Survey and detection of *Banana bunchy top virus* in Java

SM Lestari and SH Hidayat

1Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, IPB Darmaga Campus, Bogor 16680 Indonesia

E-mail: srihendarstutihidayat@gmail.com

**Abstract.** *Banana bunchy top virus* (BBTV) is one of the most important virus causing disease on banana plantation. Infection of BBTV is indicated by unique symptoms involving yellowing and curling of leaf margin, and stunting of plant. Research was conducted to determine the distribution of banana bunchy top disease (BBTD) in West Java (Bogor and Sukabumi), Central Java (Pati and Magelang), and East Java (Malang and Banyuwangi) based on symptom variations and disease severity. Field samples was then confirmed by polymerase chain reaction (PCR) detection method using four specific primers, each amplified different region of BBTV genome, i.e CP1/2 which amplified coat protein gene in DNA S, mRep1/2, BBT1/2, and BBTV-F/-R which amplified replication gene in DNA R in different region. Symptoms and incidence of BBTD was varied across banana plantation in Java. The most common symptoms observed in the field includes chlorotic, green mosaic, narrow, and upright appearance of the leaves, and stunting of the plant. Incidence of BBTV was found more severe in West Java than those in Central Java and East Java. All primers used in the detection method was successfully amplified the target DNA fragment. DNA R primers were more sensitive than DNA S primers and the better amplification result was achieved using BBT1/2 and BBTVR-F/-R primers.

1. Introduction

Banana bunchy top disease (BBTD) caused by *Banana bunchy top virus* (BBTV) is one of the most severe and widespread virus limiting production of banana and plantain (*Musa* spp.) [1]. BBTV is transmitted by banana aphid *Pentalonia nigronervosa* in a persistent, circulative, non-propagative manner [2]. The common symptoms of BBTD involves plant stunting, leaf chlorosis, leaf blight, marginal yellowing of the younger leaves, dark green, dot-dash streaks along the veins, petioles and midribs, and shortened internodes [3,4]. Infection of BBTV can contribute up to 100% yield loss in the Old World, especially in Asia Pacific and lesser extent in the Africa continent [5].

Incidence of BBTD in Indonesia has been reported in Sumatra (West Sumatra, North Sumatra, Riau, Lampung, and South Sumatra), West Java, East Java, and Bali [4,6,7,8,9]. However, little is known on disease severity and its effect on different banana cultivars. Symptoms of BBTV infection were observed on the common banana cultivars, such as Muli, Nangka, Raja, Kepok, Ambon, and Tanduk [6]. It is widely known that Southeast Asia, including Indonesia, is one of the center of origin of the...
genus *Musa*, and more than 200 varieties of banana are grown in many areas, from low land to high land [10]. Therefore, it is necessary to study the distribution of BBTV in Indonesia in order to understand the pathosystem which may help for development of mitigation strategy to protect banana germplasms in Indonesia.

One important component for conducting an efficient disease survey is the availability of detection method, in this case BBTV detection method. Serological method by ELISA (enzyme-linked immunosorbent assay) using monoclonal or polyclonal antibodies is commonly used for BBTV detection [5]. However, this method requires continuous availability of BBTV antibodies which nowadays is not easy to obtain. Nucleic acid-based detection method, for instance PCR (polymerase chain reaction), nucleic acid spot hybridization, and real time PCR were more popular for BBTV detection recently. Furthermore, Loop-mediated isothermal amplification (LAMP) and rolling circle amplification (RCA) have also been used for BBTV diagnostic [5,11]. In the present study, we discussed the use of PCR using four different primer pairs to detect BBTV from field samples.

2. Material and Methods

2.1 Field survey and samples collection

Surveys were conducted in West Java (Bogor and Sukabumi), Central Java (Pati and Magelang), and East Java (Malang and Banyuwangi) on October 2017 to January 2018. Leaf samples were collected from symptomatic plants for virus analysis in the laboratory. The samples were categorized into three groups based on the severity of the symptoms, i.e mild, intermediate and severe infection (Table 1). Top leaves of infected plants was cutted and stored in plastic bags. The samples was stored as dried samples by adding silica gel or as wet samples by keeping them in -80°C.

| Symptoms severity | Symptom description |
|-------------------|---------------------|
| Mild              | Limited vein clearing and dark green streaks on the lower part of lamina and on petiole. No significant reduction of lamina width. |
| Intermediate      | Vein clearing, upturned leaf, chlorotic, and ragged margins. Significant reduction in petiole length, distance, and lamina width. |
| Severe            | Brittle lamina with upturned, chlorotic, and ragged margins, sometimes with necrotic symptom. Leaves failed to emerged, giving a clear bunched appearance. |

2.2 Virus detection

Field samples was subjected for BBTV detection by polymerase chain reaction (PCR) using four specific primers, each amplified different region of BBTV genome, i.e. coat protein gene in DNA S using CP1/2, different regions of replication genes in DNA R using mRep1/2, BBT1/2, and BBTV-F/-R (Tabel 2). Total genomic DNA was isolated using the protocol developed by Doyle and Doyle [12] with modification. The PCR reaction consists of 1 µl genomic DNA as template, 12.5 µl Master mix (MyTaq™ HS Red Mix Bioline), 10 µM each of forward and reverse primer, 25 mM MgCl₂, and dH₂O to make a final volume of 25 µl. DNA amplification was conducted in thermal cycler (Applied Biosystems 9700 PCR System) and the amplification products was subjected to electrophoresis in a 1.2% agarose gel in 1x TBE buffer at 50 volts for 50 minutes. The agarose gel was stained using ethidium bromide and visualized under UV transilluminator.

| Target DNA | Primer | Sequence (5’ → 3’) | Amplicon size | Reference |
|------------|--------|--------------------|---------------|-----------|
| DNA-S      | CP1/F  | CCCGGGAGAATACTTCACTGGGCTATGATT  | 1083 bp       | [13]      |
|            | CP2/R  | CCCGGGCTTCACCTTGACACCAACAGCAT   |               |           |
3. Results and Discussion

3.1 Result

3.1.1 Incidence and severity of BBTD in Java

Present survey demonstrated the diversity of banana cultivars grown in Java. A total of 126 samples were taken from three provinces consisting of 51 cultivars. The most cultivar diversity was found in East Java (24 cultivars), followed by Central Java (18 cultivars) and West Java (9 cultivars) (Table 3). Most of the cultivars are known to have triploid genome (AAA, AAB, and ABB), although some cultivars with diploid genome (AA) was also found during the survey. The most common symptoms observed in the field include yellowing, slightly chlorotic margins along the new leaves, chlorotic, green mosaic, narrow and upright appearance of the leaves, and stunting of the plant. Infected plants produce no fruit or a reduce bunch with no market value. Incidence of BBTV was found more severe in West Java (10% to 80%) than those in Central Java (4.8% to 76.2%) and East Java (24.0% to 40.0%) (Table 4). Symptom severity was varied among different location in Java. More severe symptom was observed in Sukabumi, West Java whereas intermediate and mild symptom was found more in Malang, East Java and Magelang, Central Java, respectively. The correlation between symptom severity and the type (cultivar) of banana remain to be tested, although in the present study the severe symptom was commonly found in cv. Mas (AA genome) while mild symptoms were found in cv. Kepok (ABB genome) (Table 4).

Table 3. Survey location and the common banana cultivars grown in Java

| Survey location | Regency | Local name | Cultivar | Genome |
|-----------------|---------|------------|----------|--------|
| West Java       | Sukabumi| -Mas kirana| AA       |
|                 |         | -Cavendish | AA       |
|                 |         | -Barangan  | AA       |
|                 |         | -Raja Bulu | AAB      |
|                 | Bogor   | -Lampung   | AA       |
|                 |         | -Uli       | AA       |
|                 |         | -Raja      | AAB      |
|                 |         | -Apu/Awak/Kepok siam | ABB |
|                 |         | -Nangka    | AAA      |
| Central Java    | Pati    | -Putri/Muli| AA       |
|                 |         | -Mas       | AA       |
|                 |         | -Raja      | AAB      |
|                 |         | -Ambon     | AAA      |
|                 |         | -Raja bulu | ABB      |
|                 |         | -Raja nangka | AAA   |
|                 |         | -Kepok     | ABB      |
Table 4. Disease severity of banana bunchy top disease in Java

| Province   | Survey location | Total No. of sample | No. of sample showing disease severity (%) | Mild | Intermediate | Severe |
|------------|-----------------|---------------------|------------------------------------------|------|--------------|--------|
| West Java  | Sukabumi        | 10                  |                                          | 10.0 | 10.0         | 80.0   |
|            | Bogor           | 8                   |                                          | 37.5 | 25.0         | 37.5   |
| Central Java | Pati           | 31                  |                                          | 35.5 | 16.1         | 48.4   |
| East Java  | Malang          | 25                  |                                          | 76.2 | 4.8          | 19.0   |
|            | Magelang        | 21                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
|            | Malang          | 25                  |                                          | 24.0 | 40.0         | 36.0   |
3.1.2 Detection of BBTV using four specific primers

All primers used in the PCR method was successfully amplified the target DNA fragment, i.e. 1081 bp and 240 – 350 bp for DNA S and DNA R, respectively (Figure 1). However, some primers gave more successful amplification results than the others. The number of samples successfully amplified using CP1/2 and mRep 1/2 primers were in the range of 42.9% to 69.2% and 90.5% to 100%, respectively. In contrast, DNA target were always successfully amplified using BBT 1/2 and BBTVR-F/-R primers (Table 5). In general, DNA R primers was more efficient than DNA S primers, and the better amplification result was achieved using BBT1/2 and BBTVR-F/-R primers. However, positive amplification was obtained from negative control samples when using DNA R primers, which indicated false positive reaction (Figure 1).

![Figure 1](image_url)

**Figure 1.** Amplification of *Banana bunchy top virus* using four specific primers, i.e. CP1/2, mRep1/2, BBT1/2, and BBTVR-F/-R. M: marker DNA (1 kb), C+: positive control, C-1 and C-2: negative control, S: field samples.

| Symptom severity | Total No. of sample | No. of sample successfully amplified (%) | CP1/2 | mRep 1/2 | BBT 1/2 | BBTVR-F/-R |
|------------------|---------------------|----------------------------------------|-------|----------|---------|------------|
| Mild             | 21                  |                                        | 42.9  | 90.5     | 100     | 100        |
| Intermediate     | 13                  |                                        | 69.2  | 100      | 100     | 100        |
| Severe           | 26                  |                                        | 69.2  | 96.1     | 100     | 100        |

3.2 Discussion

Banana and plantains cultivation is widely distributed throughout the world, especially in the tropics region. The major banana growing countries include those in south and southeast Asia and the Pacific (37%), tropical Africa (30%), Central and South America and the Carribean (26%). Based on the history of distribution and spread of banana tree, four regions were known as the center of origin of *Musa* species, i.e. (1) northeast India, north Myanmar, southeast China, (2) peninsular Malaysia, west
Indonesia, (3) Philippines, and (4) New Guinea and adjacent islands [16]. High diversity of wild varieties of banana were reported from those regions. Diseases such as BBTD should be managed carefully in order to preserve and conserve the diversity of banana in this region. The survey showed that BBTV infection was spread in Java. A total of 51 banana cultivars were recorded, and BBTD was easily observed in the field infecting all the cultivars with various degree of severity. Severe symptoms were commonly found in cv. Mas (AA genome) while mild symptoms were found in cv. Kepok (ABB genome). According to [17] banana plants having B type genotype tend to have resistance response to BBTV infection. Evaluation of banana cultivars in Democratic Republic of Congo showed that the highest BBTV incidence was observed on ‘Poyo’ genotype (AAA). Out of 40 genotypes of banana tested, only 8 genotypes [Musa balbisiana type Tani (BB), ‘Kayinja’ (ABB), ‘FHIA-03’ (AABB), ‘Prata’ (AAB), ‘Gisandugu’ (ABB), ‘Pisang Awak’ (ABB), ‘Saba’ (ABB) and ‘Highgate’ (AAA, Gros Michel subgroup)] did not show any visible symptoms after 28 months after trial. This result supported the previous report which indicated that banana genotypes with one or two B genoms are more tolerant to BBTD [18]. Moreover Furuya et al. (2012) demonstrated that banana cv. Itobasho (Musa balbisiana var. liukiuensis) with BB genome have resistance to BBTV infection. This information is valuable especially for developing resistant varieties as a strategy to control BBTD.

Another important strategy to control BBTD is the availability to detect BBTV infection accurately. PCR method has been widely used for BBTV detection following optimization of the method due to some difficulties. Leaf extract of banana, grapevine and peanut has been observed have inhibitory factors on PCR method. The nature cell wall (thickness and composition), the presence of polysaccharides (e.g. starch) and reactive secondary metabolites (e.g. phenolics), the tissue type infected (e.g. phloem) and the type of viral infection (systemic or localized) may have an effect. Oxidation of banana extract (browning of supernatant) was correlated with an increasing inhibition of PCR [19]. Two specific primers, CP1/2 and mRep 1/2 primers were able to amplify BBTV isolates from sub-Saharan Africa (SSA) including those from Congo, Benin and Pakistan [11,13,20,21]. Another specific primer of BBTV, BBT1/2 primers were able to amplify BBTV isolate from Australia, Egypt, Western Samoa, Taiwan, Vietnam, Burundi, Congo and Rwanda [14,22]. The fourth specific primer of BBTV, BBTV R F/R primers were able to amplify BBTV isolates from the Kingdom of Tonga in South-west Pacific Ocean [15]. All four specific primers of BBTV was successfully amplified BBTV fragments from leaf samples collected from banana field in Java. However, DNA R primers (mRep 1/2, BBT1/2 and BBTV-F/R) amplified DNA target more frequently than CP1/2 primers which amplified DNA S as the target. This result might be influenced by the shorter size of target DNA for DNA R primers (~241 - 350 bp) than those of DNA S primers (~ 1083 bp). Based on our experiences, it is recommended to use DNA R primers for detection of BBTV from field samples. The use of DNA S primers is more suggested for advance analysis of BBTV sequences.

4. Conclusion

Banana bunchy top disease is one of the most important disease of banana in Java. Typical disease symptoms were observed in all banana cultivars commonly grown in West, Central, and East Java. Incidence of BBTD in West Java was more severe than those in Central Java and East Java. PCR-based detection method is recommended to confirm BBTV infection from field samples. Four specific primers, CP1/2, mRep1/2, BBT1/2, and BBTVR-F/-R, were able to amplify the DNA target, although DNA R primers (mRep1/2, BBT1/2, and BBTVR-F/-R) were more efficient than DNA S primer (CP1/2).

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