



Molecular Characterization and Mechanical Transmission of a Papaya Ringspot Virus Isolate from Sleman Regency, Indonesia

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Abstract

Viral diseases severely affect papaya (*Carica papaya* Linnaeus) production in Indonesia, but information on papaya ringspot virus (PRSV) isolates from severe outbreaks in Sleman Regency remains limited. This study aimed to identify the causal virus and evaluate its mechanical transmissibility to selected cucurbit hosts. A composite leaf sample from five symptomatic papaya plants was tested for potyvirus and begomovirus using universal primers. The potyvirus amplicon was sequenced and analyzed using BLASTn, phylogenetic analysis, and pairwise nucleotide identity analysis based on a 462 bp trimmed partial coat protein region. Mechanical transmission was tested on cucumber (*Cucumis sativus*), melon (*Cucumis melo*), and bitter melon (*Momordica charantia*), followed by symptom observation and RT-PCR confirmation. This study identified the virus as PRSV-P, registered as PRSV-SMN-TJ, accession number PX927038. Disease incidence after inoculation ranged from 50% to 100%. Phylogenetic and nucleotide identity analyses placed the PRSV-SMN-TJ isolate within the same cluster as Indonesian and Thailand PRSV isolates. This study was based on a single composite field sample and a limited host transmission assay; therefore, broader sampling, complete diagnostic controls, and quantitative pathogenicity assessment are needed. Nevertheless, these findings provide baseline information for PRSV surveillance and management planning in papaya-growing areas.

Keywords: Mechanical transmission, PCR, phylogenetic analysis, plant virus disease, Sanger sequencing

Introduction

Papaya (*Carica papaya* Linnaeus) is a fruit crop native to the tropical regions of the Americas and is now widely cultivated in tropical and subtropical areas worldwide (Koul et al., 2022). In Indonesia, papaya production centers are mostly found in East Java, Central Java, West Java, Lampung, West Sumatra, and East Nusa Tenggara Provinces (CBSI, 2025). Indonesia has diverse papaya germplasm and superior varieties that support papaya development programs

(Budiyantri et al., 2022). However, previous evaluation of several papaya varieties showed variable responses to different PRSV isolates, indicating that isolate-level information remains important for disease surveillance and management planning (Harmiyati et al., 2015). Despite its economic importance, papaya production in Indonesia is frequently constrained by viral diseases, particularly papaya ringspot disease, which can reduce plant vigor,

fruit quality, and field productivity (Premchand et al., 2025; Harahap et al., 2025).

Papaya ringspot virus (PRSV, *Potyvirus papayanuli*) is one of the most destructive viruses infecting papaya worldwide. This virus belongs to the genus *Potyvirus*, family *Potyviridae*, and has a positive-sense single-stranded RNA genome of approximately 10.3 kb with a single open reading frame encoding a large polyprotein (Saleem et al., 2021; Jyotika et al., 2024). According to Umer et al. (2022), PRSV is classified into two infectivity types, namely PRSV-P and PRSV-W. PRSV-P infects plant species in the families Caricaceae, Cucurbitaceae, and Chenopodiaceae, enabling it to cause disease in both papaya and cucurbit crops. Meanwhile, PRSV-W is confined to species within the Cucurbitaceae and Chenopodiaceae, thereby limiting its infection to non-papaya hosts (Riska et al., 2023b; Magar et al., 2024). This host-associated distinction is epidemiologically important because PRSV-P may threaten papaya cultivation and nearby cucurbit crops, while PRSV-W remains an important virus of cucurbit crops in several regions, including Asia (Tran et al., 2025).

PRSV is a globally distributed plant pathogen that limits papaya production in many tropical and subtropical regions (Umer et al., 2022). The virus is not seed-borne but transmitted mechanically or primarily by aphid vectors in a non-persistent manner. Several of the most efficient vectors are the green peach aphid (*Myzus persicae*), melon/cotton aphid (*Aphis gossypii*), and cowpea aphid (*A. craccivora*), with transmission rates of 56%, 48%, and 30%, respectively (Harmiyati et al., 2022). Because non-persistent aphid transmission can occur rapidly, PRSV is difficult to manage once infected plants and vector populations are present in the field. Therefore, accurate detection, molecular identification, isolate characterization, and continuous surveillance

are important for PRSV management (Jyotika et al., 2024; Premchand et al., 2025).

Besides PRSV, begomoviruses such as papaya leaf curl virus (PaLCuV, *Begomovirus caricae*) and ageratum yellow vein virus (AYVV, *Begomovirus ageravenae*) have also been associated with viral diseases of papaya in several regions, including Indonesia (Srivastava et al., 2015; Sutrawati et al., 2021; Helina et al., 2024). Because field symptoms caused by different virus groups may overlap, begomovirus testing was included in this study to exclude possible begomovirus infection or mixed infection in symptomatic papaya plants. This diagnostic consideration is also relevant because recent studies have reported mixed viral infections in cucurbit crops such as melon in Indonesia (Sutrawati et al., 2025).

Previous studies have reported PRSV infection in several papaya-growing regions of Indonesia, including Aceh, Java, Bali, and North Sumatra (Hidayat et al., 2012; Farida et al., 2022; Riska et al., 2023b; Harahap et al., 2025). In Bogor District, Riska et al. (2023b) provided biological and molecular characterization of PRSV, showing the relevance of combining molecular detection with biological information to understand PRSV isolates in Indonesia. Recent Indonesian reports indicate that PRSV-P remains an important viral pathogen of papaya and that isolate-level molecular data are still needed to clarify its distribution, genetic relatedness, and potential movement among regions (Riska et al., 2023b; Harahap et al., 2025). Recently, a large papaya field in Sleman Regency, Special Region of Yogyakarta, was almost completely destroyed by a viral-like disease, resulting in severe systemic symptoms, including leaf mosaic, leaf chlorosis, and ring spots on leaves and fruits.

However, the causal virus associated with this severe field outbreak, its molecular relationship with previously reported PRSV isolates, and its ability to be mechanically transmitted to selected cucurbit hosts had not

been clearly documented. Therefore, this study aimed to identify molecularly the causal virus associated with severe papaya disease in Sleman Regency, and evaluate its mechanical transmissibility to cucumber (*Cucumis sativus*), melon (*Cucumis melo*), and bitter melon (*Momordica charantia*).

Methods

Sample Collection

In response to a report by farmers of the destruction of their papaya field (7°38'20.3"S 110°21'24.2"E, alt. 450 MSL) by viral-like disease, a survey was conducted in November 2025. The field was located in Sleman Regency, Special Region of Yogyakarta, Indonesia, and covered about 5,000 m². A composite leaf sample from five randomly selected plants showing typical viral symptoms such as leaf spots, mosaic, chlorosis, and stunting was collected then taken to the Virology Room of Phytopathology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada, and kept in -20°C to be used in the molecular identification and mechanical transmission experiment.

Virus Identification

To detect possible mixed infection in the symptomatic papaya plants, the collected leaf sample was extracted to obtain DNA and RNA templates. Total DNA extraction was carried out using the Genomic DNA Mini Kit Plant (GeneAid Biotech Ltd., Taiwan), while total RNA extraction was performed using the Genomic RNA Mini Kit Plant (GeneAid Biotech Ltd., Taiwan). The concentration and purity of the extracted DNA and RNA were not assessed before molecular analysis. Reverse transcription was performed using the ReverTra Ace kit (Toyobo, Japan) according to the manufacturer's instructions. The total reaction volume for reverse transcription was 10 µL, consisting of 3.5 µL RNase-free H₂O, 2 µL 5× RT Buffer, 1 µL dNTP mixture, 0.5 µL oligo primer, 0.5 µL RNase inhibitor, 0.5 µL ReverTraAce, and 2 µL RNA

template. The reverse transcription program was set at 42 °C for 20 min, followed by 99 °C for 5 min.

The cDNA was then amplified by PCR using a universal primer pair, namely U341-Forward (5'-CCGGAATTCATGRTITGGTGYATIGAIAAYGG-3') and Poty1-Reverse (5'-GGATCCCGGGTTTTTTTTTTTTTTTTTV-3'), which amplifies 600–800 bp of the 3'-terminal region of potyviruses (Langeveld et al., 1991). The extracted DNA was tested by PCR using the universal primer pair SPG1-Forward (5'-CCCCGKGGTGC GGWRAATCCAT-3') and SPG2-Reverse (5'-ATCCVAAYWTYCAGGGAGCT-3'), which amplifies approximately 900 bp of the partial AC1 and AC2 genes of begomoviruses (Li et al., 2004).

The total reaction volume used for PCR was 40 µL, consisting of 12 µL ddH₂O, 2 µL of 10 pmol µL⁻¹ forward primer, 2 µL of 10 pmol µL⁻¹ reverse primer, 20 µL ready mix (Bioline, Germany), and 4 µL cDNA or DNA template. The PCR cycling conditions for the U341/Poty1 primer pair were as follows: pre-denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min; followed by final extension at 72 °C for 10 min (Santosa et al., 2024). Meanwhile, the PCR cycling conditions for the SPG1/SPG2 primer pair were as follows: pre-denaturation at 96 °C for 3 min; 35 cycles of denaturation at 96 °C for 1 min, annealing at 59 °C for 1 min, and extension at 72 °C for 1 min; followed by final extension at 72 °C for 10 min (Defitra et al., 2025). PCR was performed without positive and negative controls.

The PCR amplification products were visualized by electrophoresis on a 1% agarose gel stained with Florosafe DNA Staining (1st BASE, Malaysia). A total of 4 µL of a 1 kb DNA marker was loaded into one well, and 4 µL of each PCR product was loaded into the other wells. Electrophoresis was performed at 50 V for 50 min. The single amplicon of the expected size

was sent to the Integrated Laboratory for Research and Testing (LPPT) at Universitas Gadjah Mada for bidirectional Sanger sequencing. After sequencing, the forward and reverse sequences were manually aligned and edited to resolve sequence inconsistencies using MEGA12 software (Kumar et al., 2024). The edited sequence was then subjected to BLASTn analysis on the NCBI website (<https://blast.ncbi.nlm.nih.gov>) to identify the virus species. The nucleotide sequence of the isolate obtained in this study was deposited in NCBI GenBank and assigned an accession number.

Phylogenetic and Percentage Identity Analyses

The sequence of the isolate obtained in this study was aligned together with selected representative corresponding sequences of PRSV available in the NCBI GenBank database. The alignment was performed using the ClustalW algorithm implemented in MEGA12 software (Kumar et al., 2024), and the aligned sequences were trimmed to the same length for phylogenetic analysis based on the 462 bp partial coat protein region. Based on the best-fit substitution model search, the Tamura 3-parameter (T92) model (Tamura, 1992) was selected for phylogenetic tree reconstruction using the Maximum Likelihood (ML) method in MEGA12, with rates among sites modeled as Gamma distributed with invariant sites (G+I). The statistical support for tree branching was estimated using 1,000 bootstrap replicates. Three isolates of zucchini yellow mosaic virus (ZYMV), another potyvirus infecting cucurbit hosts, were used as the outgroup. Pairwise nucleotide identity percentages among the analyzed isolates were calculated using Sequence Demarcation Tool software (SDT v1.2) (Muhire et al., 2014; Topkaya et al., 2025).

Mechanical Transmission

One gram of symptomatic papaya leaves that had tested positive for PRSV infection by

PCR was ground using a mortar and pestle. Five milliliters of potassium phosphate buffer (0.01 M, pH 7.0) was then added to the ground tissue. The sap extract was filtered through cotton, and 400-mesh carborundum was used as an abrasive during inoculation. Four plants each of cucumber (*Cucumis sativus*), melon (*Cucumis melo*), and bitter melon (*Momordica charantia*) were prepared for mechanical inoculation. Inoculation was performed by gently rubbing the inoculum sap onto the leaves of test plants at the two-true-leaf stage. After inoculation, the plants were left for 10 min and then rinsed with distilled water. The inoculated plants were transferred to a greenhouse maintained at 25–27 °C and incubated for four to five weeks. The plants were observed for symptom type, time to symptom appearance, disease incidence, and symptom severity (Zahra et al., 2024). Composite leaf samples from each inoculated host species were tested molecularly for PRSV infection as described previously.

Variables Observed and Analysis

The observed variables in the mechanical transmission assay were symptom type, time to symptom appearance, disease incidence, symptom severity, and RT-PCR confirmation.

- Symptom type was recorded based on visible systemic symptoms appearing on newly developed leaves, including chlorosis, mottle, mosaic, leaf curling, or other visible abnormalities.
- Time to symptom appearance was recorded as the number of days post inoculation (dpi) when the first visible systemic symptom appeared.
- Disease incidence was calculated as the percentage of symptomatic plants from the total number of inoculated plants for each host species using the following formula:

$$I = \frac{S}{T} \times 100 \dots\dots\dots (1)$$

Where: I = Disease incidence (%), S = number of symptomatic plants, T = number of inoculated plants

- Symptom severity was assessed descriptively and was reported using qualitative descriptions, such as mild chlorosis, mottle, or curling, rather than a quantitative disease severity index.
- RT-PCR confirmation was performed on composite leaf samples collected from inoculated plants of each host species, following the molecular detection procedure described previously.

Results

Sample Collection

Leaf samples of five symptomatic papaya plants from different spots within the field were collected as a composite. The samples exhibited clear and systemic viral symptoms such as leaf mosaic, leaf chlorosis, and ring spots on leaves and fruits. Some plants even showed stunted growth. Heavy weed infestation was observed on most of the field but the aphid vector was not observed during survey (Figure 1).

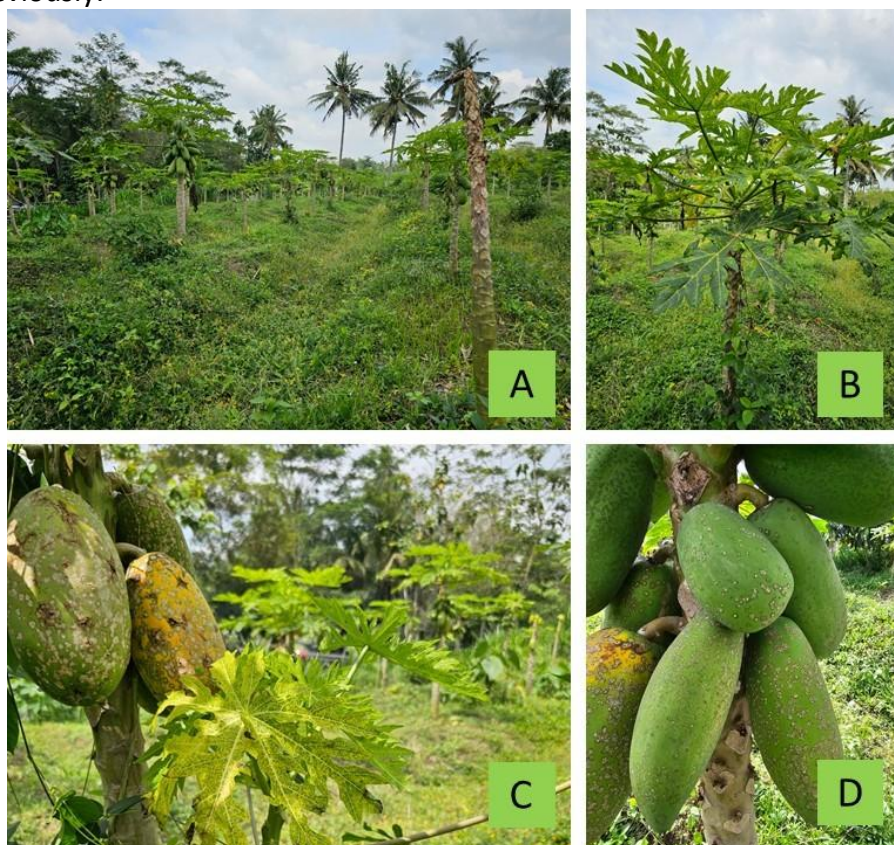


Figure 1. Symptoms on papaya plants in the observed field in Sleman Regency, Indonesia: A. General view of the field, B. Chlorosis and mosaic on papaya leaves, C. Chlorosis and mosaic on papaya leaves, and ringspots on fruits, D. Ringspots on fruits

Virus Identification

The tested sample was positive for infection by a potyvirus by forming the targeted PCR band of around 700 bp, and tested negative for begomovirus infection

since the targeted band was not amplified (Figure 2). Partial genome sequencing and the subsequent BLAST nucleotide analyses further specified PRSV as the virus causing disease on papaya in the observed field.

Partial coat protein (CP) + 3'-tail sequence of the PRSV-SMN-TJ isolate was listed in NCBI GenBank under accession number PX927038.

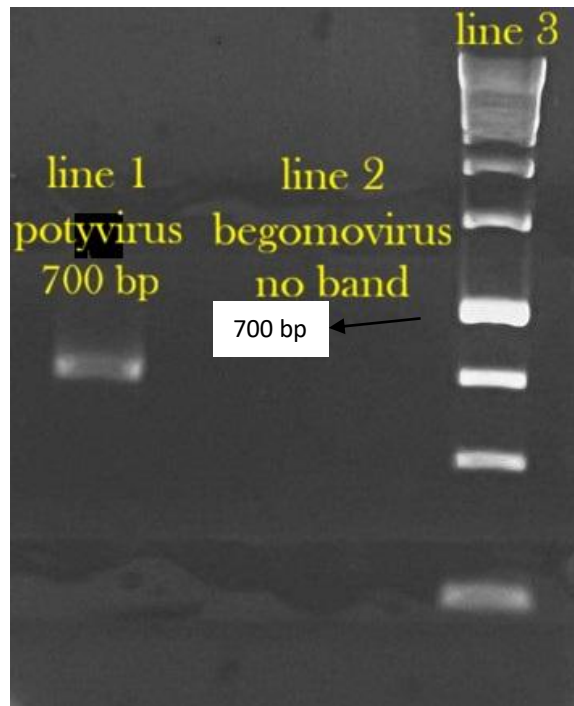


Figure 2. Results of PCR amplification visualized on agarose gel electrophoresis. Lane 1: potyvirus-positive amplicon (~700 bp); Lane 2: no begomovirus-specific amplicon; Lane 3: 1 kb DNA marker.

Phylogenetic Analysis

A phylogenetic tree based on the 3'-end of coat protein gene sequences (462 bp) was constructed using MEGA12, which showed that the PRSV-SMN-TJ isolate clustered with 16 PRSV isolates from Indonesia, Thailand, China, Malaysia, Taiwan, the Philippines, Vietnam, and Brazil (Figure 3). The PRSV-SMN-TJ isolate had the closest genetic relationship with the Chonburi2 (AY010716) and Ratchaburi (AY010721) isolates from

Thailand, which were identified as PRSV-P. Phylogenetic tree analysis revealed that the PRSV-SMN-TJ isolate was distantly related to three ZYMV isolates, including GK (LC498620) on *Cucurbita maxima* from Indonesia, MN364667 on *Citrullus lanatus* from Brazil, and TW-TN3 (NC_003224) on *Luffa cylindrical* from Taiwan. These three isolates were separated from the main clade and formed a new clade as an outgroup in the phylogenetic tree (Figure 3).

Pairwise identity analysis using SDT software showed that the PRSV-SMN-TJ isolate had nucleotide identities ranging from 94.47%–95.54% with isolates 4-DS-2022 (LC861752), PRSV IDN Nganjuk (LC311783), 1-MD-2022 (LC861751), PRSV IDN Kulon Progo (LC311781), PRSV IDN Brebes (LC311784), PRSV IDN Subang (LC311782), and Bali (LC223115) from Indonesia, and nucleotide identities ranging from 98.91%–99.35% with isolates Ratchaburi and Chonburi2 from Thailand (Figure 4). Therefore, the SDT calculation is consistent with the clustering of the phylogenetic tree.

Mechanical Transmission

Leaves of the inoculated bitter melon plants started to show curling and mottle symptoms 14 days post inoculation (dpi), with disease incidence of 100%. Chlorosis and mottle were observed on leaves of cucumber and melon (Figure 5). RT-PCR test confirmed PRSV infection in the inoculated three cucurbit species (Table 1).

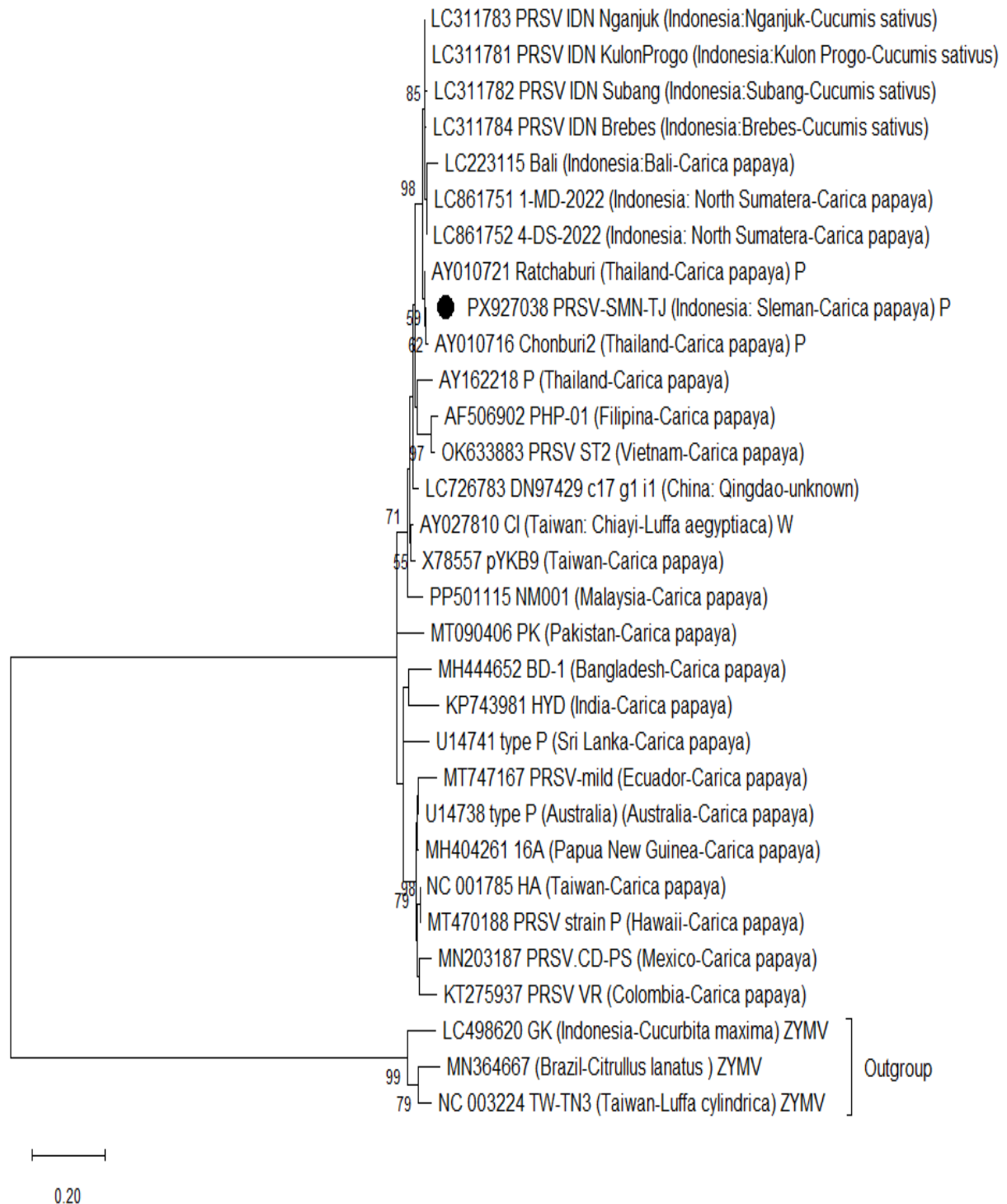


Figure 3. Figure 3. Phylogenetic relationship of the PRSV-SMN-TJ isolate based on the 462 bp partial coat protein region. The maximum-likelihood tree was constructed using MEGA12 with the Tamura 3-parameter model and 1,000 bootstrap replicates. Bootstrap values >50% are shown. The isolate from this study is indicated by a black dot, and zucchini yellow mosaic virus (ZYMV) isolates were used as the outgroup

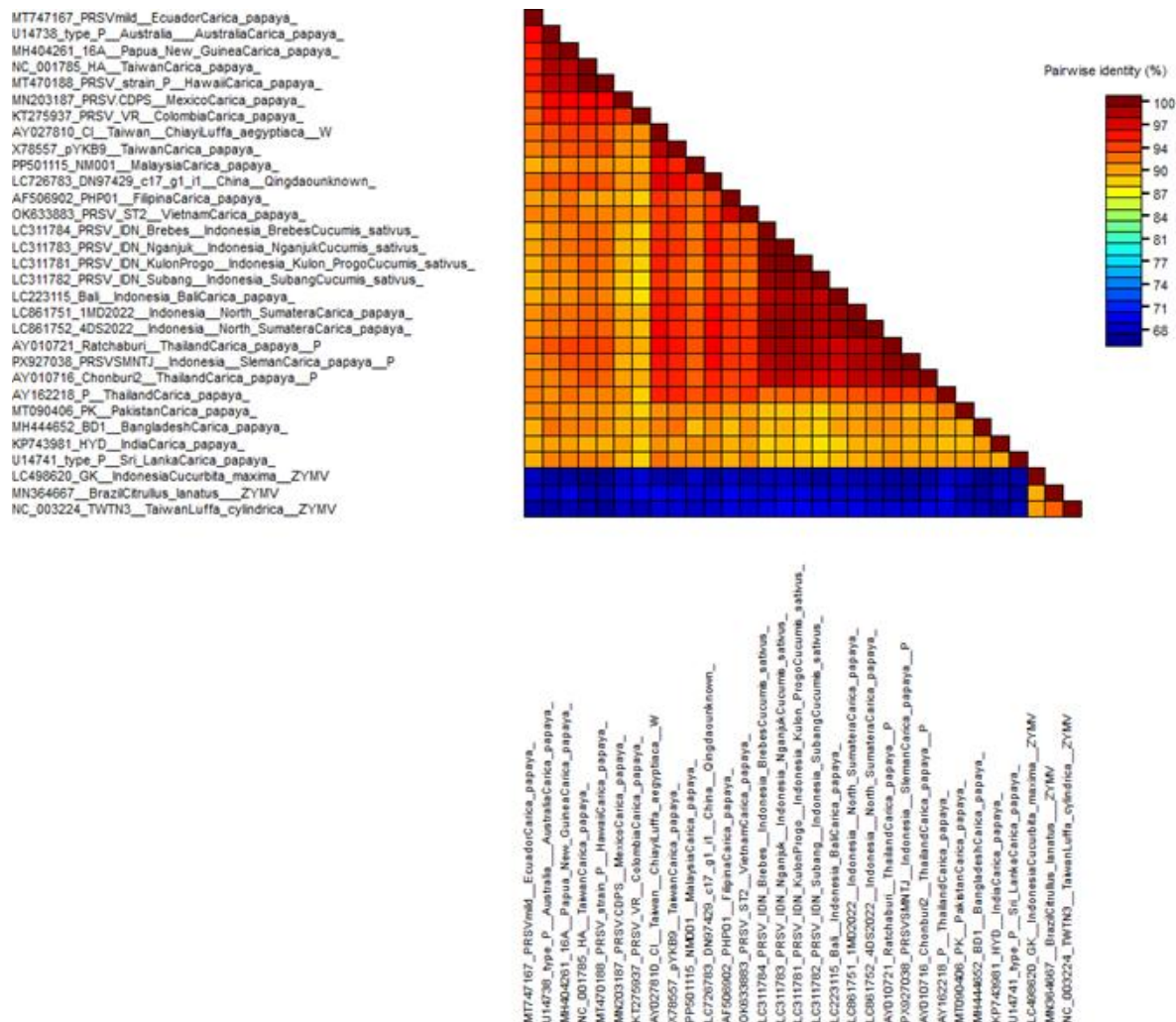


Figure 4. Schematic view of the results of percentage identity analysis by SDT v1.2 software on the obtained papaya ringspot virus isolate compared to other 27 isolates in the NCBI GenBank database based on 462 bp partial coat protein region

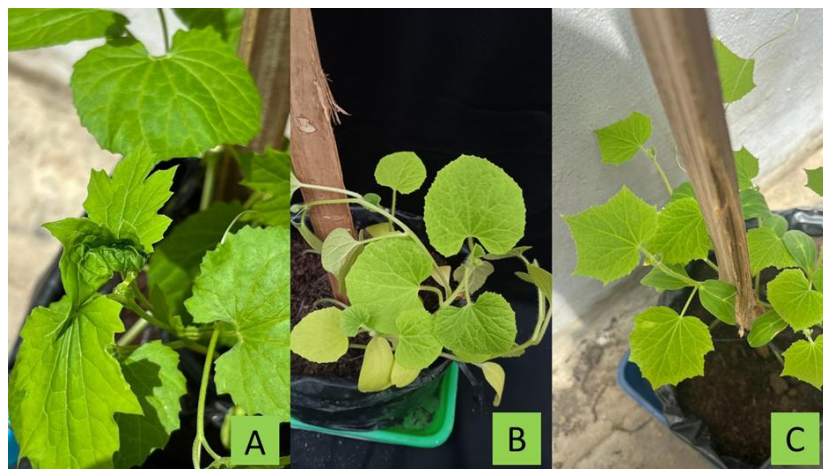


Figure 5. Symptoms on the test plants that were mechanically inoculated with PRSV-SMN-TJ isolate. A. Leaf curling and mottle on bitter melon, B. Chlorosis and mottle on melon, C. Chlorosis and mottle on cucumber

Table 1. Results of mechanical transmission experiment on three cucurbit species

Test plant	No. inoculated	No. symptomatic	Disease incidence (%)	Time to symptom appearance	Symptom description	RT-PCR result
Cucumber (<i>Cucumis sativus</i>)	4	2	50	14 dpi	Chlorosis, mottle	+
Melon (<i>Cucumis melo</i>)	4	2	50	14 dpi	Chlorosis, mottle	+
Bitter melon (<i>Momordica charantia</i>)	4	4	100	14 dpi	Curling, mottle	+

Discussion

This study confirmed that the severe viral-like disease observed in papaya plants from Sleman Regency was associated with papaya ringspot virus (PRSV). The field symptoms included leaf mosaic, chlorosis, stunting, and ring spots on fruits (Figure 1). Molecular detection further supported this finding, the symptomatic papaya sample produced an approximately 700 bp amplicon with the universal potyvirus primer pair, whereas no amplification was obtained with the begomovirus primer pair (Figure 2). The results revealed that the tested sample was infected by a potyvirus.

The symptoms observed in this study were consistent with typical PRSV-P infection in papaya. Infected plants showed leaf mosaic and chlorosis, and ring spots on fruits (Figure 1), which are commonly reported as characteristic symptoms of PRSV infection. Typical PRSV-P foliar symptoms include mosaic, chlorosis, vein clearing, abnormal leaf elongation and narrowing, and severe leaf malformation resembling shoestring-like structures. In addition, characteristic oily streaks or greasy lesions may develop on stems and petioles, while infected fruits commonly exhibit concentric ring spots, which represent the hallmark symptom of the virus infection (Hidayat et al., 2012; Thamrin,

2020). Similar symptom expression has also been reported in Indonesian papaya plants infected by PRSV, including isolates from Java and other papaya-growing regions (Farida et al., 2022; Riska et al., 2023b; Harahap et al., 2025). Therefore, the field symptoms documented in Sleman support the molecular detection results and are consistent with previous reports of PRSV infection in papaya.

The negative result for begomovirus detection is also important because several viruses may induce overlapping symptoms in papaya. Papaya leaf curl virus and other begomoviruses have been associated with papaya diseases in Indonesia and other regions, and mixed viral infection should be considered when diagnosing viral-like symptoms in the field. In this study, begomovirus testing was included to exclude possible begomovirus infection or mixed infection in the symptomatic papaya sample. The absence of a begomovirus-specific amplicon (Figure 2) revealed that begomovirus infection was not detected in the tested composite sample.

The identification of PRSV in Sleman adds further evidence to the current distribution record of PRSV in Indonesia. The first documented case of PRSV infecting papaya in Indonesia may have occurred in Aceh Besar Regency, Nanggroe Aceh

Darussalam (Hidayat et al., 2012). Since then, PRSV infection has been reported in several regions, including Sleman, Tabanan, Boyolali, Bogor, Medan, Kebumen, Purworejo, Bantul, Nganjuk, and Malang (Nurhantoro et al., 2018; Fauziyah et al., 2020; Farida et al., 2022; Riska et al., 2023b; Harahap et al., 2025). Additionally, PRSV infection in cucumber has been identified in the Special Region of Yogyakarta, West Java, East Java, and Central Java (Listihani et al., 2018). The present finding provides additional isolate-level information from Sleman Regency and supports the need for continuous surveillance of PRSV in papaya-growing areas.

Phylogenetic analysis based on the 462 bp partial coat protein region placed the PRSV-SMN-TJ isolate within the same clade as PRSV isolates from Indonesia and Thailand (Figure 3). Pairwise nucleotide identity analysis further showed that PRSV-SMN-TJ shared 98.91–99.35% nucleotide identity with the Ratchaburi and Chonburi2 isolates from Thailand and 94.47–95.54% identity with several Indonesian isolates (Figure 4). This pattern indicates a close molecular relationship between PRSV-SMN-TJ and several previously reported PRSV isolates, particularly those from Thailand. These groupings were similar to other Indonesian isolates reported by Riska et al. (2023b), suggesting possible regional relatedness or shared ancestry among isolates.

The phylogenetic result also supports the classification of PRSV-SMN-TJ as a PRSV-P-related isolate because it was obtained from papaya and clustered with known PRSV-P isolates (Figure 3). PRSV-P is known to infect papaya and several cucurbit hosts, whereas PRSV-W is generally associated with cucurbit hosts and does not infect papaya (Riska et al., 2023b; Magar et al., 2024). In this study, PRSV-SMN-TJ also showed molecular relatedness to some PRSV-W-associated

sequences, but this result should be interpreted with caution because the analysis used a short partial coat protein region. Therefore, the data are sufficient to indicate molecular relatedness among the analyzed PRSV isolates.

Mechanical transmission showed that PRSV-SMN-TJ was transmissible to the three tested cucurbit hosts. Symptoms appeared at 14 days post-inoculation in cucumber, melon, and bitter melon. Disease incidence was 50% in cucumber, 50% in melon, and 100% in bitter melon, with chlorosis and mottle observed on cucumber and melon and curling and mottle observed on bitter melon (Table 1, Figure 5). RT-PCR confirmation of inoculated host samples further supported the association of the observed symptoms with PRSV infection (Table 1). These findings indicate that PRSV-SMN-TJ can be mechanically transmitted to selected cucurbit hosts under the conditions of this assay.

The ability of PRSV isolates to infect cucurbit hosts has been reported previously and may vary among isolates. Riska et al. (2023a) showed that PRSV isolates from different Indonesian regions differed in their ability to infect cucurbit hosts and in the symptoms they induced. Such variation may be associated with isolate characteristics, host species, and environmental conditions. In the present study, bitter melon showed the highest disease incidence, whereas cucumber and melon showed lower incidence (Table 1). This trend suggests that the cucurbit hosts differed in their response to mechanical inoculation with PRSV-SMN-TJ.

Field conditions may have contributed to the severe disease observed in the papaya field, but this study did not directly test agronomic or epidemiological factors. Heavy weed infestation was observed in the field (Figure 1), but the weed species were not identified or tested for PRSV infection. At

most, the observation suggests that poor field sanitation may support conditions favorable for virus persistence or vector activity, as also emphasized in PRSV management studies (Riska et al., 2023a). Similarly, the cultivar identity and resistance status of the papaya plants were not confirmed.

This study provides molecular evidence of PRSV infection in a severely affected papaya field in Sleman and preliminary evidence that the PRSV-SMN-TJ isolate can be mechanically transmitted to selected cucurbit hosts. Broader field sampling, complete diagnostic controls, quantitative pathogenicity assessment, and expanded host testing are needed to better understand the epidemiological significance of PRSV-SMN-TJ in papaya-growing areas.

Conclusions

This study identified identified the virus as PRSV-P, registered as PRSV-SMN-TJ, accession number PX927038. Disease incidence after inoculation ranged from 50% to 100%. Phylogenetic and nucleotide identity analyses placed the PRSV-SMN-TJ isolate within the same cluster as Indonesian and Thailand PRSV isolates. This study was based on a single composite field sample and a limited host transmission assay; therefore, broader sampling, complete diagnostic controls, and quantitative pathogenicity assessment are needed. Nevertheless, these findings provide baseline information for PRSV surveillance and management planning in papaya-growing areas.

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Declaration

Author contribution

Meyrin Novia Vadilah is the main contributor of this paper, Adyatma Irawan Santosa is the corresponding author, and Fhilia Raga Cipta Wida, Rossa Wulandari, Alimah Nur Hasanah, Fatwa Annisa Yustisia, Carreista Ashila Shafa, Fadzilah Nur Hidayah, and Hanum Nilamsari are co-authors. All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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