Molecular Biology Reports (2024) 51:119
https://doi.org/10.1007/s11033-023-09029-5

BRIEF REPORT

First report of *Ageratum yellow vein virus* infecting papaya in Lampung, Indonesia

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Received: 2 August 2023 / Accepted: 2 November 2023

Abstract

Background Papaya (*Carica papaya*) is a tropical fruit of great economic and nutritional importance, loved for its sweet and delicious flesh. However, papaya cultivation faces serious challenges in the form of *Begomovirus* attacks. *Begomoviruses* are a group of viruses that pose a serious threat to plants worldwide. Including papaya, *Begomovirus* has become a significant threat to papaya production in various parts of the world and has been identified in several regions in Indonesia.

Methods DNA was extracted from seven samples representing different papaya growing areas using a Plant Genomic DNA Mini Kit. Genomic DNA from the samples was subjected to PCR using universal primers of AC2, AC1, SPG1 and SPG2. The PCR products then sequenced using the dideoxy (Sanger) approach. The obtained sequence then compared to the gene bank using BLAST software available at NCBI. Multiple sequence alignment and phylogenetic tree construction were analyzed using the MEGA11 program.

Results Detection based on viral nucleic acid in papaya plants in Pesawaran, Lampung Province with seven sampling points using universal primers SPG1/SPG2 showed positive results for *Begomovirus* infection with visible DNA bands measuring ± 900 bp. Direct nucleotide sequencing using SPG1/SPG2 primers for the AC2 and AC1 genes of the *Begomovirus* and confirmed by the BLAST program showed that papaya samples were infected with *Ageratum yellow vein virus* (AYVV). The phylogenetic results show that AYVV from papaya samples has a close relationship with the AYVV group from several other countries, with 98% homology.

Conclusion In the papaya cultivation area in Pesawaran, Lampung province, it was identified as *Begomovirus*, *Ageratum yellow vein virus* (AYVV) species and is closely related to the AYVV group from several other countries. Overall, our study further suggests that *Ageratum* acts as an alternative host and reservoir for *Begomovirus*.

Introduction

Papaya is one of the important horticultural crops in Indonesia. Since 2017, the papaya production in Indonesia has increased rapidly, from 875.112 tons in 2017 to 1.168.266 tons in 2021. However, several viruses have been reported to attack papaya plants, including the *Potyvirus* group, such as *Papaya ring spot virus strain P* (PRSV-P) [1] and the *Begomovirus* group, including *Papaya leaf curl virus* (PalCV), *Papaya leaf crumple virus* (PalCrV), *Chilli leaf curl virus* (ChilCuV), *Tomato leaf curl New Delhi virus* (ToLCuNDV), and *Croton yellow vein mosaic virus* (CYVMV) [2]. The first reported virus attack on papaya in Indonesia was *Papaya ring spot virus strain P* (PRSV-P), which was found in Nanggroe Aceh Darussalam Province [3]. Today, PRSV attacks have spread to almost all provinces in Sumatra and Java [4].

After reports of PRSV in Indonesia, there were reported the presence of *Begomovirus* group that attacks papaya plants in Bengkulu Province [5]. The symptoms of papaya plants attacked by *Begomovirus* are yellowing, malformations of the leaves, and sometimes brown spots. This report indicates that the *Begomovirus* has entered Indonesia. *Begomovirus* is transmitted by whitefly (*Bemisia tabaci*) [6]. Lampung and Bengkulu are geographically very close, so

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Published online: 16 January 2024

it is necessary to detect the presence of this virus in papaya plants in Lampung Province.

Materials and methods

To identify the *Begomovirus* in papaya, DNA was extracted from seven samples representing different papaya growing areas using a Plant Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan), following the manufacturer's instructions. Genomic DNA from the samples was subjected to PCR using universal primers for *Begomovirus* which amplify parts of AC2 and AC1, as well as SPG1 (5′CCCCKGTGCGWRAATCCAT-3′) and SPG2 (5′ATCCVAAYWTYCAGGGAGCTAA-3′) which amplify nucleotides encoding *Begomovirus* transcriptional activator protein (TrAp) and replication-associated protein (Rep) genes [7]. PCR was performed with an initial denaturation cycle of 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 50 °C for 15 s, extension at 72 °C for 10 s, and final extension at 72 °C for 5 min. The PCR products were then checked by using electrophoresis in 1% agarose gel. The PCR products were then sequenced using the dideoxy (Sanger) approach.

The obtained sequence then compared to the gene bank using BLAST software available on the National Center For Biotechnology Information (NCBI) website, www.ncbi.nlm.nih.gov. This analysis was used to determine the degree of kinship between our isolates and the *Begomovirus* strains that have been registered in the *GenBank database* (NCBI). Furthermore, several strains were selected with a similarity percentage close to 100%. Multiple sequence alignment and phylogenetic tree construction were analyzed using the MEGA11 program. The phylogenetic tree was arranged using the UPGMA algorithm, with grouping stability using bootstrap analysis with 1000 replications.

Samples that have been detected using PCR are then subjected to pathogenicity tests using three types of plants to confirm the symptoms that arise. Incoculum plants and healthy plants were placed in a glass cover which had been infested with *Bemisia tabaci* imago. A positive result of the pathogenicity test is indicated by the appearance of symptoms on healthy plants that are the same as those on inoculum plants.

Result and discussion

Observations in areas of papaya plantations in Pesawaran, Lampung Province showed that in seven sampling points the yellowing disease of papaya was detected. Among these, there were seven samples of papaya plants infected with *Begomovirus*,

Infections that occur in papaya plants will cause several symptoms that are expressed through plant parts such as leaves and other plant parts. Papaya leaves show symptoms of mosaic, leaf curl, yellowing, and malformations in papaya plants (Fig. 1). In gardens with more severe symptoms in papaya plants are the leaves that fall cause the papaya plants to die over time (Fig. 2). In addition, several *Bemisia tabaci* vector insects were found around symptomatic papaya plants. The results of these observations indicated that there was a spread of yellow virus (*Begomovirus*) in papaya plants in Lampung Province.

Observations of papaya fields in Pesawaran, Lampung Province showed that *Begomovirus* attacks had different intensities. Papaya fields in Pesawaran have an incidence of around 50–100%, while the severity of disease in the field ranges from 20 to 95%. This phenomenon showed that the plant could produce fruits while infected by this virus, although in a small yield (Fig. 3) .

Detection based on viral nucleic acid in each category of papaya plants in Pesawaran using universal primers SPG1/SPG2 showed positive results for *Begomovirus* infection with visible DNA bands measuring ± 900 bp (Fig. 4).

Ageratum yellow vein virus (AYVV) was found in the papaya samples through direct nucleotide sequencing with SPG1/SPG2 primers for the AC2 and AC1 genes of *Begomovirus*. This was confirmed by the BLAST analysis of 97.52%. The phylogenetic results show that AYVV from papaya samples are closely related to AYVV groups from several other countries with 98% homology (Fig. 4). There are 23 base pairs that are the same out of 235 base pairs. Phylogenetic analysis also showed that the AYVV of tomato plant samples in Lampung Province were in the same cluster as AYVV from Japan (KC677733), Indonesia (AB100305), Nepal (KC282641), Taiwan (DQ866134), Thailand (JN809811), and Vietnam (MW012407). Interestingly, most of the AYVV found in several countries are AYVV that attack *Ageratum* and several types of Solanaceae plants such as tomatoes, eggplants, and chilies. Meanwhile, what is found in Indonesia is AYVV which attacks papaya plants with a disease incidence rate of almost 100%. Thus, this finding poses a serious threat to papaya cultivation in Indonesia. Not only threatening Indonesia, this finding can also threaten papaya-producing countries such as India, Brazil, China, Mexico, and several other countries [8]. The main host of AYVV is *Ageratum*, a cosmopolitan weed that is often found around cultivated fields and is mostly attacked by jaundice caused by *Begomovirus*. This suggests that *Ageratum* acts as an alternative host and reservoir for *Begomovirus*.

A pathogenicity test was carried out to confirm that the symptoms present in the inoculum plants were the same as those inoculated with *Begomovirus* using the *Bemisia tabaci* vector. Pathogenicity test results on three types of plants showed that the symptoms of the inoculums

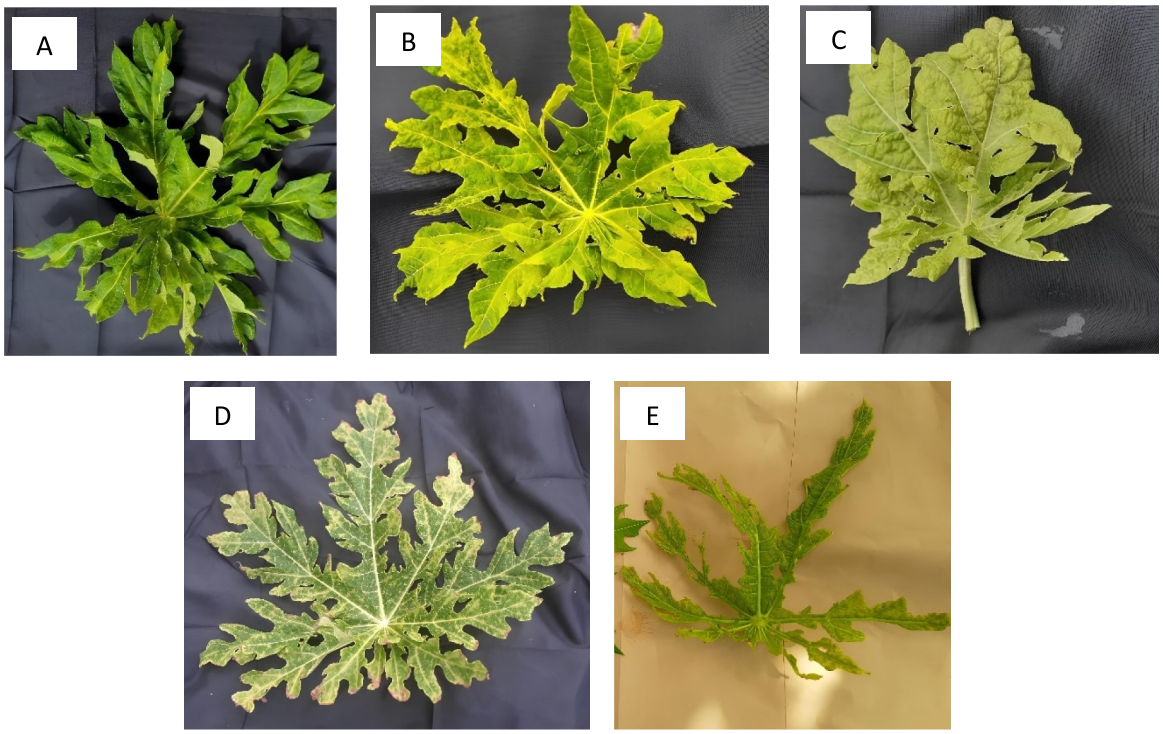

Fig. 1 Symptoms on papaya leaves infected with *Begomovirus; Mottle* **A** *Yellowing* **B** and **C** Mosaic **D** and shoe string **E**

Fig. 2 Comparison of papaya fields on *Begomovirus* attack with low **A** and high **B** attack rates

occurred in the inoculated plants. Symptoms that appear can be seen in chili leaves that curl (Fig. 5A), papaya leaves with malformations (leaves do not grow properly), curly leaves (Fig. 5B), and eggplant leaves with chlorosis yellow spots (Fig. 5C), in severe infections causing stunted plants. There are five plants that are used to confirm symptoms. in the three types of plants, five out of five plants showed symptoms of disease (100% infected).Further tests were not carried out due to research limitations.

Begomovirus infection in plants will generally cause symptoms such as leaf curling, yellowing, mosaicism, and stunting [9].

Disease epidemics are influenced by the active role of insect vectors, the adequacy of inoculum sources in the field, the use of susceptible varieties, and environmental factors suitable for the development of insect vectors and

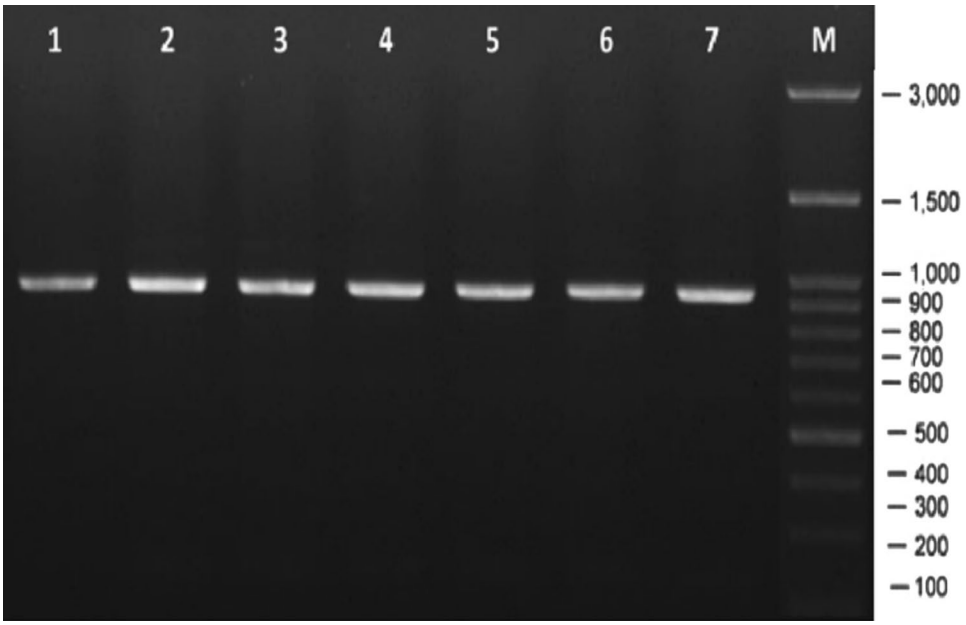

Fig. 3 Results of DNA amplification using SPG1/SPG2 primers on papaya leaf samples from several districts/cities in Lampung Province. Markers (M); sample 1 (1); sample 2 (2); sample 3 (3); sample 4 (4); sample 5 (5); sample (6), and sample 7 (7)

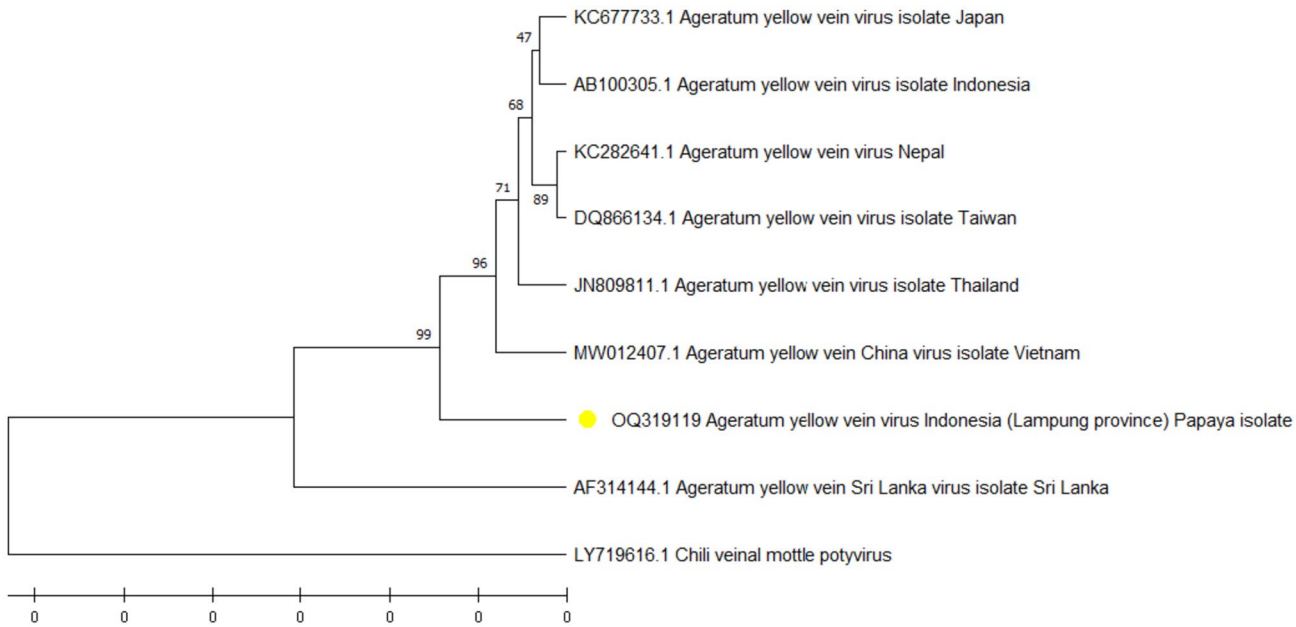

Fig. 4 Phylogenetic tree constructed using UPGMA Algorithm from AC2 and AC1 Gene

pathogens. Based on the research that has been done, information about the spread of *Begomovirus* in various plants is needed, considering that this virus easily moves between plants from various families. Therefore, accurate detection using molecular techniques is one way to obtain information on the spread of the virus, so that control strategies can be applied to minimize the spread of the virus in the field.

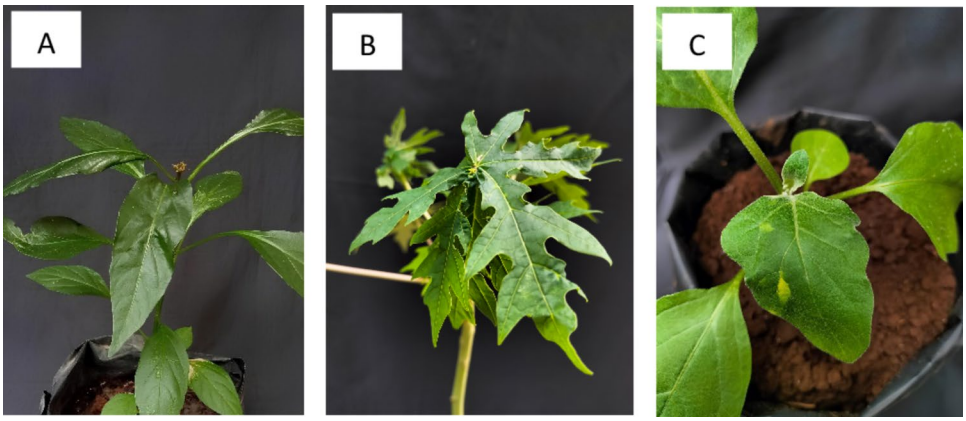

Fig. 5 Symptoms in plants inoculated with *Begomovirus*; on chili plants **A** on papaya plants **B** on eggplant **C**

Acknowledgements The authors would like to thank the farmers who giving permission for our research, laboratory and field technicians who helped the research process.

Author contributions Designed experiment, interpreted data, wrote and edited manuscript: SH, HMA. Carried out sample collection, field experiment, and serological test: SP, PL. Designed experiment, provided germplasm collection, and analysed data: MN, AA. Performed data analysis and molecular test, wrote manuscript: LD.

Funding This research was funded by a BLU grant from the Lampung University with a contract number 765/UN26.21/PN/2023.

Data availability Sequence *Begomovirus* isolate Papaya (Isolate Pesawaran, Lampung) reads were submitted to the Short Read Archive of NCBI: OQ319119.

Declarations

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval This article does not contain studies with humans or animals.

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