banana bunchy top virus (genus Babuvirus, family Nanoviridae) is a virus with isometric virions 18-20 nm in diameter, which occurs in the phloem tissues of banana leaf and incites symptoms such as leaf chlorosis, vein clearing, leaf atrophy and dwarfing (Wu and Su 1990). It has a multicomponent genome, consists of at least six components of circular single-stranded DNA, each of 1-1.1 kb at full length (Burns *et al.* 1995; Harding *et al.* 1993). The DNA components originally named DNA-1-6 and recently renamed DNA-R, -U3, -S, -M, -C and -N, respectively according

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to their putative functions (Vetten *et al.* 2005). All components (exception of DNA-R) are transcribed a single gene encode the replication-associated protein, coat protein, intercellular transport protein, retino-blastoma binding protein and nuclear shuttle protein (Wanitchakorn *et al.* 1997; Yu *et al.* 2012). Further, BBTV virions have a single coat protein of approximately 20 kDa (Thomas and Dietzgen 1991).

BBTV is endemic in many banana producing countries in Asia, the Pacific and Africa (Elayabalan *et al.* 2015; Stainton *et al.* 2015). In-depth molecular studies on evolution and global distribution of BBTV reveals evidences that BBTV likely originated in Southeast Asia then circulating elsewhere in the world. Further, their sequence analysis separated into two genetically distinct taxonomic groups of BBTV are known (Asian and South Pacific) though both have the same vector and produce the same disease symptoms in *Musa*. The Asian group contains isolates from China, Japan and South-east Asia countries, and the South Pacific group comprises isolates from the rest of the world (including Egypt, India, Sub Saharan Africa and Australia) (Furuya *et al.* 2004, 2006; Selvarajan *et al.* 2010; Stainton *et al.* 2015; Wickramaarachchi *et al.* 2016; Wanitchakorn *et al.* 2000; etc.).

There are wide variation in banana varieties response in terms of incidence and severity, but no variety is considered resistant (Ferreira *et al.* 1997). Some preliminary studies showed that dessert bananas are more susceptible than cooking bananas; and genotypes with not less than two A genomes are more susceptible, or genotypes with at least one or two B genomes tend to be more tolerant to BBTV (Hapsari and Masrum 2012; Niyongere *et al.* 2011). Symptoms development was affected by plant age and plant height. Incubation period for each cultivar varied so that the emergence of symptoms in each cultivar will vary as well (Ferreira *et al.* 1997; Hooks *et al.* 2008). Yet, BBTV can persist naturally in banana plants causing no apparent symptoms of the disease or the plants remain healthy-looking and symptomless. It can be act and serve as a reservoir, become a source of inoculums on the spread of BBTV. So far, no natural source of BBTV immunity is known (Thomas 2008), and nowadays is becoming a subject of researchers and horticulturists interests worldwide.

The control of BBTV is difficult, once the virus is established in an area. Rapid and complete eradication to the infected plants by uprooting all bananas in the fields or injecting with glyphosate then leave fallow for months is the primary manual method to control the disease (Lepoint *et al.* 2014). Thus, monitoring strategies and the capacity for early diseases identification and to deploy rapid eradication responses, also production of healthy planting materials are vital (Anandhi *et al.* 2007; Hapsari and Masrum 2012; Lepoint *et al.* 2014; Mahadev *et al.* 2013). Biosecurity controll with strict quarantine by government legislation in Australia and few countries proven to be effectively controlled the disease, although considered very resource-intensive (Cook *et al.* 2012).

The presence of BBTV in banana plants can be early detected through some methods such as Southern and dot-blot hybridizations (Xie and Hu 1995), serological test of Enzyme-Linked Immunosorbent Assay/ELISA (Kadam *et al.* 2017), monoclonal antibody (Wu and Su 1990), and molecular tools with Polymerase Chain Reaction/PCR based detection using variable options of techniques and markers (Burns *et al.* 1995; Harding *et al.* 1993; Hafner *et al.* 1995; Priani *et al.* 2010; Stainton *et al.* 2015; etc.). The serologial tests are convenient but expensive and the use of this technique is limited in detection sensitivity with very low concentrations of BBTV. Whilst, PCR based detection is considered more sensitive, easy, economic and rapid for indexing propagules also to identify various BBTV strains (Abdelkader *et al.* 2004; Anandhi *et al.* 2007; Xie and Hu 1995).

This study was aimed to confirms the BBTV infection of some symptomatic and symptomless banana accessions from Indonesia through PCR based detection using specific primers of banana bunchy top virus gene for coat protein (CP). The CP encoded by the DNA-S component of the BBTV has been used for virus detection because of its important role in viral particles packing and disease establishment and has been consistently associated with BBTV in Asian countries group (Arumugam *et al.* 2017). In addition, the viral coat protein sequences also analyzed and aligned with those already reported in Genbank for phylogenetic analysis, particularly isolates from Asian countries. The banana accessions were selected from previous preliminary study (Hapsari and Masrum 2012) also from the new collections for screening purpose. The findings of this study hopefully will be usefull for broader applicability of further banana breeding program particularly for mitigation, selection and evaluation of banana with BBTV resistance.

2. Materials and Methods

2.1. Plant Materials and BBTV Symptoms Morphological Observation

Plant materials used in this study was ten selected banana accessions collection of Purwodadi Botanic Garden, National Research and Innovation Agency (BRIN) which located in a low land-dry area of Pasuruan District, East Java, Indonesia. It comprised of two wild banana species i.e. *M. acuminata* var. *rutilifes* (AAw) and *M. balbisiana* (BBw); and eight banana cultivars representing four genomic groups AAcv, AAA, AAB and ABB (Table 1). The type and severity symptoms were recorded visually according to previous preliminary research (Hapsari and Masrum 2012), as follows: (0) Negative, indicated by healthy looking plant with no visual symptoms of BBTV; (1) Slight BBTV infected, indicated by slight marginal chlorosis and leaf narrowing, with 1 to 2 infected plants per cluster; (2) Moderate BBTV infected, indicated by moderate marginal chlorosis, leaf narrowing, twisting, distorting and stunting, with 3 to 4 infected plants per cluster; and (3) Severe BBTV infected, indicated by severe marginal chlorosis, leaf narrowing, twisting, distorting, stunting and necrosis, with all or more than 4 infected plants per cluster.

2.2. Molecular Confirmation for BBTV Resistance

Molecular confirmation was conducted by PCR assay using a primer pair of banana bunchy top virus gene for coat protein (BBTV CP). Molecular analysis was done at Plant Physiology Laboratory of Biology Department, University of Brawijaya, Malang, Indonesia. Whole genome DNA was isolated from fresh young leaf sample, one individual per banana accession number. The leaf sample was taken during the morphological observation. The DNA extraction was conducted using Promega Wizard® Genomic DNA Purification Kit (Madison, WI, USA) following the manufacturer's protocols for plant.

PCR amplification was accomplished using oligonucleotide pair of BTVCPF (5'-GCTAGGTATCCG AAGAAATC-3') and BTVCPR (5'-TCAAACATGATA TGTAATTC-3') referred from previous studies by (Burns *et al.* 1995; El-Dougdoug and El-Shamy 2011; Harding *et al.* 1993; Hazaa *et al.* 2006; Kavino *et al.* 2011). PCR reactions were conducted in a 30 µl volume contains of 15 µl of DreamTaq Green PCR Master Mix (2x) from Thermo Scientific, California, USA (Taq DNA polymerase, 2x DreamTaq Green buffer, 0.4 mM each of dNTPs and 4 mM $MgCl_2$), 3 µl of 5 pmol each of forward and reverse primers, 3 µl of DNA template (5-25 µg/ml) and 6 µl of nuclease-free water. PCR thermal cycling program consists of heat shock temperature at 94°C for 3 minutes; followed by 30 cycles of denaturation for 45 seconds at 94°C, annealing for 45 seconds at 50°C, and extension for 2 minutes at 72°C. Final extension carried out for 10 minutes at 72°C.

Qualitative confirmation was conducted through electrophoresis separation on 1.5% agarose gel, stained with 1 µg/ml of Ethidium bromide in TBE buffer, then visualised under UV light. Generuller 100 bp DNA ladder (Thermo Scientific, California, USA) were used to estimate the sizes of amplified DNA fragments. Banana sample which indicated by the presence of amplified band on agarose gel was

Table 1. Banana accessions identity and BBTV morphological symptoms observation result

Coll. number	Cultivar name/species	Locality	Genomic group	Consumption type	BBTV symptoms
XXIV.D.19	Pisang Berlin	Purwosari, Pasuruan, East Java	AAcv	Dessert	Severe
XXIV.E.75	Pisang Candi	Pasrepan, Pasuruan, East Java	AAB	Cooking	Severe
XXIV.D.42	Pisang Billa	Batu Lanteh, Sumbawa, West Nusa Tenggara	AAA	Dessert	Moderate
XXIV.D.7	Pisang Morosebo	Sarangan, Madiun, East Java	AAA	Dessert	Severe
XXIV.E.100	Pisang Mas Kripik	Senduro, Lumajang, East Java	AAcv	Dessert	Slight
XXIV.D.17	Pisang Ebung	Siman, Ponorogo, East Java	ABB	Cooking	Negative
XXIV.D.12	Pisang Cici/*Musa acuminata* var. *rutilifes*	Krawak, Tuban, East Java	AAw	Seeded, not for comsuption	Negative
XXIV.D.1	Pisang Klutuk Ijo/*Musa balbisiana*	Purwosari, Pasuruan, East Java	BBw	Seeded, not for comsuption	Negative
XXIV.D.106	Pisang Madu	Batuputih, Sumenep, Madura Island, East Java	AAA	Dessert	Negative
XXIV.D.103	Pisang Moseng	Ketapang, Sampang, Madura Island, East Java	AAA	Dessert	Negative

considered as positively infected by BBTV, while the absent of amplified band was considered negatively infected. Further, to identify the taxonomic position of BBTV CP gene sequences of each isolates and for phylogentic study, the amplified products were then gel extracted and purified prior to direct sequencing at 1st BASE Laboratories Sdn Bhd, Malaysia using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA.

2.3. DNA Sequences Analysis

The BBTV CP gene sequences results were then evaluated using ABI sequences Scanner v.10. Basic Local Alignment Search Tool (BLASTn) program in National Center for Biotechnology Information (NCBI) GenBank was employed to search the potential references of homologue sequences, then they were retrieved for further analysis (Table 2). Multiple sequences alignments were conducted using ClustalW program followed by visual adjustment at MEGA6.06 software. Phylogenetic analyses were performed based on evolution model of Kimura 2 parameter (K2P) using Neighbor Joining (NJ) algorithm with 1000 bootstrap replications. Pairwise distance analysis was also carried out to generate genetic similarities.

3. Results

3.1. BBTV Symptoms Morphological Observation

Results of BBTV morphological observation on banana accessions examined showed five out ten accessions were positively infected with varied severity levels from slight, moderate to severe; comprised of Pisang Berlin (AA), Candi (AAB/AAA),

Table 2. List of accession numbers of BBTV DNA for coat protein of this study and from GenBank

Accession number	Locality	Region	Host	Size (nt)
MK905697 (This study)	Indonesia: East Java	South East Asia	*Musa* cv. Candi (AAB/AAA)	450 1058
AB848106	Indonesia: Sumatra island	South East Asia	*Musa* cv.	1058
AB186927	Indonesia: Central Java	South East Asia	*Musa* cv.	1058
KM607538	Indonesia	South East Asia	*Musa* cv. Cavendish (AAA)	1058
KM607522	Philippines: Los Banos	South East Asia	*Musa* cv. FHIA (AAA)	1058
KM607523	Philippines: Los Banos	South East Asia	*Musa* cv. Lakatan (AA)	1058
AB250958	Philippines: Negros island	South East Asia	*Musa* cv.	513
FJ787433	Philippines: Laguna	South East Asia	*M. textilis* (ABTV)	513
FJ787435	Philippines: Quezon	South East Asia	*M. textilis* (ABTV)	1076
AB113661	Vietnam: Hanoi	South East Asia	*Musa* cv.	1076
AB113662	Vietnam: North of Hanoi	South East Asia	*Musa* cv.	1077
MF039874	Thailand: Chaiyaphum	South East Asia	*Musa* cv.	1080
KY427064	Thailand: Nongkhai	South East Asia	*Musa* cv.	1058
KM607541	Taiwan	East Asia	*Musa* cv. (severe symptom)	1058
KM607465	Taiwan	East Asia	*Musa* cv. (symptomless)	533
AB078023	Japan: Okinawa, Arakawa	East Asia	*Musa* cv.	1058
AB108450	Japan: Okinawa, Kume Island	East Asia	*Musa* cv.	513
MF688998	China: Yunnan	East Asia	*M. acuminata*	513
AF330706	China: Zhangzou	East Asia	*Musa* cv.	513
AF246122	China: Guangzhou	East Asia	*Musa* cv.	1058
KX779467	China: Sanya, Hainan	East Asia	*Musa* cv.	1058
KX779469	China: Nanning, Guangxi	East Asia	*Musa* cv.	1075
KM607534	China	East Asia	*Musa* cv.	1075
KM607536	China	East Asia	*Musa* cv.	513
EU190965	India: Assam	South Asia/Indian subcontinent	Wild *Musa* sp.	513
KF246091	India: Chennai	South Asia/Indian subcontinent	*Musa* cv. Karpooravalli ABB	513
JX171699	India: Coimbatore	South Asia/Indian subcontinent	*Ensete superbum*	528
GU085262	India: Tamil Nadu	South Asia/Indian subcontinent	*Musa* cv. Virupakshi (AAB)	1075
FJ859738	Pakistan: Sindh	South Asia/Indian subcontinent	*M. acuminata*	1075
KM607512	Srilanka	South Asia/Indian subcontinent	*Musa* cv. Mysore (AAB)	513
KT22018	Egypt: Cairo	North Africa	*Musa* cv.	474
JN204226	Burundi: Nyanza-Lac	East Africa	*Musa* cv.	474
JN204232	Rwanda: Rumbumba	East Africa	*Musa* cv.	474
JN204237	DR Congo: Ruvyimvyi	Central Africa	*Musa* cv.	1075
KM607514	Hawaii	Pacific Islands	*Musa* cv. Williams (AAA)	401
KY322774	Hawaii	Pacific Islands	*Heliconia* sp.	

Billa (AAA), Morosebo (AAA) and Mas Kripik (AA); and the others were negative (Table 1, Figure 1A-E). Meanwhile, Pisang Ebung (ABB) was apparently healthy with no visual symptoms and may produced normal fruit bunch (Figure 1F). Both wild species *M. acuminata* var. *rutilifes* and *M. balbisiana* in this study were also considered negatively infected (Figure 1G-H). Pisang Madu (Figure 1I) and Moseng (Figure 1J) were considered as new field collections in Purwodadi collected from Lumajang and Madura Island, therefore during the observation showing slight infection and healthy looking plant with no visual symptoms, respectively. Yet, according their genomic group those cultivars were supposed to be susceptible to BBTV.

3.2. BBTV Detection Based on PCR Confirmation

The PCR assay was conducted to all ten isolates of banana accessions examined, with the BBTV symptomatic accessions served as positive control. Surprisingly, the PCR was successfullly amplified to all isolates both BBTV symptomatic and symptomless as indicated by the presence of BBTV CP fragments on agarose gel (Figure 2). PCR result showing two different sizes of amplicons both partial and full length of DNA-S (Figure 2). An isolate of Pisang Candi (lane 2) was showing amplicons at approximately ±500 bp, whereas the others were ±1000–1100 bp. The repeated PCR with the same condition also showing consistant amplicons result.

3.3. Evaluation Results of BBTV CP Sequences

Unfortunately, the sequencing of nine isolates with full length amplicons (±1,000-1,100 bp) include Pisang Berlin, Billa, Morosebo, Mas Kripik, Ebung, Cici, Klutuk Ijo, Madu, and Moseng have resulted DNA sequences with very bad trace score (≤15), low signal strength and very short contiguous read length ranged 0-34 nucleotides (nt). The total sequences length were varied 875 to 1120 (nt). The identity search of the sequences using NCBI BLASTn tools showed that some accessions were 9 to 66% homologued with *M. acuminata* or *M. balbisiana* genome DNA bacterial artificial chromosome (BAC) clone, instead of BBTV CP DNA-S sequences as the target. Therefore, those nine isolates were failed for further analysis. Meanwhile, sequencing of Pisang Candi amplicon has resulted DNA sequences in a total length of 484 nt, with high trace score (46) and signal strength. Further, the NCBI BLASTn homology search was showed similarity (99%) to the reported BBTV CP gene sequences from around the world isolates. Hence, only the BBTV sequences of Pisang Candi (to 450 nt medium contiguous read length) will be proceed for

Figure 1. The BBTV symptoms of ten banana accessions: (A) Pisang Berlin, (B) Pisang Candi, (C) Pisang Billa, (D) Pisang Morosebo, (E) Pisang Mas Kripik, (F) Pisang Ebung, (G) *M. acuminata* var. *rutilifes*, (H) *M. balbisiana*/Klutuk Ijo, (I) Pisang Madu, and (J) Pisang Moseng

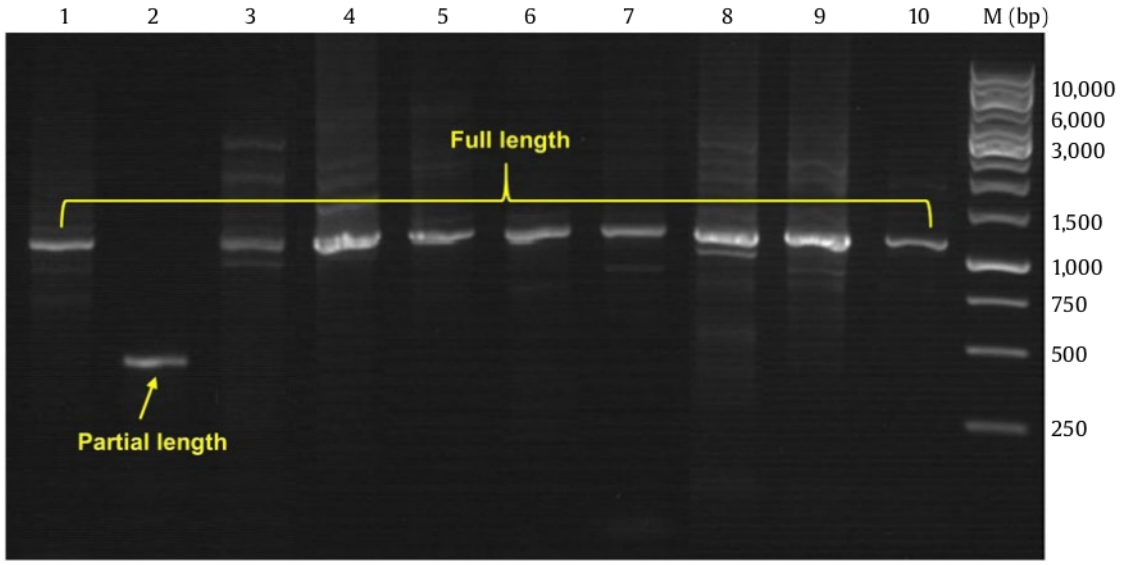

Figure 2. Electrophoregram BBTV molecular confirmation of ten banana accessions: (1) Pisang Berlin, (2) Pisang Candi, (3) Pisang Billa, (4) Pisang Morosebo, (5) Pisang Mas Kripik, (6) Pisang Ebung, (7) Pisang Cici/*M. acuminata* var. *rutilifes*, (8) Pisang Klutuk Ijo/*M. balbisiana*, (9) Pisang Madu, and (10) Pisang Moseng

further phylogenetic analysis along with other selected potential references of homologue sequences.

3.4. Characteristics of BBTV CP Sequences and Phylogenetic Analysis

About 35 accessions of homologues BBTV CP gene sequences from GenBank were retrieved comprises of isolates from Asia, Africa and the Pacific countries both at full length and partial sequences. Some different hosts species were also employed such as *M. textilis* (Abaca Bunchy Top Virus/ABTV), *Ensete superbum* (Enset Bunchy Top Virus/EBTV), and *Heliconia* sp. (Heliconia Bunchy Top Virus/HBTV) (Table 2). The total aligned and selected BBTV CP gene sequences length of 36 accessions was 450 nt. Of those, 385 positions (85.55%) were identified as conserved regions (invariable/monomorphic) and 65 positions (14.44%) were variable sites. Further, about 39 positions (8.67%) of the variable positions were potentially parsimony informative and 26 positions (5.78%) were singleton variables. The nucleotide composition were high in A+T bases (59%) and low in G+C bases (41%). Sequences pairwise distance analysis of all isolates showed 92.47 to 100% genetic similarities.

Phylogenetic analysis of CP gene sequences of BBTV including ABTV and other hosts in this study delineates isolates of the viruses into two large groups supported by strong bootstrap value, i.e. the Asian group (East Asia and South-east Asia) and the South Pacific group (South Asia/Indian subcontinent, Africa and the Pacific) (Figure 3). Further, the sequences analysis within Asian group was considered more diverse and less conserved than the South Pacific group. BBTV CP sequences of Asian group comprises of conserved region 409 nt, variable sites 41 nt, parsimony informative sites 24 nt, and singletone sites 17 nt; with 95.18 to 100% genetic similarity. Whilst, BBTV CP sequences of South Pacific group contains of conserved region 424 nt, variable sites 26 nt, parsimony informative sites 6 nt, and singletone sites 20 nt; with 96.46 to 100% genetic similarity. In particular, BBTV CP sequences analysis of Pisang Candi (East Java–Indonesia) isolate was clustered in Asian group, and very close related to the isolates from Central Java–Indonesia, Hainan–China, Arakawa–Japan, and Quezon–Phillipines (ABTV) with maximum similarity (99.71%). Pisang Candi isolate has the farthest relation to isolates from Tamil Nadu–India with a minimum similarity of 94.03%.

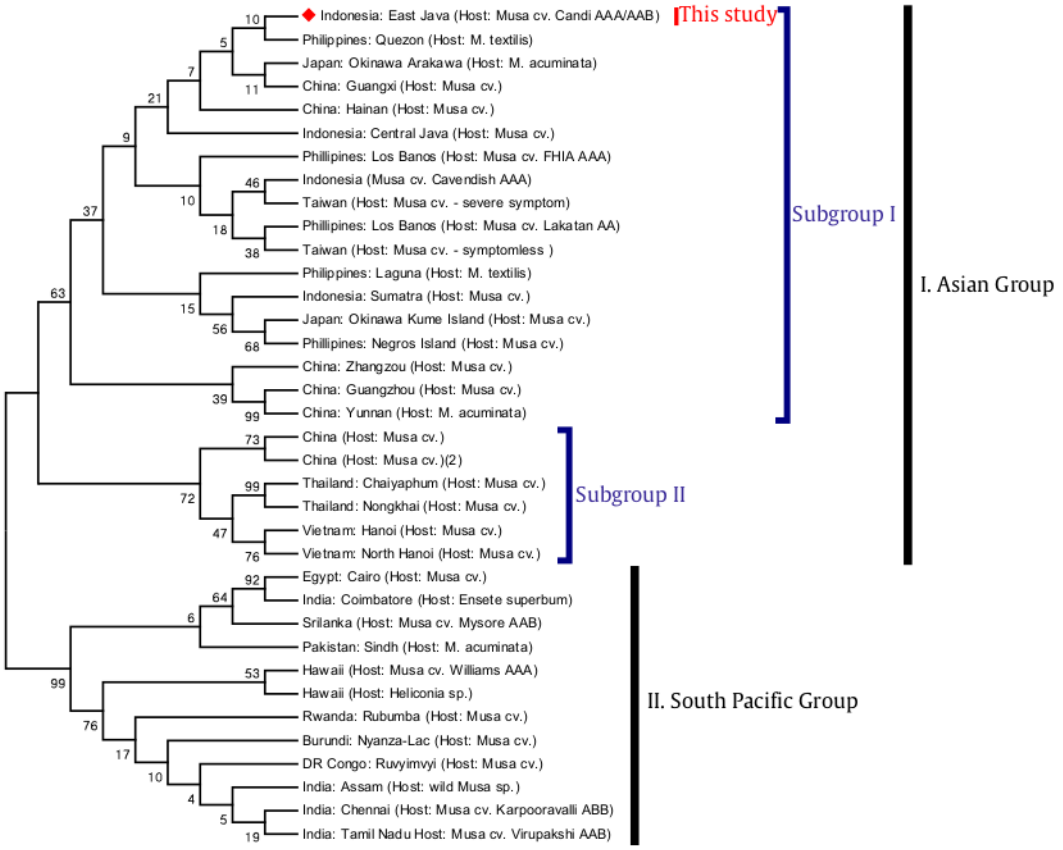

Figure 3. Bootstrap consensus phylogenetic tree using Neigbour Joining algorythm of BBTV isolates

4. Discussion

The morphologically infected accessions were mostly categorized as dessert bananas with genome composition of not less than two A genomes i.e. AA, AAA, and AAB. Unless, Pisang Candi which considered as plantain or cooking type. According to morphological approach, Pisang Candi was identified as AAB genome (Valmayor *et al.* 2000) whereas molecularly identified as AAA (Hapsari *et al.* 2015). This finding was in accordance to preliminary studies in which dessert bananas are more susceptible than cooking bananas; and genotypes with not less than two A genomes are more susceptible; or at least genotypes with one or two B genomes tend to be more tolerant to BBTV (Hapsari and Masrum 2012; Niyongere *et al.* 2011). The B-genome primarily contributes to the strength of the plant and tolerance of biotic and abiotic stress factors (Davey *et al.* 2013).

Banana wild relatives are valuable resources for future genetic improvement for many desirable traits includes disease resistancies (Wu *et al.* 2016). Some studies reported the potential resistancies of *M. acuminata* sub-species including *malaccensis*, and *burmannica/burmannicoides*, and *M. balbisiana* to fungal diseases (Chen *et al.* 2019; Fullerton 2002; Javed *et al.* 2004). *M. balbisiana* also considered to be more drought tolerant (Hapsari 2014; Vanhove *et al.* 2012). Morphologically, both wild species *M. acuminata* var. *rutilifes* and *M. balbisiana* in this study were considered negatively infected (Figure 1G-H). It may become promising source of BBTV resistancies, particularly the wild *M. balbisiana* and need further studies.

Based on the morphological symptoms on field, the BBTV isolates are classified into two types as S-type and R-type. The S-type or severe strain results in distinct bunched atrophy of leaves, dwarfness, no

yield and causes 90% of total destruction. The R-type or rough strain remains symptomless and does not affect yield (Das *et al.* 2007). Hence, BBTV isolates in severely infected banana accessions of Pisang Berlin (Figure 1A), Candi (Figure 1B), and Morosebo (Figure 1D) in this study were included as S-type, since they barely produce fruit bunch at all. Nonetheless; BBTV isolates in moderately, slightly and negatively infected in this study cannot be determined yet, need further observation and analysis. In addition, the BBTV severity symptoms was affected by plant age and plant height when BBTV infection occured also duration of the infection (Hooks *et al.* 2008). However, regular management practices such as eradication/prunning to infected clumps also may biased the negative result and the severity level.

The mophological symptoms induced by BBTV are similar to those caused by abiotic factors and other vascular diseases (Abdulkader *et al.* 2004). Since, Purwodadi is located at low land and dry area with limited water during dry season, the BBTV symptoms were sometimes complicated with the water deficiency symptoms such as stunting, rosetting, and a cessation of fruiting. Furthermore, selection of disease free planting material is important in *ex-situ* banana collection. Identification of BBTV infection in the planting material/suckers is difficult as the symptom development is not visible at the early stage of infection. Likewise, the symptomless accessions can be act and serve as a virus reservoir, become a source of inoculums on the spread of BBTV (Thomas *et al.* 2008). Hence, a reliable diagnosis of BBTV infections using molecular approach is important.

PCR results qualitatively confirms that the symptomless banana accessions were considered positively infected (or false negative) (Figure 2). Study by Stainton *et al.* (2015) also reported the presence of BBTV on symptomless bananas. The expected amplicon using the BTVCP primers in this study supposed to be approximately ±500 bp, however interestingly the PCR result showing two different size of amplicons both partial (±500 bp) and full length of DNA-S (±1,000–1,100 bp). The different sizes of amplicons produced presummable due to specific BBTV DNA-S characteristics of each cultivar. Some DNA-S BBTV CP primers available may amplified at full length or partial sequences. Previous studies by Abdulkader *et al.* (2004), El-Dougdoug and El-Shamy (2011), and Hazaa *et al.* 2006 to Egyptian banana isolates; Mahadev *et al.* (2013), Shilpa *et al.* (2016), Arumugam *et al.* (2017) to Indian isolates, Aquino *et al.* (2005) to Phillipines isolates, Niyongere *et al.* (2015) to African isolates were produced expected amplicons approximately of ±500 bp (partial length). Whilst, some studies such as Yu *et al.* (2012) to Chinese isolates, Amin *et al.* (2008) to Pakistan isolates; Selvarajan *et al.* (2010) to Indian isolates, Stainton *et al.* (2015) to various isolates around the world, Wickramaarachchi *et al.* 2016 to Sri Lankan isolates, Furuya *et al.* (2004) and Chiaki *et al.* (2015) to Indonesian isolates were using primers with expected amplicons of approximately ±1,000–1,100 bp (full length).

A PCR assay had the advantage of amplifying the target nucleic acid present even at very low level, therefore it can be used to detect the BBTV at early stages of infection before the expression of morphological symptoms. However, there are some of potential problems in PCR assay for diagnostic purposes include false negatives due to reaction failure of bad quality DNA, contains high phenolic compounds so that even though the target viral DNA is present but still fail to amplify. False positives also may appear due to DNA contamination symptoms (Arumugam *et al.* 2017). Pisang Madu and Moseng as the new collections with no visual morphological symptoms were confirm positively BBTV infected as detected by PCR (Figure 2, lane 9 and 10, respectively). Likewise, the wild *M. acuminata* and *M. balbisiana*, also Pisang Ebung (ABB) which considered resistance accessions were revealed positive. According to this finding, it can be inferred that BBTV infected those morphologically resistant accessions were characterized under 'R' type (rough strain) of BBTV since the host remains symptomless and does not affect yield.

The sequencing of nine isolates with full length amplicons produced low quality of DNA sequences. Whilst, an isolate of Pisang Candi with shorter amplicon resulted high quality sequences. The PCR amplified products of BBTV CP gene particularly the full length amplicons should be cloned into plasmid vectors (such as pCRII, pCR2000, ptz57R/T, pTZBBTri4, pET 28a, etc.) prior to sequencing to get clean specific target sequences (Arumugam *et al.* 2017; Burns *et al.* 1995; Das *et al.* 2007; Kumar *et al.* 2017).

Typical of babuviruses, each genomic segment encodes a single open reading frame and contains the highly conserved stem-loop and major common regions (Yu *et al.* 2012). The sequences characteristic

of BBTV CP sequences of Pisang Candi aligned with 35 accessions (bananas and other host species) of homologues BBTV CP gene sequences from GenBank were considered highly conserved, low G+C content, and high genetic similarities. However, it was valuable sources and prime approach to reconstruct phylogenies for elucidating the evolutionary history of BBTV and other nano-viruses (Hu *et al.* 2007). Further, the different components of BBTV may resulted different phylogenies (Das *et al.* 2007).

The phylogenetic tree resulted from this study was in accordance to most of previous BBTV phylogeny studies such as (Furuya *et al.* 2004, 2006; Selvarajan *et al.* 2010; Stainton *et al.* 2015; Wanitchakorn *et al.* 2000; Wickramaarachchi *et al.* 2016; etc.). It was separated into two large groups, i.e. the Asian group (East Asia and South-east Asia) and the South Pacific group (South Asia/Indian subcontinent, Africa and the Pacific) (Figure 3). Further, the sequences analysis within Asian group was considered more diverse and less conserved than the South Pacific group. Wanitchakorn *et al.* (2000) and Stainton *et al.* (2015) were reported the high degree of divergence in the sequence of CP gene of Asian group than South Pacific group.

The Asian group was comprised of BBTV isolates from Asian countries except South Asia/Indian subcontinent countries; and separated into two sub-groups supported by moderate bootstrap value. Subgroup I comprises of isolates from Indonesia (includes Pisang Candi isolate from this study), Phillipines, Taiwan, Japan, and some parts of China (South and Southwest of China). Whereas, subgroup II consists of limited isolates from Thailand, Vietnam and parts of China (Figure 3). Hence, this phylogenetic finding lead to the assumption that BBTV in Asia was spreads through two routes, i.e. via East Asia and Southeast Asia islands/archipelago and via Southeast Asia mainland/Indochina. Meanwhile, isolates from South Asia (India, Pakistan, and Srilanka) were close related to isolates from Africa (Egypt, DR Congo, Burundi and Rwanda) and clustered in South Pacific group with isolates from Pacific Island (Hawaii) (Figure 3).

Other host species include *M. textilis, E. superbum,* and *Heliconia* sp. were shared high babuvirus CP sequences with BBTV isolates from banana both wild and cultivars (*M. acuminata, M. balbisiana, M. acuminata* x *M. balbisiana*). Bunchy top diseases those three differest hosts species are showing similarity symptoms, the transmission of the diseases were carried by the same agents of black aphid *P. nigronervosa*, the inoculation of BBTV to those species also resulted to bunchy top infection; thus led to the conclusion that the diseases were caused by the same virus (Furuya *et al.* 2006; Hamim *et al.* 2017; Sharman *et al.* 2008; Selvarajan and Balasubramanian 2013). However, isolates from those different hosts species may represent distinct strains of babuvirus, therefore need further study.

ABTV from the Phillipines in this study were clustered with Asian group–subgroup I, and shared the closest relation 100% similarity with BBTV isolates from Hainan–China, and the farthest relation within Asian Group 96.18% with BBTV isolates from Nongkhai–Thailand. This study was supported by Furuya *et al.* (2006), that virus isolated from abaca plants showed more than 99% of homologies with BBTV. Whilst, according to Sharman *et al.* (2008), ABTV and BBTV isolates shared only 79 to 81% amino acid sequence identity for the putative CP and 54 to 76% overall nucleotide sequence identity across all components and they were also shown to be serologically distinct.

Meanwhile, the CP gene sequence of EBTV isolate were clustered in South Pacific group and shared 97.96 to 100% sequence identity, closest related to BBTV isolate from Egypt and farthest related to BBTV isolate from Hawaii. Selvarajan and Balasubramanian (2013) was firstly reported of natural occurrence of bunchy top disease in *Ensete* in India, and pressumably that EBTV isolate would have originated from hill regions of Tamil Nadu. Likewise, Hamim *et al.* (2017) were firstly confirming the presence of the virus in *Heliconia* plants with symptoms of chlorotic leaves and green flecking of the veins. It has shorter sequences 401 nt and shared 98 to 99% identities to the Hawaiian and other Pacific isolates of BBTV. In this study, HBTV isolate was shared highest genetic similarity 99.71% to BBTV isolate from Hawaii, and clustered in South Pacific group with similarities 97.06 to 99.71%. Further, HBTV isolate and EBTV isolate has sequences identity of 99.42%.

This study highlighted that both symptomatic and symptomless banana plants examined were molecularly confirmed positive infected as indicated by the occurrences of BBTV CP fragments of all isolates on agarose gel. The BBTV in symptomless accessions were considered R-type and the propagules may serve as a reservoir of inoculums source for the

spreads. Interestingly, the oligonucleotide primer was amplified the DNA-S component at two different sizes i.e. ±500 bp and ±1,000-1,100 bp. Sequencing of the amplicons have resulted in a length of 484 to 1120 nt. However, sequences evaluation showed only Pisang Candi isolate which has high trace score and homologue with BBTV CP gene sequences (similarity 99%) for further analysis. The amplicons should be cloned into plasmid vectors prior to sequencing to get clean specific target sequences.

BBTV CP sequences were considered highly conserved. Phylogenetic analysis delineates isolates of the viruses into two large groups, i.e. the Asian group (East Asia and South-east Asia) and the South Pacific group (South Asia/Indian subcontinent, Africa and the Pacific). The BBTV evolutionary history in Asia was pressumably spreads through two routes, i.e. via East Asia and Southeast Asia islands/archipelago and via Southeast Asia mainland/Indochina. Further, this study also highlighted that other host species (*M. textilis*, *E. superbum*, and *Heliconia* sp.) were shared high babuvirus CP sequences with BBTV isolates from banana both wild and cultivars (*M. acuminata, M. balbisiana, M. acuminata* x *M. balbisiana*). ABTV from the Phillipines in this study were clustered with Asian group, whilst EBTV and HBTV isolate were clustered in South Pacific group. Isolates from those different hosts species may represent distinct strains of babuvirus, therefore need further study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Author's Contribution

All authors (LH, RFR, SS) declare that LH is the main author of this manuscript. Conceptualization, LH; Conduct fieldwork, labwork and data collection, LH; Data analysis and interpretation, LH; Writing the original draft manuscript, LH; Review and edit the final manuscript, LH, RFR, SS; All authors read and approved the final manuscript.

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References

Abdelkader, H.S., Abdel-Salam, A.M., El Saghir, S.M., Hussein, M.H., 2004. Molecular cloning and expression of recombinant coat protein gene of banana bunchy top virus in *E. coli* and its use in the production of diagnostic antibodies. *Arab. J. Biotech.* 7, 173-188.

Amin, I., Qazi, J., Mansoor, S., Ilyas, M., Briddon, R.W., 2008. Molecular characterization of banana bunchy top virus (BBTV) from Pakistan. *Virus. Gen.* 13, 191-198. https://doi.org/10.1007/s11262-007-0168-y

Anandhi, J., Vijila, C., Viswanath, G.S., Lokeswari, T.S., 2007. Screening banana plants for banana bunchy top virus with primers specific to Indian isolates. *J. Plant Dis. Prot.* 114, 101-107. https://doi.org/10.1007/BF03356715

Arumugam, C., Kalaimughilan, K., Kathithachalam, A., 2017. Banana bunchy top viral coat protein (CP) gene expression studies at molecular lvel in Hill Banana cv. Sirumalai (AAB). *Int. J. Curr. Microbiol. Appl. Sci.* 6, 398-411. https://doi.org/10.20546/ijcmas.2017.606.047

Aquino, V.M., de Leon, T.B., Colle, M.G., Romo, G.A., 2005. Sequence diversity of banana bunchy top virus (BBTV) coat protein gene in the Philippines. *In: The 27th NAST Annual Scientific Meeting. Philippine Agriculture 2020: A Strategy for Poverty Alleviation, Food Security, Global Competitiveness, Sustainability, Justice and Peace 13-14 July 2005. Vol 27 Issue No. 1.* Republic of the Philippines: National Academy of Science and Technology Philippines, pp. 57-63.

Burns, T.M., Harding, R.M., Dale, J.L., 1995. The genome organization of banana bunchy top virus: analysis of six ssDNA components. *J. Gen. Virol.* 76, 1471-1482. https://doi.org/10.1099/0022-1317-76-6-1471

Chen, A., Sun, J., Matthews, A., Armas-Egas, L., Chen, N., Hamill, S., Mintoff, S., Tran-Nguyen, L.T.T., Batley, J., Aitken, E.A.B., 2019. Assessing variations in host resistance to *Fusarium oxysporum* f sp. *cubense* race 4 in *Musa* species, with a focus on the subtropical race 4. *Front Microbiol.* 10, 1062. https://doi.org/10.3389/fmicb.2019.01062

Chiaki, Y., Nasir, N., Herwina, H., Jumjunidang, Sonoda, A., Fukutomo, T., Nakamura, M., Iwai, H., 2015. Genetic structure and diversity of the banana bunchy top virus population on Sumatra Island, Indonesia. *Eur. J. Plant. Pathol.* 143, 113-122. https://doi.org/10.1007/s10658-015-0669-9

Cook, D.C., Liu, S., Edwards, J., Villalta, O.N., Aurambout, J.P., 2012. Predicting the benefits of banana bunchy top virus exclusion from commercial plantations in Australia. *PLoS ONE.* 7, e42391. https://doi.org/10.1371/journal.pone.0042391

Das, D., Simon, L., Shankarappa, K.S., Narayanaswamy, P., Reddy, L., Rangaswamy, K.T., 2007. Cloning, sequencing and identification of a banana bunchy top virus isolate from Bangalore. *Plant Viruses.* 1, 189-192.

Davey, M.W., Gudimella, R., Harikrishna, J.A., Sin, L.W., Khalid, N., Keulemans, J., 2013. A draft *Musa balbisiana* genome sequence for molecular genetics in polyploid, inter- and intra-specific *Musa* hybrids. *BMC Genomics.* 14, 683. https://doi.org/10.1186/1471-2164-14-683

Elayabalan, S., Subramaniam, S., Selvarajan, R., 2015. Banana bunchy top disease (BBTD) symptom expression in banana and strategies for transgenic resistance: a review. *Emir. J. Food. Agric.* 27, 55-74. https://doi.org/10.9755/ejfa.v27i1.19197

El-Dougdoug, Kh.A., El-Shamy, M.M., 2011. Management of viral disease in banana using certified and virus tested plant material. *African J. Microbiol. Res.* 5, 5923-5932. https://doi.org/10.5897/AJMR11.966

[FAO] Food and Agriculture Organization, 2010. *FAOSTAT Database*, June 2010 ed. Food and Agriculture Organization of the United Nations, Rome.

Ferreira, S.A., Eduardo, E., Trujillo, E., 1997. Banana Bunchy Top Virus. Plant Disease 12. College of Tropical Agriculture and Human Resources. University of Hawaii at Manoa, Honolulu, Hawaii. Available at: https://scholarspace.manoa.hawaii.edu/server/api/core/bitstreams/939f79af-ba21-4dbd-a210-5f8a5730e346/content. [Date accessed: 1 July 2019]

Fullerton, R.A., 2002. Pathogenic variability in *Mycosphaerella fijiensis* and its implications for disease resistant bananas and plantains. *In: Acorbat. Memorias XV reunioin. Realizada en Cartagena de Indias, Colombia. 27 de Octobre al 02 Noviembre 2002.* Medellin (COL): Asociacion de Bananeros de Colombia Augura. pp. 39-48.

Furuya, N., Somowiyarjo, S., Natsuaki, K.T., 2004. Virus detection from local banana cultivars and the first molecular characterization of banana bunchy top virus in Indonesia. *J. Agri. Sci.* 49, 75-81.

Furuya, N., Dizon, T.O., Natsuaki, K.T., 2006. Molecular characterization of banana bunchy top virus and cucumber mosaic virus from Abaca (*Musa textilis* Nee). *J. Agri. Sci. Tokyo. Univ of Agric.* 51, 92-101.

Hafner, G.J., Harding, R.M., Dale, J.L., 1995. Movement and transmission of banana bunchy top virus DNA component one in bananas. *J. Gen. Virol.* 76, 2279-2285. https://doi.org/10.1099/0022-1317-76-9-2279

Hamim, I., Green, J.C., Borth, W.B., Melzer, M.J., Wang, Y.N., Hu, J.S., 2017. First report of banana bunchy top virus in *Heliconia* spp. on Hawaii. *Plant Disease.* 101, 2153. https://doi.org/10.1094/PDIS-02-17-0205-PDN

Hapsari, L., Masrum, A., 2012. Preliminary screening resistance of *Musa germplasms* for banana bunchy top disease in Purwodadi Botanic Garden, Pasuruan, East Java. *Buletin Kebun Raya.* 15, 57-70.

Hapsari, L.. 2014. Wild *Musa* species collection of Purwodadi Botanic Garden: inventory and its morpho-taxonomic review. *J. Trop. Life Sci.* 4, 70-80. https://doi.org/10.11594/jtls.04.01.12

Hapsari, L., Wahyudi, D., Azrianingsih, R., Arumingtyas, E.L., 2015. Genome identification of bananas (*Musa* spp.) from East Java assessed by PCR-RFLP of the Internal Transcribed Spacer ribosomal DNA. *Int. J. Biosci.* 7, 42-52. https://doi.org/10.12692/ijb/7.3.42-52

Hapsari, L., Lestari, D.A., 2016. Fruit characteristic and nutrient values of four Indonesian banana cultivars (*Musa* spp.) at different genomic groups. *AGRIVITA J. Agr. Sci.* 38, 303–311. https://doi.org/10.17503/agrivita.v38i3.696

Hapsari, L., Kennedy, J., Lestari, D.A., Masrum, A., Lestarini, W., 2017. Ethnobotanical survey of bananas (Musaceae) in six districts of East Java, Indonesia. *Biodiversitas* 18, 160–174. https://doi.org/10.13057/biodiv/d180123

Harding, R.M., Burns, T.M., Hafner, G., Dietzgen, R.G., Dale, J.L., 1993. Nucleotide sequence of one component of the banana bunchy top virus genome contains a putative replicase gene. *J. Gen. Virol.* 74, 323-328. https://doi.org/10.1099/0022-1317-74-3-323

Hazaa, M.M., El-Dougdoug, Kh.A., El-Maaty, S.A., 2006. Eradication of banana viruses from naturally infected banana plants. Production of certified banana plants and virus tested. *J. Appl. Sci. Res.* 2, 714-722.

Heslop-Harrison, J.S., Schwarzacher, T., 2007. Domestication, genomics and the future for banana. *Ann. Bot.* 100, 1073–1084. https://doi.org/10.1093/aob/mcm191

Hooks, C.R.R., Wright, M.G., Kabasawa, D.S., Manandhar, R., Almeida, P.P.P., 2008. Effect of banana bunchy top virus infection on morphology and growth characteristics of banana. *Appl. Biol.* 153, 1–9. https://doi.org/10.1111/j.1744-7348.2008.00233.x

Hu, J.M., Fu, H.C., Lin, C.H., Su, H.J., Yeh, H.H., 2007. Reassortment and concerted evolution in banana bunchy top virus genomes?. *J. Virol.* 81, 1746–1761. https://doi.org/10.1128/JVI.01390-06

Javed, M.A., Chai, M., Othman, R.Y., 2004. Study of resistance of *Musa acuminata* to *Fusarium oxysporum* using RAPD markers. *Biologia Plantarum.* 48, 93-99. https://doi.org/10.1023/B:BIOP.0000024281.85427.6d

Kadam, S., Gokhale, N.B., Sawardekar, S.V., Joshi, M.S., Kelkar, V.G., 2017. Standardization of protocol and detection of BBTV for tissue cultures plantelts of banana cv Safed velchi through sandwich elisa techniques. *Plant Cell Biotech. Mol. Biol.* 18, 570-574.

Kavino, M., Harish, S., Kumar, N., Saravanakumar, D., Samiyappan, R., 2011. Molecular characterisation of coat protein and nuclear shuttle protein genes of Banana bunchy top virus from Western Ghats in India. *Arch. Phytopathol. Plant Prot.* 44, 405-411. https://doi.org/10.1080/03235400903092933

Kumar, P., Arun, V., Lokeswari, T.S., 2017. Cloning of BBTV (banana bunchy top virus) components and screening of BBTV using functionalized gold nanoparticles. *Biotech.* 7, 225. https://doi.org/10.1007/s13205-017-0849-8

Lepoint, P., Staver, C., Ajambo, S., Rietveld, A., Kumar, P.L., Hanna, R., Iskra-Caruana, M.L., with contributions from presenters and work group rapporteurs CGIAR. 2014. Workshop Report: Strategic Planning Workshop on Recovering Banana Production in BBTD Affected Areas: Community and Farm Household Approaches. Bujumbura, Burundi 20-25 January 2014. CGIAR research program on roots, tubers and bananas.

Mahadev, S.R., Thamilarasan, S.K., Kathithachalam, A., 2013. PCR detection of banana bunchy top virus (BBTV) at tissue culture level for the production of virus-free planting materials. *Int. Res. J. Biological. Sci.* 2, 22-26.

Niyongere, C., Ateka, E., Losenge, T., Blomme, G., Lepoint, P., 2011. Screening *Musa* genotypes for banana bunchy top disease resistance in Burundi. *Acta. Hort. (ISHS).* 897, 439-447. https://doi.org/10.17660/ActaHortic.2011.897.60

Niyongere, C., Lepoint, P., Losenge, T., Blomme, G., Ateka, E.M., 2015. Towards understanding to diversity of banana bunchy top virus in the Great Lakes region of Africa. *African J. Agr. Res.* 10, 7012-709. https://doi.org/10.5897/AJAR2014.8862

Pinili, M.S., Nagashima, I., Dizon, T.O., Natsuaki, K.T., 2013. Cross-transmission and new alternate hosts of banana bunchy top virus. *Trop. Agr. Develop.* 57, 1-7.

Priani, R.A., Somowiyarjo, S., Hartono, S., Subandiyah, S., 2010. Detection and differentiation of banana bunchy top virus using PCR-RFLP detection techniques. *Jurnal Perlindungan Tanaman Indonesia.* 16, 1–5.

Selvarajan, R., Sheeba, M.M., Balasubramanian, V., Rajmohan, R., Dhevi, N.L., Sasireka, T., 2010. Molecular characterization of geographically different banana bunchy top virus isolates in India. *Indian J. Virol.* 21, 110-116. https://doi.org/10.1007/s13337-010-0020-1

Selvarajan, R., Balasubramanian, V., 2013. Natural occurrence of banana bunchy top virus in *Ensete superbum* in India. *Indian. J. Virol.* 24, 97–98. https://doi.org/10.1007/s13337-012-0123-y

Sharman, M., Thomas, J.E., Skabo, S., Holton, T.A., 2008. Abaca bunchy top virus, a new member of the genus *Babuvirus* (family Nanoviridae). *Arch. Virol.* 153, 135-147. https://doi.org/10.1007/s00705-007-1077-z

Shilpa, B.S., Moger, N., Vani, A., Rabinal, C., Hedge, R.V,, Byadagi, A.S., 2016. Cloning and expression of banana bunchy top virus (BBTV) coat protein gene in *E. coli*. *Elixir Agriculture*. 94, 40000-40004.

Stainton, D., Martin, D.P., Muhire, B.M., Lolohea, S., 2015. The global distribution of Banana bunchy top virus reveals little evidence for frequent recent, human-mediated long distance dispersal events. *Virus Evolution*. 1, vev009. https://doi.org/10.1093/ve/vev009

Thomas, J.E., Dietzgen, R.G., 1991. Purification, characterization and serological detection of virus-like particles associated with banana bunchy top disease in Australia. *J. Gen. Virol.* 72, 217-224. https://doi.org/10.1099/0022-1317-72-2-217

Thomas, J.E., 2008. Banana bunchy top virus, in: Mahy, B.W.J., Regenmortel, M.H.V.V. (Eds.), *Desk Encyclopedia of Plant and Fungal Virology*. Elsevier Academic Press, Oxford, pp. 94-100. https://doi.org/10.1016/B978-012374410-4.00636-1

Valmayor, R.V., S.H., Jamaluddin, B., Silayoi, S., Kusumo, L.D., Danh, O.C., Pascua, R.R.C., Espino, 2000. *Banana Cultivar Names and Synonyms in Southeast Asia*. International Network for the Improvement of Banana and Plantain–Asia and the Pacific Office. Los Banos, Laguna, Philippines.

Vanhove, A.C., Vermaelen, W., Panis, B., Swennen, R., Carpentier, S.C., 2012. Screening the banana biodiversity for drought tolerance: can an *in vitro* growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. *Front. Plant Sci.* 3, 176. https://doi.org/10.3389/fpls.2012.00176

Vetten, H.J., Chu, P.W.G., Dale, J.L., Harding, R., Hu, J., Katul, L., Kojima, M., Randles, J.W., Sano, Y., Thomas, J.E., 2005. Nanoviridae, in: Faquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), *Virus taxonomy, VIIIth Report of the ICTV*. Elsevier Academic Press, London, pp. 343-352.

Wanitchakorn, R., Harding, R.M., Dale, J.L., 1997. Banana bunchy top virus DNA-3 encodes the viral coat protein. *Arch. Virol.* 142, 1673-1680. https://doi.org/10.1007/s007050050188

Wanitchakorn, R., Harding, R., Dale, J., 2000. Sequence variability in the coat protein gene of two groups of banana bunchy top isolates. *Arch. Virol.* 145, 593-602. https://doi.org/10.1007/s007050050049

Wickramaarachchi, W.A.R.T., Shankarappa, K.S., Rangaswamy, K.T., Maruthi, M.T., Rajapakse, R.G.A.S., Ghosh, S., 2016. Molecular characterization of banana bunchy top virus isolate from Sri Lanka and its genetic relationship with other isolates. *Virus Disease*. 27, 154-160. https://doi.org/10.1007/s13337-016-0311-2

Wu, R.Y., Su, H.J., 1990. Purification and characterization of banana bunchy top virus. *J. Phytopathol.* 128, 153-160. https://doi.org/10.1111/j.1439-0434.1990.tb04261.x

Wu, W., Yang, Y.L., He, W.M., Rouard, M., Li, W.M., Xu, Meng, Roux, N., Ge, X.J., 2016. Whole genome sequencing of a banana wild relative *Musa itinerans* provides insights into lineage-specific diversification of the *Musa* genus. *Nature*. 6, 31586. https://doi.org/10.1038/srep31586

Xie, W.S., Hu, J.S., 1995. Molecular cloning, sequence analysis, and detection of banana bunchy top virus in Hawaii. *Phytopathology*. 85, 339-347. https://doi.org/10.1094/Phyto-85-339

Yu, N.T., Zhang, Y.L., Feng, T.C., Wang, J.H., Kulye, M., Yang, W.J., Lin, Z.S., Xiong, Z., Liu, Z.X., 2012. Cloning and sequence analysis of two banana bunchy top virus genomes in Hainan. *Virus Genes*. 44, 488-494. https://doi.org/10.1007/s11262-012-0718-9

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