Survey of *Banana bunchy top virus* on non-cultivated bananas in West Java

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Abstract. Banana is a horticulture crop originated from Southeast Asia. Therefore, the genetic diversity of bananas is presumably high in Indonesia. Banana bunchy top disease caused by *Banana bunchy top virus* (BBTV) has been reported occurred in several regions in Indonesia, especially in the banana cultivation. A survey was conducted in April 2019 in West Java to make documentation on the types of bananas that grow unattended and to monitor BBTV infection. Banana cultivars with various types of genomes was found during the survey, i.e. "Mas", "Uli", and "Rejang" (type AA); "Ambon", "Muli", and "Urang" (type AAA); "Kapas" and "Raja" (type AAB). Besides, wild bananas were also found, i.e. "Caukole" in Cianjur and "Tongka Langit" in Tasikmalaya with genome types AAw and T, respectively. Leaf samples from infected plants were collected and taken to the laboratory for detection of BBTV using polymerase chain reaction (PCR) method. Specific DNA band of BBTV around 240 bp was successfully amplified from all leaf samples using mRepF/mRepR specific primer pairs. The results of this survey indicated that BBTV has spread in the field and has the potential to be a source of disease inoculum if no immediate control efforts are made.

1. Introduction
Banana bunchy top disease (BBTD) is considered as one of the most important diseases in cultivated banana plants. The disease is caused by infection of *Banana bunchy top virus* (BBTV), a member of Babuvirus. Besides through infected seedlings and suckers, BBTV transmission mainly occurs through its insect vector, i.e. banana aphid *Pentalonia nigronervosa* (Hemiptera: Aphididae) [1]. The incidence of BBTV has been widely reported in Asia, Australia, Africa and South Pacific regions. BBTV infection has also spread widely in banana plantations in Indonesia, including those in Bali, Yogyakarta and Lampung [2][3][4]. Incidence of BBTD in West Java in Bogor, Cianjur, Subang, Sumedang and Pandeglang has also been reported [5]. Based on field observations and experiments in the screenhouses, several banana cultivars such as ‘Muli’, ‘Jackfruit’, ‘Raja’, ‘Ambon’ and ‘Kepok’ are known to be susceptible to BBTV.

Disease management strategies for BBTD should be focusing on the effort to suppress the source of inoculum in the field. This can be effectively done when a BBTV-resistant cultivar is available.
Efforts to find sources of resistance to BBTV have been carried out through the selection of germplasms. However, a source of resistance to BBTV has not been found in banana cultivars that have long been cultivated in Indonesia. Therefore, it is necessary to conduct more extensive exploration to find sources of resistance, for example among wild banana species. There are two types of bananas from the Musa group, i.e. *Musa acuminata* and *M. balbisiana* which is believed to be the ancestors of today’s cultivated banana [6]. Indonesia is known as one of the centre of origin for world bananas, so it has a high diversity of germplasm, both wild and cultivated bananas [7].

Wild banana *M. acuminata* grows throughout Indonesia from Aceh to Papua, with various local names, such as pisang hutan, pisang monyet, awaikeken, pisang rimbo, pisang tengkayak, caukole, pisang cicielas, unti darek, punti lampung, nukanuibo [8]. Meanwhile, wild banana species *M. balbisiana* is spread in India, Burma, Thailand, China, Taiwan, Japan to the Philippines [9][10] and rarely found in Indonesia. Studies on the potential of Indonesian wild banana *M. acuminata*, especially as a source of resistance to disease have increased since it was known that wild bananas have resistance to disease [11][12][13].

This paper describes the survey work conducted in several areas in West Java to monitor BBTV infections from bananas that grow wild or uncultivated.

2. Materials and methods

2.1. Field survey and sampling

The survey was conducted in April 2019 in several areas in West Java, including Cianjur, Cimahi, Garut, Tasikmalaya and Subang Regencies. The focus of the study was (1) to explore wild banana species; (2) to observe the symptoms of BBTV infection in wild banana plants, and also banana cultivars that grow along roadsides, in paddy fields or other crop cultivation areas, and the middle of protected forest areas. Infection of BBTV may cause various symptoms, involving stunted plants, shrunken leaves, small leaves that are arranged at the top of the plant (rosette), bleaching of leaf veins and chlorosis. Leaf samples from plants showing symptoms were collected and brought to the laboratory to confirm BBTV infection using the PCR detection method.

2.2. Virus detection by PCR method

2.2.1. Total DNA extraction. Total DNA was extracted from banana leaf samples using CTAB method according to Doyle and Doyle (1987). Leaves (0.1 g) were ground using pistil and mortar with the addition of liquid nitrogen, then 500 µL of extraction buffer containing 1% 2-β mercaptoethanol was added. Plant extract was then put into a 2 mL microtube and incubated in a water bath at 65 °C for 1 h, turning it every 10 min to assist the lysis process. After 1 h, the tube containing the plant extract was removed, let stand for 2 min and 500 µL of the Chloroform: Isoamil alcohol (24: 1) mixture was added and then it was reversed for 5 min so that it was well mixed. Furthermore, centrifugation was carried out at a speed of 12 000 rpm for 15 min. The supernatant was transferred to a new tube, and 1/10 of potassium acetate (CH₃COOK) was added to the total volume. Furthermore, 2/3 of the total volume isopropanol was added, stand overnight at -20 ºC and then centrifuged at 12 000 rpm for 10 min. After centrifugation, the liquid was discarded, and 500 µL of 70% ethanol was added. The tubes were then centrifuged at 8 000 rpm for 5 min. The liquid is discarded, and the DNA precipitate (pellet) is dried in laminar airflow by placing the tube upside down on the tissue for one h. Pellet DNA can be resuspended by adding 50 µL of TE buffer 1x (10 Mm Tris-HCl pH 8.0 Mm EDTA).

2.2.2. DNA amplification. One PCR reaction composed of 0.5 µL MgCl₂ 25 mM, 8 µLddH₂O, 12.5 µL 2x MyTaq™ HS Red Mix Bioline, 1 µL F primer 10 µM, 1 µL R primer 10 µM and 2 µL DNA template. Amplification of DNA was performed using a specific mRepF / mRepR primer [14](Table 1) in GeneAmp® PCR System 9700 (Applied Biosystems USA). Amplification cycles are as follow: 94 ºC for 5 min for pre-denaturation, followed by 35 cycles of denaturation (94 ºC for 30 sec), annealing (55 ºC for 45 sec), and extension (72 ºC for 1 min), and post extension at 72 ºC for 5 min.
Amplicons were then visualized on 1% agarose gel using electrophoresis in 0.5x TBE (Tris Borate EDTA) buffer.

**Table 1.** Specific primers for amplification of BBTV

| Target DNA | Primer | Sequence (5’ to 3’) | Amplicon size (bp) | Reference |
|------------|--------|---------------------|-------------------|-----------|
| DNA-R*     | mRepF  | GCGTGAAACGCACAAAAAGGCC | 240               | [14]      |
| DNA-R*     | mRepR  | GCATAAGGTGTCAAACCTTCCTC |                  |           |

*DNA-R, encodes a replication initiation protein

3. Results and discussion

3.1. Variation of banana cultivars

A total of 12 banana cultivars were identified during the survey (Table 2). Two types of wild banana from the *M. acuminate* group, with the local names ‘Caukole Merah’ and ‘Caukole Hijau’ were found on a hillside in the Gunung Gede Pangrango National Park area in Cianjur Regency. The characters that distinguish the two types of wild banana include the colour of the leaves and the shape of the heart. Outside the Gunung Gede Pangrango National Park area, several types of cultivated banana cultivars grow unattended were found, i.e. ‘Mas’, ‘Uli’, ‘Ambon Kuning’, ‘Ambon Hijau’, ‘Kapas’ and ‘Raja Nangka’. Cultivars ‘Mas’ and ‘Raja Nangka’ were also found in Subang District in the research station of The Indonesian Tropical Fruit Research Institute.

**Table 2.** Banana cultivars found in the survey location in West Java

| Location (Regency) | Cultivar      | Local name | Genome type |
|--------------------|---------------|------------|-------------|
| Cianjur            | Mas           | AA         |             |
|                    | Uli           | AA         |             |
|                    | Ambon Kuning  | AAA        |             |
|                    | Ambon Hijau   | AAA        |             |
|                    | Kapas         | AAB        |             |
|                    | Raja Nangka   | AAB        |             |
|                    | Caukole Merah | AAw        |             |
|                    | Caukole Hijau | AAw        |             |
| Cimahi             | Muli          | AAA        |             |
|                    | Raja Nangka   | AAB        |             |
| Garut              | Uli           | AA         |             |
|                    | Ambon Kuning  | AAA        |             |
|                    | Muli          | AAA        |             |
| Tasikmalaya        | Rejang        | AA         |             |
|                    | Urang         | AAA        |             |
|                    | Muli          | AAA        |             |
|                    | Raja Nangka   | AAB        |             |
|                    | Kapas         | AAB        |             |
|                    | Tongka Langit | T          |             |
| Subang             | Mas           | AA         |             |
|                    | Raja Nangka   | AAB        |             |

Banana plants that grow unattended in the area along Jalan Raya Bandung consist of ‘Muli’ and ‘Raja Nangka’ in Cimahi Regency; and ‘Uli’, ‘Ambon Kuning’ and ‘Muli’ in Garut Regency. Another type
of wild banana found during the survey was ‘Tongka langit’ which grows in the rice fields in Tasikmalaya Regency. In the same area, several cultivars were found growing unattended, consisting of ‘Rejang’, ‘Urang’, ‘Muli’, ‘Raja Nangka’ and ‘Kapas’.

Cultivated bananas are the result of evolution from wild banana species, which are characterized by the level of ploidy and genome. Several types of bananas with the AA diploid genome are ‘Mas’ and ‘Bangkahulu’; AAA triploid genome are ‘Ambon’ and ‘Barangan’. In addition, several cultivars were cultivated from hybridization between _M. acuminata_ and _M. balbisiana_, for example the type of banana with the AAB triploid genome (‘Raja’, ‘Tanduk’), ABB (‘Kepok’, ‘Siem’), and tetraploid AAAB (‘Castrali’/‘Tarali’/‘Ustrali’). More or less 200 names of local cultivars are found in Indonesia. Some of them have been characterized molecularly, but almost all of them have never undergone genetic improvement or breeding. Several cultivars of Indonesian banana have also been studied and utilized in studies of their potential resistance to the pathogen [8][15].

### 3.2. Symptoms of BBTD in the field

Symptoms of BBTV infection in the field can be distinguished into three categories, i.e. mild, moderate and severe symptoms (Fig1). Mild symptoms involve limited vein clearing, dark green streaks on the lower part of the lamina and the petiole, but there is no significant reduction of the lamina width. Moderate symptoms involve noticeable vein clearing, chlorotic, and ragged margins of the leaf; significant reduction in petiole length, distance, and lamina width. Unique symptoms of BBTV is shown when infection is severe, involving brittle lamina, chlorotic, and ragged margins, sometimes with necrotic symptom; leaves failed to emerge, giving a bunched appearance; often with stunted plant growth. Mild symptoms were found in ‘Mas’, ‘Uli’, and ‘Kapas’ cultivars; moderate symptoms were found in cultivars ‘Urang’, ‘Ambon Kuning’, and ‘Ambon Hijau’; meanwhile, severe symptoms were found in ‘Tongka Langit’ and ‘Rejang’ cultivars. Symptom severity of BBTV infection is influenced by several factors, including the response of the plant and the phase of plant growth when infected. Plants infected in the early growth phase tend to show more severe symptoms than plants infected at a later stage of growth [16]. Furthermore, [17] stated that plants showing mild or moderate symptoms might have a resistance mechanism to BBTV.
Figure 1. Symptom severity of banana bunchy top disease at survey sites in West Java. ‘Caukole Merah’ wild banana shows no symptoms (A); mild symptoms on cv. Mas (B) and cv. Kapas (C); moderate symptoms on cv. Urang (D) and cv. Ambon Kuning (E); severe symptoms on cv. Tongka Langit (F) and cv. Rejang (G)
3.3. Detection of BBTV

The specific primers used to detect BBTV from field samples successfully amplified the target DNA band measuring 240 bp (Fig 2). This result confirmed BBTV infection in symptomatic plants. The identity of BBTV infecting banana plants in the survey area in West Java needs to be further characterized through the analysis of the nucleotide sequence of these amplified DNA fragments. However, the confirmation of BBTV infection on samples mainly collected from non-cultivated banana plants indicated the widespread of the disease. Infection of these plants may occurred through transmission by aphids vector or banana suckers as propagative materials.

![Amplification of specific BBTV DNA fragments from banana leaf samples using mRepF/mRepR primers. Visualization on 1.5% agarose gel in 0.5x TBE. 100 bp DNA marker (M), positive control (K +), healthy plants (K -), leaf samples from West Java (no. 1 to 14)](image)

**Figure 2.** Amplification of specific BBTV DNA fragments from banana leaf samples using mRepF/mRepR primers. Visualization on 1.5% agarose gel in 0.5x TBE. 100 bp DNA marker (M), positive control (K +), healthy plants (K -), leaf samples from West Java (no. 1 to 14)

4. Conclusion

BBTV infection has been confirmed from wild banana (‘Tongka langit’) and several types of cultivated banana that grow unattended in Cianjur, Cimahi, Garut, Tasikmalaya and Subang Regencies. These results indicate that BBTD has spread in the field and has the potential to become a source of disease inoculum if no control measures are taken immediately.

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