# FIRST REPORT OF *Begomovirus* INFECTION ON PAPAYA IN BENGKULU, INDONESIA

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#### ABSTRACT

*First report of* **Begomovirus** *infection on papaya in Bengkulu, Indonesia.* A field survey was conducted during 2019, we found a severe systemic yellow mosaic, striped green mosaic on leaves petiole, green spots on the fruit of papaya, leaf malformation, and stunting symptoms on three papaya cultivation area in Rejang Lebong, Kepahiang, Bengkulu Tengah, and Seluma, Bengkulu Province, Indonesia. A begomo-like virus was inferred to be the possible cause of the virus-disease-like symptoms. The study aimed to identify the causal of those typical symptoms on papaya. PCR using universal primer for transcriptional activator protein (TrAp) and replication-associated protein (Rep) gene of *Begomovirus* successfully amplified the DNA fragments of 900 bp in all 10 detected samples, except for samples with leaf malformation and stunting symptoms. It is indicating that those typical symptoms on papaya is associated with *Begomovirus* infection, while the causal of leaf malformation and stunting is unknown yet. This work is the first report of *Begomovirus* infected papaya in Indonesia. Severe disease incidence caused by this pathogen was observed on papaya plants in Bengkulu Province that was in the range of 42–100%. This finding is a precious information to be used for identification, and characterization the species of the virus, determining control strategies against the disease.

Key words: leaf malformation, polymerase chain reaction, stunting, yellow mosaic

### **INTRODUCTION**

Papaya (*Carica papaya*) is one of the important fruit and widely cultivated in Indonesia. At least 20 plant viruses species have emerged in tropical and subtropical regions infecting papaya (Tennant *et al.*, 2007). The severity of disease symptoms varies widely from mild to severe; reducing plant vigor, yield, and impaired fruit quality. Numerous *Begomovirus* and *Potyvirus* have been characterised from papaya in India. The study of *Papaya leaf curl virus* (PalCV) was reported in 1998 (Saxena *et al.*, 1998). Another reports of *Begomovirus* infecting papaya i.e. *Papaya leaf crumple virus* (PalCrV), *Chilli leaf curl virus* (ChilCuV), *Tomato leaf curl New Delhi virus* (ToLCuNDV), *Croton yellow vein mosaic virus* (CYVMV) (Chang *et al.*, 2003; Singh-Pant *et al.*, 2012) have been documented. There have not been many reports about diseases caused by viral infections in papaya plants in Indonesia. Previously, the causal of ringspot disease on papaya by *Papaya ring spot virus* strain P (PRSV-P) have been reported in Indonesia. First report of PRSV infection on papaya in Nanggroe Aceh Darussalam Province (Hidayat *et al.*, 2012). It was also reported in Medan, Bogor, and Bali, with disease incidence reaching 100% (Hidayat *et al.*, 2013; Harmiyati *et al.*, 2015; Nuhantoro *et al.*, 2017). Furthermore, Listihani *et al.* (2018) reported the present of PRSV-P on cucumbers in Java.

The diseased papaya leaves found in Bengkulu was different to those found in other area in Indonesia. During field survey in January to October 2019 at seven papaya cultivation areas in 4 districts in Bengkulu Province (Figure 1), we found a systemic symptom of yellow mosaic, upward leaf curling, and stunting, dark green and light green striped mosaic patterns on the petiole, and green spots on papaya fruit in almost all locations. The symptoms were typically caused by plant pathogenic viruses. The symptom found on papaya leaves in Bengkulu showed severe mosaic with a distinctive yellow color indicating that it may caused by different virus(es). The study aimed to identify the causal virus of severe yellow mosaic on papaya plants in Bengkulu Province. This information is important in developing appropriate control measures that would prevent the spread of the virus to another area where the disease is not yet present.

# **MATERIALS AND METHODS**

**Research Site**. A field survey was conducted from January to October 2019 at Rejang Lebong, Kepahiang, Bengkulu Tengah, and Seluma, Bengkulu Province (Figure 1). Virus detection was carried out in Laboratory of Genetics and Biotechnology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu.

**Sample Collection**. Leaf samples were collected from the plants aged 8–12 months after planting. Samples were taken with purposive-random sampling according to the virus infection symptom. Papaya leaf samples showing typical of virus infection such as yellow mosaic, leaf curling, smaller leaflet, and stunting were collected from several papaya producing area in Bengkulu Province (Figure 1). The observation was conducted directly in the field with a 300–800 m<sup>2</sup> area. The disease incidence was determined by counting the number of plants that show the symptoms from 30% of the plant population. Three typical symptomatic samples were taken for each location. The total number were 21 samples. Leaf samples were placed in plastics bags and carried to the laboratory for virus detection.

# RNA Virus Detection by RT-PCR analysis.

Detection of RNA viruses by the Reverse transcriptionpolymerase chain reaction (RT-PCR) using a pair of primers specific to the *Papaya ringspot virus* (PRSV) and universal primers for *Potyvirus*.

*Total RNA Extraction.* We extracted total RNA from 0,1 g symptomatic leaves using Promega RNA extraction kit according to protocol provided by the manufactures (Promega, USA). The total RNA extract was stored at -20 °C for further used.

Synthesis of cDNA. The reverse transcription reaction was carried out with a total reaction consisting of 1  $\mu$ l of total RNA, 1  $\mu$ L of Oligo d(T) 50  $\mu$ M, 1  $\mu$ L of dNTP mix 10 mM, and 16  $\mu$ L nuclease free water. The mix incubated at 65 °C for 5 min on waterbath, then chilled



Figure 1. Sampling location in Bengkulu to collect papaya-infected virus; Rejang Lebong, Kepahiang, Bengkulu Tengah and Seluma District.

on ice at least 1 min. Then, added 2  $\mu$ L of 10× reaction buffer (NZY, Smobio), 1  $\mu$ l of ribonuclease inhibitor (NZY, Smobio), 1  $\mu$ L of reverse transcriptase (200 units). Reverse transcription with total volume 20  $\mu$ L, then mixed gently and, incubate at 50 °C for 30 min, then inactivated the reaction by heating at 85 °C for 5 min. cDNA was stored at -20 °C for further used.

DNAAmplification for PRSV. The cDNA amplification was carry out by using specific primer for PRSV, PRSV326 (5'-TCGTGCCACTCAATCACAAT-3GTCCA-3') PRSV800 (5'and GTTACTGACACTGCCGTCCA-3') (Mohammed et al., 2012). PCR reactions were prepared in 25 µL total volume, containing go taq green master mix  $(2^{\times})$ (Thermo Fisher Scientific, Waltham, MA, USA, 10 mM each primer, 1 µL cDNA template, and ddH<sub>2</sub>O. The amplification was performed in SimpliAmp thermal cycles machine (Fisher Scientific, US) with 5 min at 94 °C for pre-denaturation, followed by 35 cycles of denaturation (1 min at 94 °C), annealing (1 min at 59 °C), and extension (1 min at 72 °C). The last cycles were followed by 72 °C for 10 min and decreased at 4 °C.

DNA Amplification for Potyvirus. The cDNA amplification was carry out by using universal primer Poty for Potyvirus, namely MJ-1 (5'-ATGGTHTGGTGTGTGYATHGARAAYGG-3') and PotyMJ-2 (5'-TGCTGCKGCYTTCATYTG-3') to amplification coat protein of *Potyvirus* with target 320 bp (Marie-Jeanne et al., 2000). PCR reactions were prepared in 25 µL total volume, containing go-tag green master mix (2×) (Thermo Fisher Scientific, Waltham, MA, USA), 10 mM each primer, 1 µL cDNA template, and ddH<sub>2</sub>O. The amplification was performed in SimpliAmp thermal cycles machine (Fisher Scientific, US) with 5 min at 94 °C for pre-denaturation for 3 min, followed by 45 cycles of denaturation (30 sec at 96 °C), annealing (1 min at 61 °C), and extension (2 min at 72 °C). The last cycles were followed by 72 °C for 10 min and decreased at 4 °C.

# DNA Virus Detection by PCR Analysis.

**Total DNA Extraction**. For the detection of DNA virus, we extracted total DNA from symptomatic leaves according to the method of Doyle & Doyle (1990) with minor modification. Leaf tissue was ground in sterile mortar in liquid nitrogen and then 500  $\mu$ L extraction buffer added to the leaf powder. The extraction buffer

contained 100 mM Tris pH 8.0, 1.4 mM NaCl, 20 mM EDTA pH 8 and 1% (v/v)  $\beta$ -mercaptoethanol. The extraction buffer was autoclaved, and 2% (v/v) polyvinylpyrrolidone (PVP) and 2% CTAB were added immediately before use. After grinding, 500 µL aliquots were transferred to a 2 mL microtube and incubate 60 minutes at 65 °C with gentle mixing to avoid the homogenate's aggregation. The extract added 500 µL of chloroform: isoamyl alcohol (24:1), and the mixture was vortexed thoroughly. Then the microtube centrifuged in high speed mini-centrifuge (Biosan, Riga Latvia) for 15 min at 13,000 rpm. The supernatant was then transferred to a new tube, and protein precipitated by adding isopropanol 2/3× volume of supernatant and centrifuged in high speed mini-centrifuge (Biosan, Riga Latvia) for 10 min at 12,000 rpm. The pellet was washed with ethanol 70% (v/v) and centrifuged in high speed mini-centrifuge (Biosan, Riga Latvia) for 5 min at 8000 rpm. The pellet was dried and resuspended by 100 µL nuclease-free water. This total DNA extract was stored at -20 °C for further use.

**DNA** Amplification. The Begomovirus genome was amplified by the PCR technique using a universal primer for transcriptional activator protein (TrAp) and replication-associated protein (Rep) gene of Begomovirus SPG1 (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2 (5'-ATCCVAAYWTYCAGGGAGCTAA-3') (Li et al., 2004). PCR reactions were prepared in 25 µL total volume, containing go taq green master mix  $(2\times)$ (Thermo Fisher Scientific, Waltham, MA, USA), 10 mM each primer, 1 µL DNA template, and ddH2O. The amplification was performed in SimpliAmp thermal cycles (Fisher Scientific, US) with 5 min at 94 °C for pre-heating, followed by 30 cycles of denaturation (1 min at 94 °C), annealing (1 min at 59 °C), and extension (1 min at 72 °C). The last cycles were followed by 72 °C for 10 min and decreased at 4 °C.

**DNA Visualization by Electrophoresis.** DNA Amplification products were analyzed on 1% agarose gel electrophoresis in 0.5X TBE buffer containing nucleic acid staining with Ethidium bromide at 50 volt in 50 min. Agarose gel electrophoresis was used to visualize the PCR products. Furthermore, visualization of the agarose gel on Gel documentation (GelDoc, Axygen) and the results of the images were stored on a computer.

### **RESULTS AND DISCUSSION**

**Disease Symptoms.** Infected plants were recognized in the field based on visual symptoms. The symptom dominantly found in each district can be seen in Table 1. Five main symptoms of yellow mosaic disease in the field were observed, i.e. 1) yellowing; 2) yellowing with the brown spot; 3) leaf malformation (Figure 2). The most symptom found in Rejang Lebong and Kepahiang District was yellowing and leaf malformation. The symptoms seen in Bengkulu Tengah and Seluma District were yellow with dark spots. This type of symptoms yellowing and yellowing with brown spot had not been reported as typical symptoms of papaya's yellowing disease in Indonesia.

Disease Incidence. The incidence of yellow mosaic disease was very high, i.e. 42.75% to 100% on calina and 0% on local varieties in 7 growing areas located at Rejang Lebong, Kepahiang, Bengkulu Tengah, and Seluma District (Table 1). Begomovirus is a member of the family Geminiviridae, which is the largest group of plant viruses. Geminiviruses are subdivided into four genera based on host range, insect vector, and genome organization. The genus Begomovirus (Subgroup III) consists of whiteflies' viruses to infect dicotyledonous plants with a monopartite or bipartite arrangement (Fauquet & Stanley, 2003). In Indonesia, Begomovirus was observed infecting pepper (Rusli et al., 1999, Trisno et al., 2009); tobacco (Hidayat et al., 2002, Trisno et al., 2014); Ageratum conyzoides (Haerani & Hidayat, 2003); eggplant (Kintasari et al., 2013); cucumber (Septariani *et al.*, 2014, Wiratama *et al.*, 2015); yard long bean (Nurulita *et al.*, 2015); and soybean (Rahim *et al.*, 2015; Sutrawati *et al.*, 2020). Sulandari (2004) reported that *Begomovirus* on pepper was named *Pepper yellow leaf curl virus* (PYLCV) and characterization of the partial genomic sequence of the PYLCV was reported by Hidayat *et al.* (2006). *Begomovirus* on cucumber was reported by Wiratama *et al.* (2015) was named as *Squash leaf curl China virus* (SLCCNV). *Begomovirus* has been reported to infect papaya in India (Reddy *et al.*, 2010) and China (Shen *et al.*, 2014).

**Identification of the Causal Agent.** RT-PCR by using a pair of specific primers to PRSV and universal primers for *Potyvirus* showed negative results (data not shown), indicating that the causal virus (es) were not PRSV or other *Potyvirus*. However, by using universal primer for *Begomovirus* the DNA fragments with size ~900 bp were successfully amplified from samples showing yellow with brown spot (Figure 3, lane 1–2), yellow mosaic (Figure 3 lane 3, 4, 5) from all locations, but no DNA amplified from samples showing stunting and leaf malformation (Figure 3, lane 7). This is indicating that *Begomovirus* with severe yellow mosaic disease and yellow with papaya's brown spot disease in Bengkulu.

Two types of *Begomovirus* in papaya that have been reported in China; *Papaya leaf curl China virus* (PalCuCNV, Acc. No. AJ558122) and *Papaya leaf curl Guangdong virus* (PalCuGDV, Acc. No. AJ558123) (Tennant *et al.*, 2007). The other member of *Begomovirus*, *Papaya leaf curl virus* (PalCV) have

Location		Variativ	Symmetry	Disease incidence
District	Sub district	Variety	Symptom	(%)*
Rejang Lebong	Curup	Calina	Yellow mosaic, yellowing, leaf malformation	100 (66/66)
		Local	No symptom	0 (0/3)
	Curup Selatan	Calina	Yellow mosaic, leaf malformation	44.70 (114/255)
		Local	No symptom	0 (0/22)
	Curup Selatan	Calina	Yellow mosaic systemic	100 (56/56)
Bengkulu Tengah	Taba Penanjung	Calina	Yellow with dark spot	100 (116/116)
Kepahiang	Kepahiang	Calina	Yellow mosaic, leaf malformation	42.75 (59/138)
		Local	No symptom	0 (0/22)
Seluma	Sukaraja	Calina	Yellow with dark spot	100 (400/400)

Table 1. Symptoms and disease incidence of yellowing disease on papaya in Bengkulu

\*Number in parenthesis showed total infected plants/total plants observed

been reported in papaya plants in Taiwan (Chang *et al.*, 2007) and India (Reddy *et al.*, 2010) with symptoms of infection in the form of curly leaves, twisted petiole, leaf enation, and stunting, produce smaller fruit and will die prematurely. Other research in China shows the

symptoms of curling and yellowing of the leaf bones in papaya plants caused by *Ageratum yellow vein virus* (AYVV) associated with a new betasatelit that infects papaya, which is then proposed as *Papaya leaf curl China beta satellite virus* (Shen *et al.*, 2014). A new

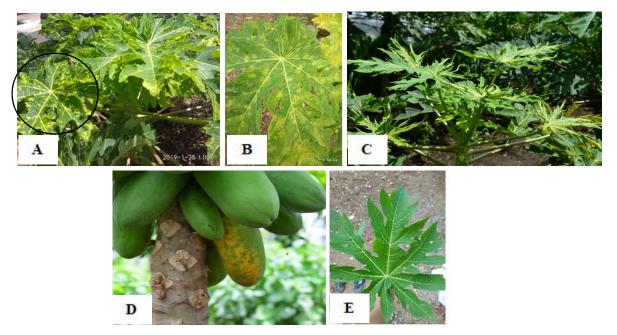


Figure 2. Symptoms of yellowing disease on papaya. (A) Yellowing; (B) Yellowing with brown spot; (C) Leaf malformation; (D) Green spot on fruit; (E) Healthy papaya leaves.

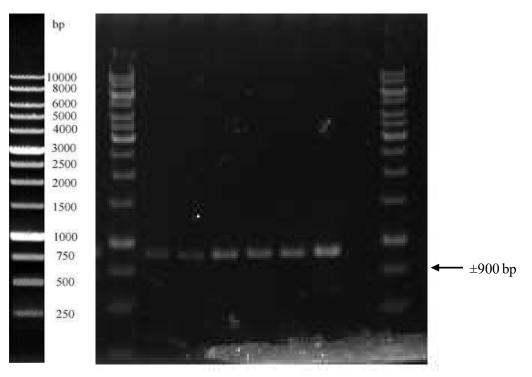


Figure 3. Visualization of *Begomovirus* amplification from leaf samples using universal primers SPG1/SPG2 on 1% agarose gel. M, 1000 bp DNA Ladder (Thermo Scientific, US); lanes 1-2, Seluma samples; 3, Bengkulu Tengah samples; 4, Rejang Lebong samples; 5, Kepahiang samples; 6, Positive control (Chilli); 7, Rejang Lebong samples.

*Begomovirus Papaya leaf curl virus* and its associated betasatellite infecting papaya in India, this virus considered as a new species of *Begomovirus* with propose the name *Papaya yellow leaf curl virus* according to ICTV guidelines for new *Begomovirus* species (Nehra *et al.*, 2019).

Symptoms on papaya leaves such as yellowing and yellowing with brown spot had not been reported as typical symptoms of papaya's yellowing disease in Indonesia. To the author's knowledge, *Begomovirus* infection on papaya has never been reported in Indonesia. This finding is the first report of *Begomovirus* infection in papaya in Indonesia.

Molecular detection confirmed the association of *Begomovirus* in the yellow mosaic disease of papaya in Bengkulu. Further study on identifying virus species, host range, insect transmission, and disease spread is significant for understanding disease development and controlling the disease. In addition, in the future it is necessary to identify the causal of stunting and leaf malformation on papaya found in the fields which may become a potential threat on papaya production in Indonesia.

## CONCLUSION

Severe systemic yellow mosaic on papaya leaves is associated with *Begomovirus* infection, while the causal of leaf malformation and stunting is unknown yet. This work is the first report of *Begomovirus* infected papaya in Indonesia. It is necessary to study the existence of *Begomovirus* infecting papaya on other area, their characterization, biological and genetic variabilities and integrated management strategies to mitigate this virus infection and distribution in Indonesia.

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