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First report of banana bunchy top disease on banana in Bengkulu

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ABSTRACT : Banana is a horticulture crop that has economic value and is widely cultivated in tropical countries. Banana production in Bengkulu province reached 259,748 quintals, then durian (110,387 quintals), tangerines (94,396 quintals) (BPS 2015). Banana bunchy top disease caused by Banana bunchy top virus (BBTV) infection is considered the most crucial virus disease affecting yield losses of a banana plantation in Asia, Africa, and the South Pacific. However, the incidence and molecular characters of BBTV has never been reported in Bengkulu. This research aims to characterize symptom variations, disease incidence, and disease severity of BBTV infection in Bengkulu and virus detection using molecular methods by polymerase chain reaction (PCR). Disease incidence of BBTV was measured based on field symptoms. The disease survey was conducted in Bengkulu city, Bengkulu Utara district, and Rejang Lebong district. The study showed that the incidence of BBTV in Bengkulu City, Bengkulu Utara, and Rejang Lebong ranged from 0% to 100%. The most common symptoms observed in the field involved vein clearing, upturned leaf, chlorotic, and ragged margins, reducing petiole length, distance, lamina width, and stunting. Banana crops that are infected with BBTV in the vegetative phase will not produce fruit. In contrast, viral infection in the generative phase causes the formation of stunted fruit that is not suitable for harvesting. Thus, the potential loss of yield due to stunted disease can reach 100%. This study's results are the first reports of BBTV infection in banana crops in Bengkulu. Disease diagnosis will form the basis of disease control strategies in banana crops.

Keywords : Bunchy top disease, disease incidence, disease severity, symptom

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INTRODUCTION

Banana is a horticulture crops that has economic value and is widely cultivated in tropical countries. Banana is one of important fruits in Bengkulu. Banana production in Bengkulu province reached 259,748 quintals, then durian (110,387 quintals), tangerines (94,396 quintals) (BPS 2015). Banana bunchy top disease (BBTD) is considered as the most important virus disease affecting banana plantation in Asia, Africa, and South Pacific (Furuya et al., 2005; Hooks et al., 2009). Banana bunchy top disease (BBTD) is caused by the *Banana bunchy top virus* (BBTV), which is a species of the genus *Babuvirus* in the family *Nanoviridae*.

BBTD is dispersed over long distances through the exchange of infected suckers and / or through non-virus indexed tissue culture (TC)-derived plantlets (Thomas & Caruana, 2000). Through these propagules, the virus is introduced into new areas where the banana aphid *Pentalonia nigronervosa* (Hemiptera, Aphididae) occurs (Almeida *et al*., 2009). This virus only transmitted persistently by its specific vector, banana aphid, *Pentalonia nigronervosa* (Hemiptera: Aphididae).

BBTV is a member of Genus Babuvirus in Family Nanoviridae, containing at least six genome components, each approximately 1000 bp size. The six genome components referred to DNA-R, DNA-U3, DNA-S, DNA-M, DNA-C, and DNA-N,encoding different types of protein (King *et al*. 2012). BBTV isolates in the world fall into two geographic phylogenetic groups, the South Pacific group (SPG) and Asian group (AG) based on genome components (Karan *et al*. 1994).

In the 1990s, the first severe outbreak of BBTD in Africa was estimated to have reduced banana production in the Nkhatabay and Nkhotakota districts of Malawi from 3500 ha to about 800 ha (Soko et al., 2009; Kumar et al., 2011). Although accurate estimates of yield losses are lacking for the Great Lakes countries of Africa, about 90% yield loss was reported in severely BBTD-infected plants of susceptible cultivars such as Poyo and AAA-Cavendish in a screening trial conducted in the Rusizi valley, in Burundi (Niyongere et al., 2011a). Because of the high destructive potential of the disease, Banana bunchy top virus (BBTV) was listed as one of the world's 100 worst invasive species and the International Plant Protection Convention included it as a pathogen to be subjected to rigorous quarantine measures (IPPC, 2010; Kumar *et al*., 2011). The incidence of BBTD in Indonesia has been reported in Sumatra, Bali, and Special Territory of Yogyakarta (Furuya *et al*. 2004; Pinili *et al*. 2011; Chiaki *et al*. 2015). However, the incidence and molecular characters of BBTV has never been reported in Bengkulu. The objective of this research was to characterize symptom variations, disease incidence, and disease severity of BBTV infection in Bengkulu, Indonesia.

MATERIALS AND METHODS

Field Survey

Survey was conducted on several banana plantations in Bengkulu city, Rejang Lebong district , and Bengkulu Utara district, Bengkulu province. Infection of BBTV was observed using purposive sampling method based on BBTV common symptoms i.e. stunting, bunched up leaves, and streaking between leave margins and midrib. Disease incidence (DI) of BBTV in each plantation was calculated using the following formula:

$$DI = \frac{\sum \text{symptomatic plants}}{\sum \text{total banana population}} \times 100\%$$

Table 1 Description of BBTV symptom severity

Symptom Severity	Symptom Description
Mild infection	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.

Based on the severity of the symptoms, the level of infection was categorized into three groups, i.e. mild, intermediate, and severe infection (Table 1).

Virus Detection by Polymerase Chain Reaction of BBTV

PCR was conducted to confirm the infection of BBTV from leaf samples. Total viral DNA was isolated from infected leaf following a procedure described by Doyle and Doyle (1987) with minor modification. Fresh tissue (0.1 g) was ground in liquid Nitrogen to powder, 500 µl of CTAB buffer (10% Cetyl-trimethyl-ammonium bromide, 0.1 M Tris-HCl pH 8, 0.05 M EDTA, 0.5 M NaCl, 1% β-mercapto-ethanol) was added, and the sap was transferred to 1.5 ml clean tube. The sap was incubated in water bath at 65 oC for 1 hr, then shaked every 10 min to separate lipid and protein. 500 µl of chloroform/ iso-amyl alcohol (24:1, v/v) was added to the liquid, then tube was vortexed for 5 min, and centrifuged at 14 000 rpm for 15 min. The supernatant was pipetted to 1.5 ml clean tube, 3 M ammonium acetate and isopropanol of 1/10 and 2/3 volume supernatant, was added respectively. The liquid was mixed gently then incubated overnight at -20 oC or 4 hr at room temperature. After incubation, the liquid was centrifuged at 12 000 rpm for 10 min to precipitate DNA and then discarded flow-through. The pellets were washed with 500 µl of 70% ethanol, centrifuged at 8000 rpm for 5 min and dried under room temperature after discarding the flow through. Dried pellets containing total DNA were dissolved in 50 to 100 µl of nuclease free water or TE buffer (pH 8) and the DNA was ready for amplification.

DNA can be used as DNA templates for further PCR amplification using two specific primer pairs for BBTV CP1/FCCCGGGAGAATACTTCACTGG GCTAT GATT and CP1/R CCCGGGCTTCACCTTGCACACCAAC A GCAT with target sequence 1083 bp (Mansoor *et.al.* 2005). Amplification of DNA was conducted based on method described by Kumar *et al*. (2011). The DNA was amplified in GeneAmp PCR system 9700 machine with 5 min at 94 ^{o}C for pre-heating, followed by 35 cycles of denaturation (30s at 94 ^{o}C), annealing (45s at 55 ^{o}C), and extension (30s at 72 ^{o}C), with final extension of 7 min at 72 ^{o}C. Amplicons was then visualized on 1% agarose gel using electrophoresis in TBE 0.5x buffer. The PCR was conducted on twelve symptomatic banana samples.

RESULTS AND DISCUSSION

Symptoms of BBTV

Symptom of BBTV can be observed on the lamina of the leaves. The initial symptoms were characterized by the appearance of dark green streak and dots on petiole and lower part of lamina, also slightly chlorotic margins along the new developing leaves. However, the dark green streak can be absent from some cultivars and severe symptoms usually developed since the first leaf of plants derived from infected planting materials (Thomas 2008). The general symptoms that observed in all banana crops in Bengkulu involved upturned leaves, chlorotic and ragged margins (figure 1 A), with leaves failed to emerge and stunting (figure 1 C). Generative plant failed to develop fruits (figure 1B)

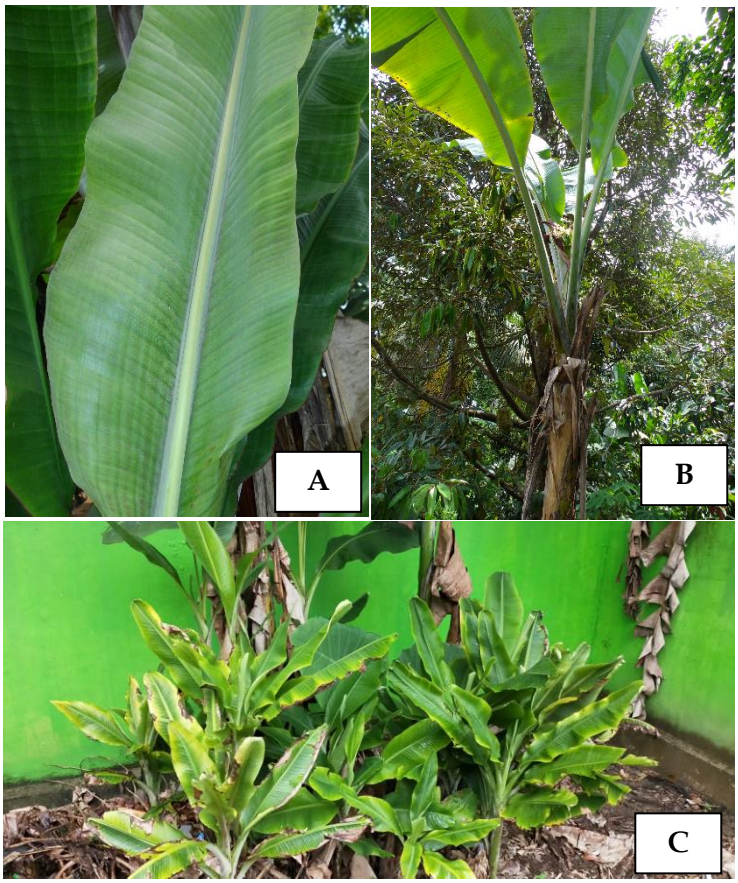

Figure 1. Cultivated banana plants showing mild symptom (A), failed to developed fruits (B), and stunting (C) of BBTV infection in several cultivation area at Bengkulu

The incidence of BBTD in banana plantation in Bengkulu ranged from 0% to 100% (table 2). The highest disease incidence was observed on 'Mas' varieties in Singaran Pati (Bengkulu city) and 'Jantan' varieties in Dusun Curup (Bengkulu Utara) with DI value of 100%, while the lower DI were observed on 'Kepok' varieties in Bengkulu city. The most common symptoms observed in the field involved vein clearing, upturned leaf, chlorotic, and ragged margins with reduction in petiole length, distance, lamina width, and stunting. Banana crops that infected with BBTV in the vegetative phase will not produce fruit, while viral infection in the generative phase causes the formation of stunted fruit that is not suitable for harvesting. Thus, the potential loss of yield due to stunted disease can reach 100%.

A study of BBTV epidemiology in Burundi, Africa indicated that incidence of BBTD significantly varied according to trial location, banana cultivar and planting materials (Niyongere *et al*. 2011). Based on this study, 'Mas' and 'Jantan' varieties is susceptible to BBTV infection, while no disease symptom on 'Kepok' vareties. Previous research in Indonesia reported that 17 out of 38 banana cultivars that grown in banana germplasm field in Yogyakarta were infected by BBTV, with 5 cultivars ('Kepok Gabu', 'Raja Entos', 'Raja Trunpong', 'Rejang' and 'Tanduk Hijau') among them were positively infected without virus symptoms (Furuya *et al.* 2004). There are no comprehensive report confirming any banana cultivars that

Table 2. Disease incidence and disease severity of BBTD in banana crops in Bengkulu

No	Survey location	Varieties	Disease incidence (%)	Disease severity
1	Muara Bangkahulu (Bengkulu city)	Mas	4/24 (16.67)	4 severe
2	Muara Bangkahulu (Bengkulu city)	Kepok	0/36 (0)	No symptom
3	Muara Bangkahulu (Bengkulu city)	Jantan	12/39 (30.77)	10 severe 2 intermediate
4	Sungai serut (Bengkulu city)	Mas	5/19 (26.31)	5 intermediate
5	Singaran Pati (Bengkulu city)	Mas	12/12 (100)	10 severe 2 intermediate
6	Ratu Samban (Bengkulu city)	Jantan	10/83 (12.05)	10 severe
7	Teluk Segara (Bengkulu city)	Jantan	5/23 (21.74)	5 intermediate
8	Dusun Curup (Bengkulu Utara)	Jantan	20/20 (100)	20 severe
9	Curup Utara (Rejang Lebong)	Mas	15/17 (88.23)	15 severe

completely resistant to BBTV infection. However, previous study have provided evidence that banana cultivars showed different response against infection of BBTV(Hooks 2008; Niyongere 2011).

Virus Detection using Polimerase Chain Reaction

BBTV was not successfully detected by PCR using specific primer. Futher optimation of PCR reaction using this primer should be conducted for the next study.

CONCLUSION

Incidence of BBTD in Bengkulu is considered high based on symptom observation. Disease incidence of BBTV in Bengkulu City, Bengkulu Utara and Rejang Lebong ranged from 0% to 100%. The most common symptoms observed in the field involved vein clearing, upturned leaf, chlorotic, and ragged margins with reduction in petiole length, distance, lamina width, and stunting. BBTV was not successfully detected by RT-PCR using specific primer. Futher optimation of PCR reaction using this primer should be conducted for the next study.

SUGGESTION

Further research on epidemiology and evaluation of the resistance of banana varieties against BBTV infection should be conducted in order to provide suitable controling methods and virus free planting materials for integrated disease management of banana bunchy top disease.

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