Agr. Nat. Resour. 56 (2022) 1019–1028

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Symptom expression and resistance of some banana cultivars to banana bunchy top virus infection

Ruth Feti Rahayuniati[a,*], Siti Subandiyah[b]

[a] *Department of Agrotechnology, Faculty of Agriculture, Universitas Jenderal Soedirman, Jl. Dr. Suparno, Karangwangkal, Central Java 53123, Indonesia*

[b] *Research Center for Biotechnology, Universitas Gadjah Mada. Jl. Teknika Utara, Yogyakarta 55281, Indonesia*

Article Info

Article history:
Received 7 March 2022
Revised 20 July 2022
Accepted 26 August 2022
Available online 21 october 2022

Keywords:
Banana,
BBTV,
Infection,
Morphology,
Resistance

Abstract

Importance of the work: Bunchy top is a damaging disease of bananas. Considering the scarcity of resistant plants, it is vital to conduct screening for the resistant cultivars

Objectives: To determine symptom expression and screen some banana cultivars for resistance.

Materials and Methods: A survey was undertaken to collect some banana cultivars with different typical symptoms. An artificial inoculation method was applied to screen the resistant cultivars of some banana cultivars. Polymerase chain reaction was used to detect the banana bunchy top virus (BBTV), while ultraviolet-visible spectrometry was used to analyze the chlorophyll**.**

Results: Several different symptoms of banana bunchy top diseases (BBTD) were identified among the banana cultivars but the most prevalent ones were stunting, narrow leaves, upright growth, yellow leaves and a rosette. Some cultivars showed brittle and rigid leaves. Molecular detection showed similar characteristics of DNA R, with a nucleotide 1104 bp long in all infected plants. Chlorophyll b was more affected by BBTV than chlorophyll a, with a mean (± SD) chlorophyll content of 0.0050 ± 0.013 g/L. The screening test of some bananas indicated that cultivar Tanduk (AAB), Klutuk Wulung (BB) and wild banana *M. acuminata* subsp. *halabanensis* (AA) had higher resistance levels than the others, where the parentheses indicate the genome. All three of these bananas had the longest incubation period, 0–20% disease incidence, 0–6.67% disease severity and 0.00–0.006 susceptibility level.

Main findings: The symptoms of BBTV varied based on the cultivar. Cultivar Tanduk, Klutuk Wulung and the wild banana subspecies *halabanensis* were resistant to BBTV. This information might be useful in banana breeding programs, primarily in the development of new, resistant cultivars.

* Corresponding author.
E-mail address: ruth.rahayuniati@unsoed.ac.id (R.F. Rahayuniati)

https://doi.org/10.34044/j.anres.2022.56.5.16

Introduction

Banana bunchy top disease (BBTD) is one of the most damaging banana virus diseases that causes production losses of up to 100%; it has spread globally, including Indonesia (Stainton et al., 2015; Qazi, 2016; Rahayuniati et al., 2021b). To date, there have been no BBTV-resistant banana plants. Accordingly, multiple studies on various banana varieties that exhibit resistance properties have been conducted in Asia and Africa (Kumar et al., 2015).

Today, the ongoing search for resistant banana varieties or sources of banana disease resistance has identified that the wild type of banana, the ancestor of the currently cultivated bananas, is an alternative source of resistance, with *Musa acuminata* Colla. and *M. balbisiana* Colla. having a scattered distribution in Southeast Asia, including Indonesia (Perrier et al., 2011; Poerba et al., 2016). There are approximately 15 varieties of *M. acuminata* from Aceh to Papua and 200 local cultivars that are still natural (Poerba et al., 2016).

Disease severity can be attributed to varying degrees of virulence, inherent factors of the host or the interaction of these two factors (Pfennig, 2001; White et al., 2020). The challenge in screening for resistance includes the fact that the host population consists of individuals with different levels of susceptibility to infection (González et al., 2019). The resistance or susceptibility of banana plants can be observed through changes or genetic expression after inoculation, in which genetic responses to a viral infection on bananas may be induced by the resistance gene, while other indices of resistance include visible symptoms, incubation period, disease incidence, disease severity and the area under the disease progress curve or AUDPC (Ngatat et al., 2017; Parac et al., 2020).

Studies on BBTV have focused on the morphological features of infected bananas, the chlorophyll content, incubation period, alternative hosts, resistance of banana and abaca, and disease distribution (Magee, 1927; Furuya et al., 2006; Hooks et al., 2008; Vishnoi et al., 2009; Pinili et al., 2013; Kumar et al., 2015; Rahayuniati et al., 2021a, b). However, these studies did not investigate different symptoms and resistance in cultivated and wild bananas. Therefore, the current research considered the following: 1) differences in the visible symptoms among banana cultivars; and 2) The resistance responses of bananas to BBTV infection. The study aimed to reveal the characteristic visible symptoms of some cultivated bananas and screen resistance against BBTV in some wild and cultivated bananas using self-reared aphids. The finding of the research might be used to develop new resistant banana cultivars to BBTV.

Materials and Methods

Sampling collection

Random sampling was performed in some districts of Indonesia: Solok in West Sumatra, Bengkulu in Bengkulu province, Manado in North Sulawesi, Halmahera in North Maluku, Salatiga in Central Java and Bantul and Kulonporogo in the Special Region of Yogyakarta. A minimum of five plants were collected for each visible symptom of seven cultivars of banana and one wild-type banana bearing typical symptoms, based on samples of cigar leaves or tissue for molecular detection and identification.

Banana bunchy top virus detection

The genome of BBTV consists of at least six components: DNA-R, DNA-S, DNA-M, DNA-C, DNA-N and DNA-U3, with being approximately 1 kb. A fragment of DNA-R was amplified for detection and molecular characterization because it not only indicates the presence of the virus but also demonstrates its ability to replicate. DNA was extracted from leaf samples using a mini-DNA kit preparation for plants from Geneaid (Geneaid Biotech Ltd, New Taipei City, Taiwan R.O.C.). All extraction procedures followed the instructions accompanying the kit. A back-to-back DNA R primer pair (Table 1) was used for polymerase chain reaction (PCR) amplification of the BBTV genome of leaf samples, according to Rahayuniati et al. (2021b).

Aphid mass rearing

Mass rearing was conducted based on Rahayuniati et al. (2021a), with some modifications. Aphids were collected from the field and directly isolated on healthy bananas until the end of the first cycle. Prior to mass propagation, molecular identification of the aphids was used to confirm they were *Pentalonia nigronervosa*. Total DNA was extracted from the aphids following the DNA extraction protocol in the Geneaid mini kit DNA kit for extraction for tissue. In total, 10 aphids were macerated using a plastic mortar in a 1.5 µL tube and added with extraction buffer, before washing using available washing solutions. For PCR detection, Bioline

MyTaq™ HS Red Mix (Meridian Bioscience, Eveleigh, New South Wales, Australia) was used as the polymerase, with two pairs of primers for aphid identification and of primers for detection of BBTV in aphids (Table 1). The sequence of aphids was compared to the same data collection in GenBank. Evolutionary analysis was conducted using the Mega 11 software (Philadelphia, PA, USA) and the maximum likelihood method with the Tamura-Nei model. The results indicated that the aphid was *P. nigronervosa*, as evidenced from its position in the phylogenetic tree (Fig. 1).

Mass rearing of the aphids was carried out on caladium (*Caladium bicolor*). Five non-viruliferous, wingless adult aphids were transferred to caladium that had been kept in screen boxes. The aphid nymphs were allowed to reproduce on the caladium and the colonies were re-tested to determine the presence or absence of BBTV in the body before being used as a vector.

Banana plant collection

The banana plants were derived from cultured tissues, suckers and seeds. In total, 50 tissue culture seedlings (at 1 mth after acclimatization) of each cultivar (cv) were obtained from the Salaman horticultural nursery in Magelang district for: cv Mas (AA), cv Cavendish (AAA), cv Raja (AAB) and cv Kepok (ABB), where the genome is given in parentheses. Twenty seeds from the hump of cv. Tanduk (ABB) were obtained from a farmer in Purworejo district. As a negative control, 20 suckers of cv. Klutuk Wulung (BB) were collected from the Banyumas district. Banana seedlings derived from seed propagation were obtained by planting wild banana seeds from South Sumatra and Bengkulu, namely *M. acuminata* subsp. *malacensis* (AA), subsp. *halabanensis* (AA) and subsp. *longipetiolata* (AA); 10 seedlings of each subspecies were maintained until aged 1 mth.

Table 1 Primers for aphid and banana bunchy top virus identification

Number	Primer	Target	Reference
1.	*Aphid identification using primer Lep.* Lep Forward: 5'-ATTCAACCAATCATAAAGATATTG G-3' Lep Reversed: 5'-TAAACTTCTGGATGTCCAAAAAAT CA-3'	700 bp	Foottit et al. (2010)
2.	*BBTV identification using primer DNA R* Forward: 5'- TTGAGAAACGAAAGGGRAGC-3' Reversed: 5'- GGTGTGCGCCTGGGAAG- 3'	1100 bp	Stainton et al. (2015)
3.	*BBTV inside primer* Forward: 5'- TGCGTGAAACGCATAAACGG-3' Reversed: 5'- TCAACTCTGCTTGCACTCTGT-'	550 bp	

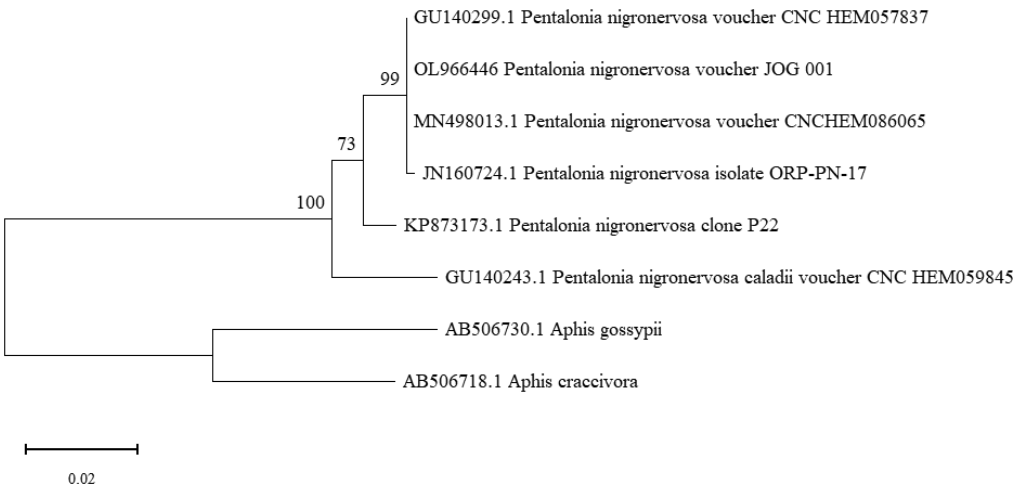

Fig. 1 Phylogenetic analysis of banana aphid (OL96646 *Pentalonia nigronervosa* voucher JOG 001), where trees constructed using maximum likelihood method and TN93-parameter model with 1,000 bootstrap replications

Transmission of banana bunchy top virus

The inoculation was carried out based on Suparman et al. (2017) and Jebakumar et al. (2018) with some modifications. Non-viruliferous aphids, aged 8–10 d, were transferred from caladium to infected bananas as the source of BBTV inoculum. After an acquisition period of 48 h, exactly 20 aphids were transferred to each recipient banana plant using a soft wet brush. Each aphid was placed on the underside of a coiled banana leaf. The inoculated plants were placed in a room covered with organza fabric in the greenhouse at 25–28 °C. After 5 d of incubation, all the test plants were sprayed with 500 ppm lambda-cyhalothrin insecticides.

Observations

Observations were made once a week. The observed parameters were the incubation period (time from inoculation to the first appearance of symptoms) and plant height (length from the base of the stem above the soil surface to the tip of plant growth). The observed early symptoms included chlorosis on new leaves that appeared after inoculation. Furthermore, the following traits were monitored: chlorosis on the leaf margins, the presence of dark green lines on the petioles or pseudo-stems, changes in the size of new leaves, rosette symptoms and plant death. In addition, the chlorophyll contents of healthy plants and infected plants were measured using a spectrophotometer (Anuradha et al., 2015). Data obtained from observations of symptom development were used to calculate the percentages of disease incidence, disease severity, AUDPC and plant susceptibility.

Chlorophyll concentration

Calculation of chlorophyll concentrations was carried out based on Arnon (1949). Four composite chlorophyll samples for spectroscopic measurement were prepared by extracting 1 g of banana leaves in 20 mL of 80% acetone. Subsequently, the samples were measured using an ultraviolet-visible spectrometer at wavelengths of 663 nm and 645 nm. The calculating chlorophyll content was determined based on Equations 1 and 2:

$$D663 = 82.64Ca + 9.27Cb \quad (1)$$

$$D645 = 16.75\ Ca + 45.6\ Cb \quad (2)$$

where D is the density of chlorophyll and Ca and Cb are the chlorophyll a and chlorophyll b amounts, respectively, measured in milligrams per liter.

$$Ca = 0.0127\ D663 - 0.00269\ D645$$
$$Cb = 0.0229\ D645 - 0.00468\ D663$$

Total chlorophyll in grams per liter is

$$C = 0.0202\ D645 + 0.00802\ D663$$

or in milligrams per liter is

$$C = 20.2\ D645 + 8.02\ D663$$

The mean and standard deviation values for chlorophyll a, chlorophyll b and total chlorophyll were calculated.

Disease incidence

Disease incidence was calculated at the end of observation (week 21). Based on the observation of visible symptoms, the disease incidence was calculated by comparing the number of symptomatic plants to the number of test plants. Calculations were made based on Rao et al. (2002) as follows:

$$\text{Disease incidence} = (\Sigma \text{ number of plants with dwarf disease symptoms}) / (\Sigma \text{ total number of test plants}) \times 100\% \quad (3)$$

The infection of BBTV was confirmed through molecular detection based on a PCR technique. Sample extraction was carried out according to the procedure in the Bioline Isolate II DNA Extraction kit (Meridian Bioscence, Eveleigh, New South Wales, Australia). In total, 10 g of the fresh banana leaf was macerated using a mortar and added with extraction buffer. BBTV was detected based on PCR. Bioline My Taq Red ready mix with two pairs of primers of DNA R was used for the polymerase (Table 1).

Disease severity

The severity of BBTD was observed weekly for visual symptoms using a four-score scale adapted from other studies (Leiwakabessy et al., 2017; Wirya et al., 2020) (Table 2) as shown in Equation 4:

$$\text{Disease severity} = \Sigma\ [(n_i \times v_i) / (N \times Z)] \times 100\%, \quad (4)$$

where n_i is the number of infected samples with score "i", v_i is the score at the time of observation, N is the total number of plants and Z is the highest score.

Table 2 Level of infection according to Leiwakabessy et al. (2017)

Score	Description of symptom
0	No symptoms.
1	Mild symptoms. Limited vein clearing and dark green streaks on lower part of lamina and petiole. No significant reduction of lamina width.
2	Intermediate symptoms. Vein clearing, upturned leaf, chlorotic and ragged margins. Significant reduction in petiole length, distance and lamina width.
3	Severe symptoms. Brittle lamina with upturned, chlorotic and ragged margins, sometimes with a necrotic symptom. Leaves fail to emerge, giving a clear bunched appearance.

Areas under disease progress curve

The AUDPC is the area under the curve of disease progression or disease severity, measured based on the comparison between disease severity 'y' with time 't'. The AUDPC value was calculated according to Shaner and Finney (1977), with slight modifications referred to in Ngatat et al. (2017) and Parac et al. (2020), according to follows Equation 5:

$$AUDPC= [(yi + yi+1)/2] \times (ti+1 – ti) \quad (5)$$

where y_i is the severity of the disease at time i and t_i is the time at moment i.

AUDPC values cannot be used directly to compare plant severity or susceptibility. Therefore, a relative AUDPC value (rAUDPC) was developed by dividing the AUDPC value by the highest AUDPC potential value (Forbes et al., 2014).

$$rAUDPC = AUDPC / [(tn-t1) \times 100]$$

To determine the value of the plant susceptibility scale, we divided the relative value of AUDPC in each cultivar by a set constant.

$$Susceptibility\ level = (rAUDPC/constant)$$

The constant value was obtained by dividing the disease severity by the rAUDPC.: Constant = (highest disease severity score/control rAUDPC)

Statistical analysis

All treatments were repeated five times, with the number of each unit of the treatment depending on the plant collection. For the disease severity data, AUDPC and susceptibility level, statistical analysis was performed using a one-way analysis of variance using the SPSS 24 software (IBM, Armonk, NY, USA). Mean comparisons were performed using Duncan's multiple range test. The tests were considered significance at $p < 0.05$.

Results and Discussion

Visible symptoms on various banana types

The characteristic symptoms of BBTV infected bananas included yellow leaf margins and dark green stripes around the midrib, rigid leaves (Fig. 2) and swollen and shiny leaf veins. Morse-code-like stripes appeared on the leaves and petioles, while on the pseudo-stem, there were dark green lines that extended as high as the plant (Fig. 2C) and the leaves became smaller than normal size. The petioles were shortened and the leaves appeared to grow in a cluster at the tip of the plant, forming a rosette pattern (Fig. 2A). On the lamina near the leaf bone, there were dark green lines that curved to form the letter "J" or were hook-like (Fig. 2B).

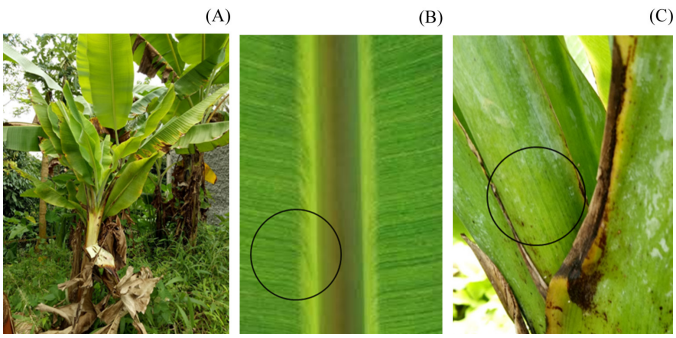

Fig. 2 General symptoms of bunchy top disease: (A) banana plants with stunting, narrow leaves, upright growth, short stalks and leaves growing in a cluster at tip of the plant (rosette symptom); (B) dark green lines on leaf lamina and curved like the letter "J" near the mother leaf bone; (C) dark green lines, long and short, like Morse code

However, these symptoms appeared differently on Uter cultivars, as chlorotic and curling leaf margins, prominent, somewhat transparent and as shiny veins and stiff and crisp leaves. In addition, the leaves became narrower and grew upright (Fig. 3). A possible reason for these symptoms on Uter banana leaves was a deficiency of complex nutrients, such as nitrogen, phosphorus, potassium, boron, sulfur and iron. A lack of nitrogen causes all parts of the leaves to turn pale green, the leaf midrib becomes reddish and growth slows down (Tejada and Gara, 2017; Torres-Bazurto et al., 2019). Since phosphorus is compulsory for plant growth, its lack causes stunted plants and makes old leaves chlorotic and saw-toothed and the leaf edges curl (Malhotra et al., 2018). The lack of K in banana plants causes the leaves to narrow (Tejada and Gara, 2017). Deficiencies of boron, sulfur and zinc contribute to the severity of the symptoms of curling, chlorosis, thickening of the leaf bones and stunted growth (Tejada and Gara, 2017; Chen and Fan, 2018). These symptoms also are attributed to cucumber mosaic virus (CMV) infection which causes a chlorotic leaf lamina, smaller leaf size, curling leaf and a shorter plant (Ahamedemujtaba et al., 2019). Therefore, multiple infections of BBTV and CMV probably occurred in the Uter banana cultivar.

Different visible symptoms also appeared in the wild bananas. *M. acuminata* subsp. s*umatrana* had leaf margins that were slightly curled or coiled with apparent necrosis, the leaf color remained dark green, the size decreased and the rosette was clearly visible at the tip of the plant. Symptoms of banana bunchy top disease on cultivar Mas were chlorotic leaves, thickening leaf bones and wavy leaves. Cultivar Kepok had an apparent rosette, a shortened petiole and a narrow leaf lamina, but no symptoms of chlorosis. Muli banana leaves infected with BBTV showed symptoms of chlorosis along the leaf margins, with leaves slightly curled along the edges, a shortened petiole, erect new leaf growth and a narrow leaf lamina. The Maraseba banana did not undergo changes in leaf color but the new leaf stalks were shorter and were clustered at the tips of the plant (Fig. 3). According to Pesti et al. (2019), differences in response to or the expression of symptoms due to BBTV infection shown by each plant are influenced by genetic factors, plant physiological changes and environmental factors.

Several different symptoms of BBTD were identified, but in general, overall stunting symptoms in the bananas were typical, with stunted plants, narrow leaves, upright growth, clustering at the tips of the plant and a rosette-like formation. Changes in the chlorophyll content in banana plant leaves infected with BBTV are related to physiological stress in the plant, which affects plant growth and yield (Hooks et al., 2008). Virus infection also triggers the occurrence of pigmentation or chlorosis on the leaves, causing yellowing symptoms (Zhao et al., 2016). Chlorosis after viral infection can also occur if carbohydrates accumulate in the leaves (Anuradha et al., 2015). Changes in the chlorophyll content are often considered a secondary effect of viral multiplication and accumulation in the cytoplasm (Zhao et al., 2016). However, the presence of viruses in the cytoplasm is important in inducing disease or the formation of plant resistance (Mandadi et al., 2013). Viruses that affect the structure and function of chloroplasts lead to changes in photosynthetic activity (Zhao et al., 2016).

Molecular detection

BBTV was detected from all disease symptoms based on PCR. Back-to-back primers of DNA R were used in polymerase, with a target of 1100 bp. A fragment of around 1100 bp in size was amplified using PCR with DNA samples from the various BBTD symptoms of some banana cultivars (Fig. 4).

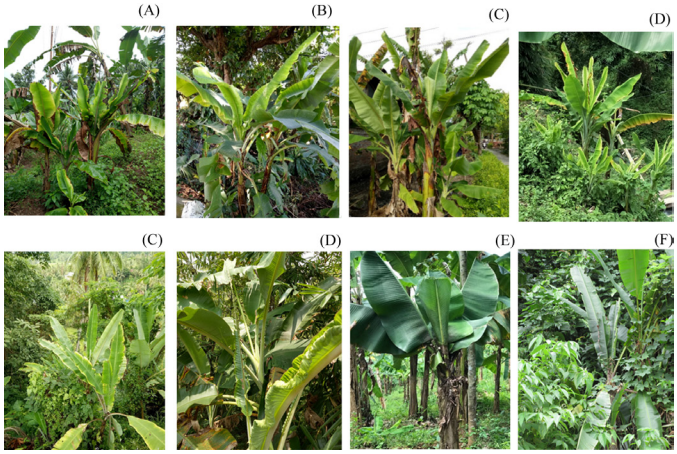

Fig. 3 Various banana bunchy top disease symptoms on banana cultivars in field with genome in parentheses: (A) cultivar (cv) Gros Michel (AAA); (B) cv Mas (AA); (C) cv Kepok (ABB); (D) cv Ketip (AAB); (E) cv Muli (AA); (F) cv Uter (AAB); (G) cv Maraseba (AAA); (H) wild banana *M. acuminata* subsp. sumatrana (AA)

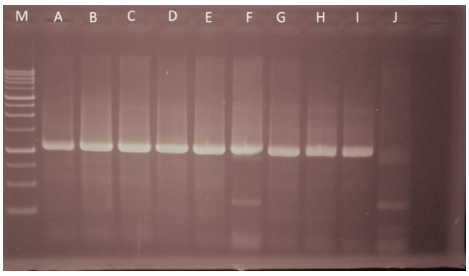

Fig. 4 Amplification of some banana cultivars, where M = marker/ladder 1 kb; A = positive control (infected banana); B = cultivar (cv) Gros Michel; C = cv Mas; D = cv Kepok; E = cv Ketip; F = cv Muli; G = cv Uter; H = cv Maraseba; I = *M acuminata* subsp. *sumatrana*; J = negative control (uninfected banana)

Sequence data analysis showed a conserved region, known as the stem-loop common region (CR-SL), on the DNA (Fig. 5). CR-SL is composed of the TATTATTAC nucleotide which probably marks the origin of the virion DNA strand (Wickramaarachchi et al., 2016). There are three non-coding sequences in the BBTV genome, namely GGGAC (F1, F2) and R (GTCCC) and another common region is the major common region (CR-M), consisting of GC-rich sequences generally located after CR-SL (Burns et al., 1995; Stainton et al., 2017; Rahayuniati et al., 2021b). According to Karan et al. (1994), CR-M is feasible for identifying the origin and affinity of new isolates.

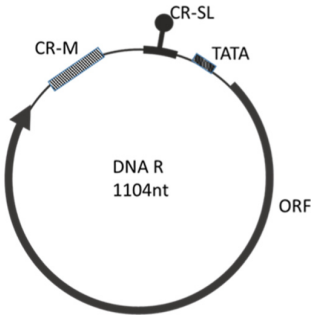

Fig. 5 Organization of banana bunchy top virus DNA R

The nucleotide of DNA R was 1104-bp long and CDS/ORF was 861 nucleotides (nucleotide numbers 103 to 963) and encoded 287 amino acids (33.6 kDa). This DNA is code Mrep protein that contributes to BBTV replication. CR-M was found at nucleotide numbers 1044 to 1058 and CR-SL at numbers 1 to 31. Poly A was formed from the nucleotide numbers 826 to 830 and the TATA box was at 52 to 55.

Resistance of some banana cultivars

BBTV resistance can be evaluated based on the susceptibility of each banana plant cultivar. The percentages of incidence and severity of disease also varied in each banana cultivar (Table 3).

Cultivar Mas was used as the reference to determine the level of plant susceptibility. This plant showed symptoms in the week 2 after inoculation, with the incidence and severity of disease reaching 100% in the week 5. Weekly disease progression was fast, with an AUDPC of 1732,222 and a maximum susceptibility level of 3. The susceptibility of cv Raja was not significantly different from that of Mas for which the incubation period ended by week 2 and the disease developed rapidly, as observed from the 1728,886 AUDPC and 2.994 susceptibility levels. Cavendish with a 2.831 susceptibility level showed symptoms of the disease in wk 3 after inoculation with 11.11% disease severity which continued to increase up to 100% in week 5. Cultivar Kepok showed slower developing symptoms than those of the Mas, Raja and Cavendish cultivars, albeit the levels of susceptibility were not significantly different. The incubation period of BBTV in Kepok was 4 wk and disease severity reached 100% in week 6.

Tanduk and Klutuk were resistant because no symptoms of the disease were observed in both cultivars. Likewise, subspecies *halabanensis* of wild banana had the longest incubation period (up to 21 wk) compared to the other wild banana (Fig. 6A). The *halabanensis* subspecies was evidently a resistant subspecies because it had a susceptibility level of 0.0006 and non-significantly different levels of vulnerability from the Tanduk and Klutuk cultivars.

Table 3 The incubation period, severity, AUDPC, susceptibility level

Tested plant	Genome	Incubation period (wk)	Disease incidence (%)	Disease severity (%)	AUDPC	Susceptibility level
Mas	AA	2±0.707^{a}	100.00±30.112^{e}	100.00±32.856^{f}	1732.222±1.581^{c}	3.000±0.100^{d}
*longipetiolata**	AA	3±1.000^{b}	40.00±12.032^{c}	40.00±13.101^{d}	566.667±7.106^{b}	0.981±0.007^{c}
*malacensis**	AA	11±0.707^{d}	60.00±30.079^{d}	60.00±23.909^{e}	416.667±7.211^{b}	0.722±0.001^{bc}
*halabanensis**	AA	21±0.707^{e}	20.00±4.364^{b}	6.67±1.456^{b}	3.333±1.000^{a}	0.006±0.002^{a}
Cavendish	AAA	3±0.707^{b}	100.00±34.350^{e}	100.00±37.277^{f}	1634.444±7.071^{c}	2.831±0.001^{d}
Raja	AAB	2±0.001^{a}	100.00±33.533^{e}	100.00±30.745^{f}	1728.886±7.106^{c}	2.994±0.002^{d}
Tanduk	ABB	asymptomatic	0.00±0.000^{a}	0.00±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
Kepok	ABB	4±0.707^{c}	100.00±35.857^{e}	100.00±35.766^{f}	1692.222±1.000^{c}	2.931±0.001^{d}
Klutuk	BB	asymptomatic	0.00±0.000^{a}	0.00±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}

AUDPC = area under disease progressive curve; * subspecies of *Musa acuminata* (wild type).
Data on disease incidence and disease severity are results of analysis at age 21 wk.
AUDPC value indicates disease progression over time. Susceptibility level refers to highest score of disease severity (3).
Values (mean ± SD) within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

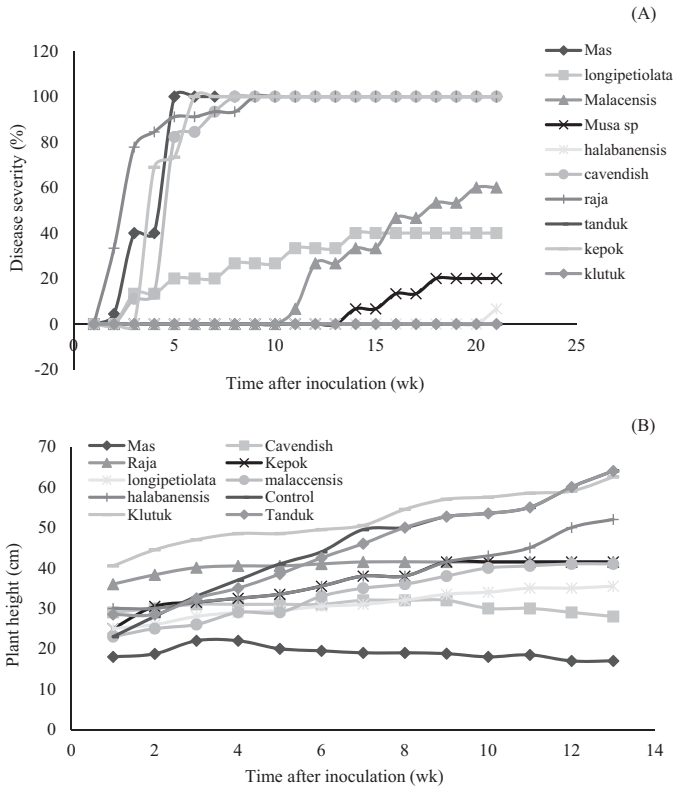

Fig. 6 (A) Severity and disease development of banana bunchy top virus on different banana varieties; (B) average growth of banana plant height

Subspecies *longipetiolata* showed slow-developing symptoms at the beginning of infection, with the incubation period lasting until week 2 after inoculation and proceeded at a slow pace. The number of symptomatic plants (4 out of 10) remained the same until the week 21 of observation, indicating the tolerant and resistant nature of the *longipetiolata* subspecies. Likewise, the *malaccensis* subspecies showed moderate resistance or tolerance to BBTV infection, as indicated by its incubation period of 10 wk.

Based on these data, it seemed that almost all banana plants could be infected by BBTV. Resistance reactions were evident in cv Tanduk from Purworejo and cv Klutuk Wulung from Banyumas. The resistance of these two plants probably occurred because BBTV was unable to infect them or there was a reaction of biochemical compounds as a result of virus infestation. Another possibility was the insect vector *P. nigronervosa* could not live and reproduce on these cultivars; consequently, the virus was not well distributed to the treated banana plants. This explanation was supported by the absence of aphids on day 5 after inoculation. According to Suparman et al. (2011), a plantain is not favorable for *P. nigronervosa*. In contrast, aphids were still found and reproduced on other banana plants 5 d after pesticide treatment. The unfavorable plants apparently prevented the aphids from transmitting BBTV to cv Tanduk and cv Klutuk Wulung.

BBTV infection affects the increase in plant height and is commonly known for the infection of other viruses (Hooks et al., 2008). Fig. 6B shows delayed growth in banana plants infected by BBTV in week 4 of observation on Mas, Cavendish, Kepok, Raja, subspecies *malacensis* and subspecies *longipetiolata*. This was in contrast to the uninfected plants that showed normal growth. Kluthuk and Tanduk did not exhibit slow growth, with even subspecies *halabanensis* continuing to grow, although more slowly than the other cultivars. This equated to slower disease development in *halabanensis* than in the other infected bananas and was indicative of the higher tolerance of *halabanensis* to BBTV. The chlorophyll content in banana species was closely related to disease severity (Table 4). There was a decreased chlorophyll content (chlorophyll b) in the plants infected with BBTV, with more severe symptoms than in uninfected (healthy) plants.

Table 4 shows the comparison of the average chlorophyll content in healthy and diseased banana plants, where chlorophyll b was apparently affected by BBTV infection of chlorophyll a. The decreased amount of chlorophyll in plants is believed to reduce photosynthetic capacity and plant growth (Hooks et al., 2008), producing chlorosis symptoms on the leaves as well as stunting plants. According to Anuradha et al. (2015) and Tanuja et al. (2019), changes in the chlorophyll content may be due to either the stimulation of chlorophyllase enzyme that can degrade chlorophyll or the influence of viruses on pigment synthesis. BBTV is thought to utilize plastid proteins and their precursors to synthesize their proteins (Eseverri et al., 2020). A low amount of chlorophyll may disrupt plant physiological processes, including photosynthesis.

Table 4 Chlorophyll content of infected and uninfected banana cultivars, where leaf samples were measured using ultraviolet-visible spectrometry at wavelengths of 663 nm and 645 nm.

Infection	Chlorophyll a (g/L)	Chlorophyll b (g/L)	Total chlorophyll (g/L)
Infected cultivars	0.033±0.001[a]	0.050 ± 0.001[a]	0.043 ± 0.004[a]
Uninfected cultivars	0.033±0.001[a]	0.053 ± 0.001[cb]	0.042 ± 0.002[a]

Values (mean ± SD, $n = 5$) in each column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Leaf discoloration is often considered a secondary result of viral infection because the virus accumulates and replicates in the cytoplasm (Zhao et al., 2016). The enumeration of the discoloration process is important to evaluate the resistance of plants to viral infections. The presence of BBTV in plants was tested based on PCR, using specific primers for DNA R BBTV. All DNA samples from the BBTV-inoculated plants tested positive based on PCR, except for the cultivars Tanduk and Klutuk; therefore, it was surmised that BBTV did not infect these two cultivars. Wild banana subspecies *longipetiolata, malacensis* and *halabanensis* also showed BBTV infection. According to Hooks et al. (2009), while all banana plants with AA/AAA genomes are highly susceptible to BBTV, plants with the B genome (AAB/ABB) are slightly susceptible or more resistant. However, the results f the current study indicated that not all banana plants with the AA/AAA genome were highly susceptible; for example, wild bananas with the AA genome had a susceptibility range of 0.006–0.981, which was much lower than for cultivated bananas. Banana plants with AAB/ABB genomes showed the same susceptibility as those with AA/AAA genomes. It was possible that some of the resistant bananas in the current study might have contained novel resistant genes in the A-type genome.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The UGM Research Directorate and Reputation Improvement Team towards World-Class Universities-UGM Quality Assurance Office (*Direktorat Penelitian UGM dan Tim Peningkatan Reputasi UGM menuju World Class Universitas-Kantor Jaminan Mutu UGM* (No. 6162/UN1/DITLIT/DIT-LIT/PT/2021). The Bill and Melinda Gates Foundation provided research funding with Prof. Assoc. Dr. John E. Thomas as the BBTV project leader (OPP1130226).

References

Ahamedemujtaba, V., Cherian, A.K., Namitha, P.M, Louis, V.I.M.I., Beena, S. 2019. Detection and biophysical characterization studies of cucumber mosaic virus causing infectious chlorosis disease of banana. J. Pharmacogn. Phytochem. 8: 2606–2611.

Anuradha, C., Selvarajan, R., Vasantha, S., Suresha, G.S. 2015. Biochemical characterization of compatible plant virus interaction: A case study with bunchy top virus-banana host-pathosystem. Plant Pathol. J. 14: 212–222. doi: 10.3923/ppj.2015.212.222

Arnon, D. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24: 1–15. doi.org/10.1104/pp.24.1.1

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.

Chen, H.-b., Fan, X.-l. 2018. Effects of magnesium remobilization and allocation on banana plant growth. J. Plant Nutr. 41: 1312–1320. doi.org/10.1080/01904167.2018.1450422

Eseverri, A., Baysal, C., Medina, V., Capell, T., Christou, P., Rubio, L.M., Caro, E. 2020. Transit peptides from photosynthesis-related proteins mediate import of a marker protein into different plastid types and within different species. Front. Plant Sci. 11: 560701. doi.org/10.3389/fpls.2020.560701

Foottit, R.G., Maw, H.E.L., Pike, K.S., Miller, R.H. 2010. The identity of *Pentalonia nigronervosa* Coquerel and *P. caladii* van der Goot (Hemiptera: Aphididae) based on molecular and morphometric analysis. Zootaxa 2358: 25–38. doi.org/10.11646/zootaxa.2358.1.2

Forbes, G., Pérez, W., Piedra, J.A. 2014. Field Assessment of Resistance in Potato to *Phytophthora infestans*. International Potato Center (CIP). Lima, Peru.

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). Jour. Agri. Sci. Tokyo Univ. of Agric. 51: 92–101.

González, R., Butković, A., Elena, S.F. 2019. Role of host genetic diversity for susceptibility-to infection in the evolution of virulence of a plant virus. Virus Evol. 5: vez024.

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

Hooks, C.R.R., Manandhar, R., Perez, EP., Wang, K-H, Almeida, R.P.P. 2009. Comparative susceptibility of two banana cultivars to banana bunchy top virus under laboratory and field environments. J. Econ. Entomol. 102: 897– 904. doi.org/10.1603/029.102.0306

Jebakumar, R.M., Balasubramanian, V., Selvarajan, R. 2018. Virus titer determines the efficiency of *Pentalonia nigronervosa* (Aphididae: Hemiptera) to transmit the banana bunchy top virus. Virus Dis. 29: 499–505. doi.org/10.1007/s13337-018-0493-x

Karan, M., Harding, R.M., Dale, J.L. 1994. Evidence for two groups of banana bunchy top virus isolates. J. Gen. Virol. 75: 3541–3546. doi.org/10.1099/0022-1317-75-12-3541

Kumar, P.L., Selvarajan, R., Iskra-Caruana, M.L., Chabannes, M., Hanna, R. 2015. Biology, etiology, and control of virus diseases of banana and plantain. In: Loebenstein, G., Katis, N.I. (Eds.). Advances in Virus Research, Vol. 91: Control of Plant Virus Diseases: Vegetatively-Propagated Crops. Elsevier Inc. Waltham, MA, USA, pp. 230–258.

Leiwakabessy, M., Nurulita, S., Hidayat, S.H. 2017. Disease incidence and molecular analysis of banana bunchy top virus in Bogor, Jawa Barat. In: International Proceedings on Tropical Horticulture 2016. Bogor, Indonesia, pp. 37–45.

Magee, C.J.P. 1927. Investigation on the bunchy top disease of the banana. Council for Scientific and Industrial Research Bulletin 30: 7–64.

Mandadi, K.K., Scholthof, K-B.G. 2013. Plant immune responses against viruses: How does a virus cause disease? Plant Cell. 25: 1489–1505. doi.org/10.1105/tpc.113.111658

Malhotra, H., Vandana, Sharma, S., Pandey, R. 2018. Phosphorus nutrition: Plant growth in response to deficiency and excess. In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (Eds.). Plant Nutrients and Abiotic Stress Tolerance. Springer Nature Singapore Pte Ltd. Singapore, pp. 171–190.

Ngatat, S., Hanna, R., Kumar, P.L., Gray, S.M., Cilia, M., Ghogomu, R.T., Fontem, D.A. 2017. Relative susceptibility of *Musa* genotypes to banana bunchy top disease in Cameroon and implication for disease management. Crop Prot. 101: 116–122. doi.org/10.1016/j.cropro.2017.07.018

Parac, E.P., Lalusin, A.G., Pangga, I.B., Cruz F.C., Sta. 2020. Characteristics of selected hybrids of abaca (*Musa textilis* Nee) with resistance to bunchy top. Philipp. Agric. Scientist. 103: 1–12.

Perrier, X., De Langheb, E., Donohuec, M., et al. 2011. Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proc. Natl. Acad. Sci. USA 108: 11311–11318. doi.org/10.1073/pnas.1102001108

Pesti, R., Kontra, L., Paul, K., Vass, I., Csorba, T., Havelda, Z., Va´rallyay, E. 2019. Differential gene expression and physiological changes during acute or persistent plant virus interactions may contribute to viral symptom differences. PLoS One 14: e0216618. doi.org/10.1371/journal.pone.0216618

Pfennig, K.S. 2001. Evolution of pathogen virulence: The role of variation in house phenotype. Proc. Biol. Sci. 268: 755–760. doi.org/10.1098/rspb.2000.1582

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. doi: 10.11248/JSTA.57.1

Poerba, Y.S., Martanti, D., Handayani, T., Herlina, Witjaksono. 2016. Musa Catalog: Collection of Banana Germplasm Gardens Biology Research Center-LIPI. LIPI Press. Jakarta, Indonesia.

Qazi, J. 2016. Banana bunchy top virus and the bunchy top disease. J. Gen. Plant Pathol. 82: 2–11. doi.org/10.1007/s10327-015-0642-7

Rahayuniati, R.F., Hartono, S., Somowiyarjo, S., Subandiyah, S., Thomas, J.E. 2021a. Characterization of the banana bunchy top virus on Sumatera (Indonesia) wild banana. Biodiversitas 22: 1243–1249. doi: 10.13057/biodiv/d220321

Rahayuniati R.F., Subandiyah. S., Hartono. S., et al. 2021b. Recent distribution and diversity analysis on the banana bunchy top virus of banana and alternative host in Indonesia. Trop. Plant Pathol. 46: 506–517. doi.org/10.1007/s40858-021-00443-3

Rao, S.A., Qureshi, M.A., Khanzada, A.L., Khanzada, M.A., Rajput, M.A., Rajput, K.I. 2002. Occurrence and incidence of banana bunchy top disease in southern part of Sindh. Pakistan J. Plant Pathol. 1: 74–75. doi: 10.3923/ppj.2002.74.75

Shaner, G., Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. Phytopathology 67: 1051–1056.

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

Stainton, D., Martin, D.P., Collings, D.A., Varsani, A. 2017. Comparative analysis of common regions found in babuviruses and alphasatellite molecules. Arch. Virol. 162: 849–855. doi.org/10.1007/s00705-016-3168-1

Suparman, Gunawan, B., Pujiastuti, Y., Arsi, Cameron, R.R. 2017. Alternatif hosts of banana aphid *Pentalonia nigronervosa* Coq. (Hemiptera: Aphididae), the vector transmitting banana bunchy top virus. J. Adv. Agric. Technol. 4: 354–359.

Suparman, Nurhayati, Setyawati, D.A. 2011. Preference and suitability of *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) to various banana varieties. J. Entomol. Indon. 8: 73–84. [in Indonesian]

Tanuja, N., Ramanathan, A., Vanitha, S., Soorianathasundaram, K., Kumar, K.K. 2019. Differential biochemical response among banana (*Musa* spp.) genotypes against banana bunchy top virus (BBTV). Curr. J. Appl. Sci. 38: 1–11. doi: 10.9734/cjast/2019/v38i630416

Tejada, J.A., Gara, G.P.P. 2017. Leaf check IT: A banana leaf analyzer for identifying macronutrient deficiency. In: Proceedings of the 3rd International Conference on Communication and Information Processing. Tokyo, Japan, pp. 458–463.

Torres-Bazurto, J., Magnitskiy, S., Sánchez, J.D. 2019. Effect of fertilization with N on height, number of leaves, and leaf area in banana (Musa AAA Simmonds, cv. Williams). Rev. Colomb. Cienc. Hortic. 13: 9–17. doi.org/10.17584/rcch.2019v13i1.8440

Vishnoi, R., Raj, S.K., Prasad, V. 2009. Molecular characterization of an Indian isolate of *Banana bunchy top virus* based on six genomic DNA components. Virus Genes 38: 334–344. doi.org/10.1007/s11262-009-0331-8

White, P.S., Choi, A., Pandey, R., Menezes, A., Penley, M., Gibson, A.K., de Roode, J., Morran, L. 2020. Host heterogeneity mitigates virulence evolution. Biol. Lett. 16: 20190744. dx.doi.org/10.1098/rsbl.2019.0744

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

Wirya, G.N.A.S., Sudiarta, I.P., Selangga, D.G.W. 2020. Disease severity and molecular identification of *Banana bunchy top virus*, infecting local banana in Bali Island. Jurnal Perlindungan Tanaman Indonesia 24: 11–16. [in Indonesian]

Zhao, J., Zhang, X., Hong, Y., Liu, Y. 2016. Chloroplast in plant-virus interaction. Front. Microbiol. 7: 1565. doi: 10.3389/fmicb.2016.01565