

Molecular identification of pineapple mealybug wilt-associated virus-1 in pineapple fruit flesh

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Abstract: Pineapple mealybug wilt-associated virus 1 (PMWaV-1) as one of the causal agents of Mealybug Wilt of Pineapple (MWP) has been identified in different regions of Indonesia, and in other countries. Those surveys mostly collected pineapple (*Ananas comosus* L.) leaf samples for molecular detection of the phloem limited PMWaV-1. Therefore, presence of the virus in flesh of fruit samples, which contain a lot of phloem, from Pemalang Regency was observed in this study to improve our knowledge on the distribution of the virus in plant tissues. As a results, RT-PCR products with single targeted band confirmed that the flesh of the two fruits also contained PMWaV-1. Subsequent BLAST and phylogenetic analyses on the sequences of two iso-lates obtained here suggested that they are identical to the isolates recovered previously from leaves of their respective plants. This report added additional information on the distribution of PMWaV-1 in pineapple plant tissues.

Keywords: phloem-restricted pathogen, phylogenetic study, plant virus, systemic infection.

Introduction

Pineapple (*Ananas comosus* L.) is one of the most popular tropical fruit commodities due to its pleasant flavor and high content of vitamins and minerals (Hossain et al., 2015). As a leading horticultural product, pineapple is valuable in the international market, and serves as an important export commodity for Indonesia with economic value of \$ 277.95 million in 2023 (CBSI, 2024). However, its productivity often fluctuates, even tends to decline over time as shown by the 2.74 million tons production in 2024 that was much lower than 3.17 million tons yielded in the previous year (CBSI, 2024). This reduction is frequently associated with abnormal growth in pineapple plants caused by various diseases, including those induced by viral pathogen.

One of the major viral diseases affecting pineapple production is Mealybug Wilt of Pineapple (MWP), which is associated with a complex of viruses collectively known as pineapple mealybug wilt-associated viruses (PMWaVs) (Dey et al., 2018). Among these, pineapple mealybug wilt-associated virus 1 (PMWaV-1) has been identified as one of the causal agents of MWP. PMWaV-1 belongs to the genus *Ampelovirus* within the family *Closteroviridae*, possessing a positive-sense single-stranded RNA genome of approximately 13.1 kb in length that contains seven open reading frames (ORFs) (Melzer et al., 2008). The virus is phloem-restricted, being confined exclusively to phloem tissues, and not spreading systemically throughout the plant (Dey et al., 2015). Therefore, transmission of PMWaV-1 is semi-persistent, involving specific interactions between the phloem-feeding mealybugs and the phloem tissues of pineapple (Carrasco-Lozano et al., 2025).

MWP incidence was estimated to be around 15.6 - 21.6% in North Tapanuli, North Sumatera Province (Hutahayan et al., 2021). PMWaV-1 has been molecularly detected in pineapple cultivated in plantations in Blitar, East Java, with phylogenetic analyses showing close clustering with isolates from Ghana, Taiwan, Mexico, China, Mauritius, Thailand, Cuba, Australia, and the United States (Valentino et al., 2023). Recent surveys by Ningrum et al. (2025) also reported PMWaV-1 occurrence in several major pineapple-producing regions across Java, including Kediri, Sleman, Bantul, Magelang, and Pemalang, indicating its widespread distribution likely driven by the movement of infected planting materials.

Infection of PMWaV-1 in pineapple plants sometimes occurs without inducing visible symptoms, making field detection difficult. Nevertheless, PMWaV-1 infection can subtly disrupt physiological processes, leading to reductions in both yield quantity and fruit quality, including decreased fruit size and weight. These effects are mainly attributed

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to the impairment of water and nutrient transport systems within the plant, which can be exacerbated under environmental stress conditions such as drought (Sether & Hu, 2001). Furthermore, molecular detection using RT-PCR has revealed that the highest viral concentrations occur in the basal leaf sheaths, crown and stem base tissues, as well as in roots adjacent to mealybug colonies (Ochoa-Martinez et al., 2016). Possible presence of PMWaV-1 in the flesh of fruit which is rich in phloem (Montero-Calderón et al., 2009), was investigated in this study to enrich our knowledge on the distribution of the virus in pineapple plant.

Materials and Methods

Sampling

Two pineapple fruits were collected during a survey in Pemalang District, Central Java in January 2025. The fruits themselves were slightly stunted and wilting, and growing on two plants showing leaf redness and slight wilting, typical of viral symptoms. Leaf and fruit samples were brought to the Phytopathology Laboratory in Universitas Gadjah Mada to be kept in a freezer at -20 °C. The leaves were shown to be positive for PMWaV-1 infection in which partial genome of the two obtained isolates were listed in NCBI GenBank with acc nos. PV543607 and PV543608, respectively (Ningrum et al., 2025). Presence of PMWaV-1 in the flesh of the two fruit samples (Figure 1) were then observed in this study.

Molecular identification of PMWaV-1 in fruit samples

Total RNAs were extracted from the flesh area of pineapple fruits (Figure 1) using a Total RNA Mini Kit Plant by following standard protocols provided by its manufacturer (Geneaid Biotech Ltd., Taiwan). Reverse transcription step to produce complementary DNAs from the RNA templates were performed with ReverTra Ace kit (Toyobo, Japan), each in a reaction volume of 10 µl consisted of 2 µl RNA, 2 µl 5× RT Buffer, 1 µl dNTP, 0.5 µl (10 pmol µl⁻¹) random primer, 0.5 µl RNase inhibitor, 0.5 µl ReverTraAce®, and completed with 3.5 µl nuclease-free water. The reverse transcription procedure was performed at 30 °C for 10 min, 42 °C for 20 min, and completed with 99 °C for 5 min.

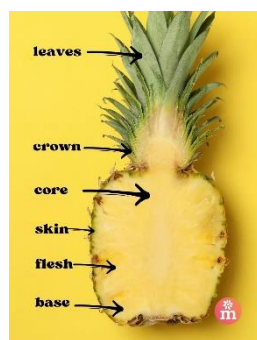


Figure 1. Parts of a pineapple fruit (McMillen, 2023)

The subsequent PCR was each done in a reaction volume of 40 µl consisted of 4 µl cDNA, 20 µl MyTaq HS Red Mix (Bioline, Germany), 2 µl (10 pmol µl⁻¹) each of the forward (225F 5'-ACAGGAAGGACAACACTCAC-3') and the reverse (226R 5'-CGCACAACCTTCAAGCAATC-3') primers for amplification of ± 590 bp of partial HSP70 gene (Sether et al., 2001), and 12 µl nuclease-free water. PCR cycles were completed with pre-denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 30 sec, and elongation at 72 °C for 1 min, and a final elongation at 72 °C for 10 min (Ningrum et al., 2025).

Five µl of the PCR products were pipetted into wells of 1% agarose gel (w/v) stained with FloroSafe DNA Stain (1st BASE, Malaysia). Electrophoresis was performed in TBE buffer (pH 7) at 100 V for 30 min. Appearance of bands with the targeted specific size was then examined with UV light on a transilluminator (Optima Inc., Japan). PCR products with positive results were delivered to the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada for Sanger two-directional sequencing. The obtained nucleotide sequences were submitted online to be compared with those already in NCBI GenBank database using 'nucleotide BLAST' software

(<https://blast.ncbi.nlm.nih.gov>) to ensure virus species identification. The sequences were then deposited in the GenBank with specific accession numbers.

Phylogenetic study

To understand positions of isolates obtained in this study among isolates in the GenBank, a phylogenetic tree was built with MEGA11 software (Tamura et al., 2021) applying Tamura 3-parameter model (Tamura, 1992) in Maximum Likelihood (ML) statistical method. Significance of each formed branch were tested with 1000 bootstrap.

Results and Discussion

Molecular identification of PMWaV-1 in fruit samples

The two fruit samples were found to be infected by PMWaV-1 following BLAST analysis on the obtained sequences from the successfully amplified PCR products (Figure 2). The two new isolates were named PMWaV1-PML-1F and PMWaV1-PML-2F, with F = fruit.

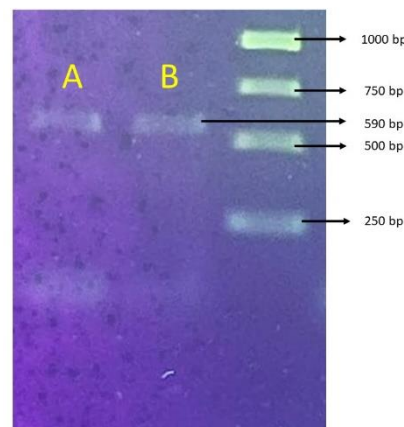


Figure 2. Result of electrophoresis of RT-PCR products with the targeted $\pm$ 590 bp showed that PMWaV-1 was detected in both flesh of pineapple fruit samples. A. PMWaV1-PML-1F and B. PMWaV1-PML-2F isolates

Some pineapple cultivations in Indonesia are concentrated in major production centers across Java, notably in Bogor, Subang, Pemalang, and Kediri—regions that also harbor the country's principal pineapple germplasm collections. Among these, Pemalang Regency in Central Java represents a key production area, with cultivation primarily centered in Belik District, particularly Beluk Village (Hadiati & Indriyani, 2008). Prasetyo et al. (2023) reported that the predominant cultivar, locally known as 'Nanas Madu,' belongs to the Queen variety, characterized by small fruit size (0.5 - 0.9 kg), spiny dark-brown leaves, and sweet, tender flesh. This cultivar is widely utilized for both fresh consumption and processing into products such as jam, sweets, and chips.

PMWaV-1 has been reported to occur ubiquitously across major pineapple-producing regions worldwide, infecting both plants exhibiting mealybug wilt symptoms and those that remain asymptomatic. Its broad geographic distribution is largely attributed to the unintentional dissemination of vegetatively propagated planting materials harboring systemic infections (Sether et al., 2001). *Dysmicoccus brevipes* serves as the primary vector across most tropical regions, including Hawaii, Asia, and South America, whereas *D. neobrevipes* is considered an important vector in certain regions, particularly in Hawaii and Southeast Asia (Gambley et al., 2008; Carrasco-Lozano et al., 2023; Carrasco-Lozano et al., 2025; Ningrum et al., 2025).

Phylogenetic study

Sequences of the two isolates obtained from the fruits were observed using BLAST analysis to be identical to the isolates retrieved from the respective plant leaf (PMWaV1-PML-1 and PMWaV1-PML-2). In agreement with this, the constructed phylogenetic tree showed that the fruit isolates shared a basal node with their respective leaf isolate (Figure 3). These results strongly indicated that the isolates from fruit and leaf are the same. Therefore, PMWaV-1 was found to be distributed into flesh of the pineapple fruit.

Although pineapple leaves are commonly taken and tested in PMWaV-1 molecular surveys (Valentino et al., 2023; Ningrum et al., 2025; Anuar et al., 2025), Ochoa-Martinez *et al.* (2016) reported that PMWaV-1 infection also spread into the basal leaf sheaths, crown and stem base tissues, and the adjacent roots. Furthermore, flesh of Nanas Madu fruits sampled from Pemalang Regency evidently also harbor the virus in this study. The virus is likely concentrated in the phloem of flesh and core of the fruits based on report by Dey et al. (2015). Subsequent BLAST and phylogenetic analyses determined that the isolates in fruit were identical to the isolates previously recovered in their respective leaves (Ningrum et al., 2025), at least in the observed genome region.

This report added growing data on the dispersion of viral particles in pineapple plant tissues. It is not recommended to restrict interregional movement of fruits since they are traded mostly for consumption not as planting materials thus its potency to disseminate viruses is minimal. Since viruses still are major obstacle in horticultural cultivation (Helina et al., 2025; Arsi et al., 2025), field sampling in other regions of Indonesia as well as full genome sequencing of Indonesian isolates are clearly needed to advance our understanding on the distribution and genomic diversity of PMWaVs in the country.

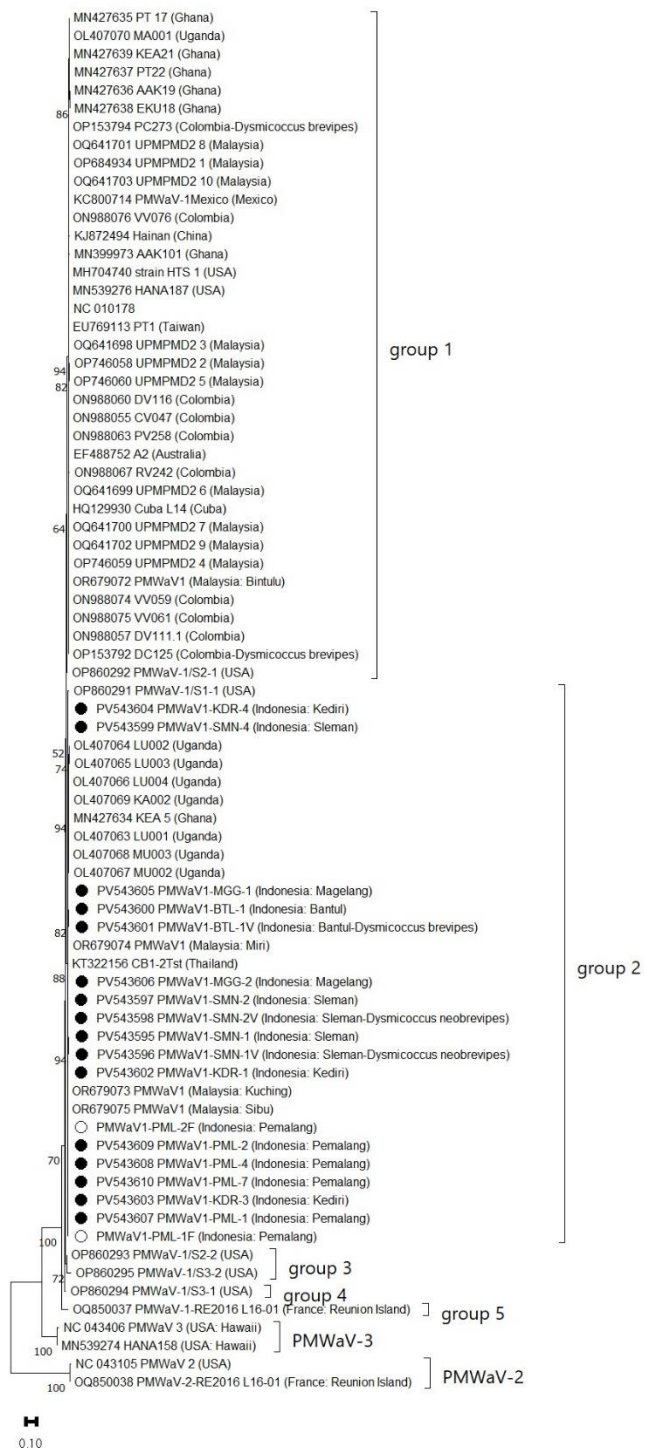


Figure 3. Phylogenetic tree constructed based on the sequences of partial HSP70 region (~500 nt) of PMWaV-1 genome. The tree was generated using MEGA11 applied Tamura 3-parameter model with 1000 bootstrap replicates and uniform rates among sites. Bootstrap values > 50% were shown. Sixteen Indonesian PMWaV-1 isolates listed in GenBank were highlighted with black dots. Two isolates from fruits obtained in this report were marked with white dots. PMWaV-2 and -3 isolates serve as out-groups

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