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Molecular detection and distribution analysis of papaya ringspot virus-p in North Sumatra, Indonesia

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ABSTRACT
The Papaya Ringspot Virus-P (PRSV-P) presents a significant threat to papaya cultivation, especially due to inter-island fruit trading, which facilitates its rapid spread. In North Sumatra, PRSV-P has caused substantial damage to papaya plantations, resulting in decreased fruit yields. This research aimed to elucidate the bio-ecological and molecular characteristics of PRSV-P in papaya, emphasizing its potential seedborne transmission route. Field assessments were performed in Deli Serdang, Dairi, and Pakpak Bharat, where symptomatic plants exhibited dark green ring spots on fruits and mosaic patterns on foliage. Samples were analyzed with DAS-ELISA, RT-PCR, and nucleotide sequencing, while a synthesized infectious PRSV-P clone was manually introduced into juvenile pepper plants to confirm pathogenicity. DAS-ELISA validated infections in leaf and seed specimens, while RT-PCR analysis identified elevated proportions of whole viral genomes. Sequence analysis revealed over 99% nucleotide and amino acid identity with PRSV-P strains from Bali and Thailand, signifying a close genetic affinity. The detection of PRSV-P in papaya seeds constitutes the initial molecular evidence of seed transmission in North Sumatra and indicates a possible method for long-distance dispersal independent of insect vectors or vegetative propagation. These findings highlight the imperative to incorporate molecular diagnostics into monitoring initiatives and to implement effective management techniques, including seed health assessments, creation of resistant cultivars, and stringent phytosanitary protocols. This study underscores the pivotal significance of seed transmission in the epidemiology of PRSV-P and its ramifications for sustainable papaya cultivation in Indonesia.

Contribution/Originality: The paper's primary contribution is finding that Papaya Ringspot Virus-P (PRSV-P) undergoes seed transmission in North Sumatra, demonstrated through molecular characterization for the first time. This novel evidence establishes a critical dispersal route, advancing understanding of PRSV-P epidemiology and informing integrated disease management strategies to safeguard papaya cultivation.

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1. INTRODUCTION

Papaya (Carica papaya) is an herbaceous fruit plant in the species Caricaceae, native to the Central and West regions of America, in the vicinity of Mexico and Costa Rica (Sharma, Bachheti, Sharma, Bachheti, & Husen, 2020). The reliable papaya, which can even be found in home gardens across Indonesia, is affected by soil and environmental conditions (Koul et al., 2022). Indonesia has bad news for the papaya industry because the production of papaya in Indonesia has recorded a decrease since 2010 (Fuentes & Santamaría, 2013). Nevertheless, Indonesia was still a major papaya producer among countries such as India, Brazil, Mexico, and Nigeria (Alara, Abdurahman, & Alara, 2022). One of the major problems in papaya cultivation is the Papaya Ringspot Virus-P (PRSV-P), a potyvirus that belongs to the family Potyviridae (Mesta et al., 2023).

The first description of PRSV-P was made in Taiwan during 1975, and it rapidly dispersed, decreasing papaya production from 41,595 tons in 1974 to 18,950 tons in 1977 (Bau et al., 2008). Production in the Philippines declined significantly, from 36,000 tons in 1981 to only 10,000 tons by 1987 (Gonsalves et al., 2004); Brazilian and Indonesian production also decreased. The decline in production is primarily due to erratic weather conditions and pest attacks, resulting in a loss of up to 85–90% due to PRSV-P infection (Mesta et al., 2023). Factors contributing to the spread of plant diseases include cultivation practices, fertilizer use, differing seeds of origin, and diverse planting systems (Chavan, Tomar, & Dhale, 2010). PRSV-P is prevalent in tropical and subtropical nations where papaya is grown, particularly across Southeast Asia (Umer et al., 2022). In North Sumatra, papaya plays a significant role, with major production zones in Deli Serdang, Langkat, and Binjai City, and recent expansion to Karo, Dairi, and Pakpak Barat. PRSV-P, initially reported in Indonesia in Aceh in 2011 and in Deli Serdang, North Sumatra, in 2013 (Farida, Damayanti, Efendi, & Hidayat, 2022), is now found across Sumatra, Java, and Bali (Budiyanti & Husada, 2023).

PRSV-P mainly infects papaya but also has secondary hosts in the Cucurbitaceae and Chenopodiaceae plant families (Datar, 2012). Detection and identification of PRSV-P can be carried out effectively using serological strategies such as DAS-ELISA and molecular techniques like RT-PCR (Joy, Johnson, & Thara, 2023). The inter-island trade of papayas poses a significant threat for the widespread spread of PRSV-P, which has already caused harm and reduced fruit production in North Sumatra. The decline in yields results from decreased photosynthetic ability and vigor in infected plants, increasing susceptibility to environmental stresses and leading to higher mortality rates. Since commercial antiviral treatments are unavailable, eradication of infected plants remains a primary control measure, although it results in substantial losses. Effective disease management strategies, based on a comprehensive understanding of the virus, are essential.

Although PRSV-P is widely reported in Indonesia, there appears to be limited scientific evidence of seed transmission in North Sumatra. Seed-borne infection, if confirmed, could offer a significant pathway for long-distance dissemination and pose a serious challenge for disease control. The present study is the first to report molecular-based detection of PRSV-P in papaya seeds from North Sumatra. The objectives were to (1) survey the distribution and incidence of PRSV-P in major papaya-growing areas, (2) detect PRSV-P in leaves and seeds using DAS-ELISA and RT-PCR, and (3) assess the genetic similarity of North Sumatran isolates with those from other regions.

2. MATERIALS AND METHODS

2.1. Selection of Sample Locations

The research was carried out by surveying symptoms and disease occurrence of PRSV-P in the field. Field surveys were conducted in three regencies (Deli Serdang, Dairi, Pakpak Bharat). In each regency, three villages with intensive papaya cultivation were purposively selected, and one plantation per village was chosen. Table 1 shows locations for sampling papaya plants.

Table 1. Locations for sampling papaya plants.

No	Location of plant sampling papaya	Variety papaya	Height
1.	Deli Serdang Regency Hamparan Perak Village, Hamparan Perak District	California	23 meters above sea level
2.	Saentis Village, Percut Sei District Sir	California	19 meters above sea level
3.	Patumbak I Village, Patumbak District	California	40 meters above sea level
4.	Dairi Regency Lau Njuhar Village, Tanah Pinem District	California	200 meters above sea level
5.	Lae Parira Village, Lae Parira District Dairi Regency	California	777 meters above sea level

No	Location of plant sampling papaya	Variety papaya	Height
6.	Village: Sitinjo 1, Sitinjo District	Calina	1080 masl
7.	Pakpak Bharat Regency, Boang Village Manalu, Salak District	Thailand	946 meters above sea level
8.	Siempat Rube I Village, District Sifour Rube	California	1086 masl
9.	Kutadame Village, Royal District	California	1090 masl

2.2. Observation of Symptoms and Incidence of PRSV-P in the Field

Observations were carried out at nine papaya planting locations, where five plant samples per research location were taken randomly. Papaya plants showing symptoms of PRSV-P disease were placed in paper bags and stored in a cool box to be taken to the laboratory. This condition helps to keep the plant samples fresh (Shivas & Beasley, 2005).

Disease observations in the field are conducted based on symptoms and disease incidence counts. The incidence of Papaya Ringspot Virus-P (PRSV-P) is calculated using the following formula:

$$K = n/N \times 100\%$$

Note:
K = PRSV-P disease incidence (%).
n = Number of papaya plants attacked by PRSV-P.
N = Number of papaya plants observed (Fajinmi, 2011).

2.3. Detection of PRSV-P using Double Antibody Sandwich - Enzyme Linked Immunoassay (DAS-ELISA) Method

Initially, a microplate was prepared by filling each well with 100 µl of PRSV-P antibody dissolved in coating buffer at a 1:100 ratio. The plate was covered with aluminum foil, moistened with a paper towel, placed in a plastic container, and incubated overnight at 4ºC. At each study site, samples from five symptomatic papaya plants were collected, including leaves and seeds. The sap was extracted by crushing the samples with General Extract Buffer (GEB) at a 1:10 ratio. The homogenate was centrifuged at 5000 rpm for 5 minutes. Plates were washed with PBST, incubated with an enzyme-conjugated secondary antibody (1:100), and developed with PNP substrate (0.5 g in 5 mL buffer). Absorbance was measured at 405 nm using an ELISA reader (Begum, Masud, Akanda, & Miah, 2016).

2.4. PRSV-P Identification via Reverse Transcription Polymerase Chain Reaction

RT-PCR was carried out using primers OYDVKBF and OYDVKBR targeting the PRSV-P coat protein gene. Cycling conditions were: 95°C for 3 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 60 s; final extension 72°C for 10 min. Products were run on 1.5% agarose gel stained with ethidium bromide and visualized under UV (Mohamed, Smith, & Lee, 2012).

2.5. Nucleotide Sequence

PCR samples from each location were delivered for genetic sequencing at FirstBASE Laboratories in Singapore. The resulting nucleotide sequences underwent comparative analysis with published PRSV-P genetic sequences accessible through GenBank using Basic Local Alignment Search Tools (BLAST). The selected nucleotide data were later modified and inspected for precision using a wide range of alignment applications such as ClustalW, BioEdit version 7.0.5, and CLC Genomics Workbench version 0.2 (Chong et al., 2011).

3. RESULTS AND DISCUSSION

3.1. Survey, Detection and Incidence of Papaya Ringspot Virus – (PRSV-P) in Papaya Plants

The survey conducted across nine diverse locations in North Sumatra uncovered a significant occurrence of Papaya Ringspot Virus-P (PRSV-P) afflicting papaya plants. As highlighted in Table 2 and Figure 1, the survey results elucidated the pervasive impact of Papaya Ringspot Virus-P (PRSV-P) across the varied regions of North Sumatra. In all nine sites surveyed, disease incidence diverged notably, with certain areas such as Hamparan Perak Village, Saentis Village, and Patumbak I Village in Deli Serdang Regency reporting complete infection rates of 100%, whereas higher altitude locales, including Kutadame Village in Pakpak Bharat Regency exhibited lower incidence rates of merely 25%.

Table 2. Incidence of Papaya Ringspot Virus-P (PRSV-P) in nine surveyed papaya plantations across North Sumatra.

No	Regency/Subdistrict/Village	Sample Papaya Garden Area	Number of papaya plants	Number of plants affected	PRSV disease incidence in each location	Altitude (masl)
1.	Hamparan Perak Village, Hamparan Perak District Deli Serdang Regency	2,000 m²	500	500	100%	23
2.	Saentis Village, Percut Sei Tuan District Deli Serdang Regency	2,400 m²	600	600	100%	19
3.	Patumbak I Village, Patumbak District, Deli Serdang Regency	2,400 m²	550	550	100%	40

No	Regency/Subdistrict/Village	Sample Papaya Garden Area	Number of papaya plants	Number of plants affected	PRSV disease incidence in each location	Altitude (masl)
4.	Lau Njuhar Village, Tanah Pinem District, Dairi Regency	10,000 m²	12,500	12,500	100%	200
5.	Lae Parira Village, Lae Parira District, Dairi Regency	5,000 m²	6250	6250	100%	777
6.	Village: Sitinjo 1 Sitinjo District, Dairi Regency	10,000 m²	12,400	12,400	100%	1080
7.	Boang Manalu Village, Salak District, Pakpak Bharat Regency	2,000 m²	500	200	40 %	946
8.	Siempat Rube I Village, Siempat Rube District, Pakpak Bharat Regency	4,000 m2	1000	500	50 %	1086
9.	Kutadame Village, Royal District, Pakpak Bharat Regency	2,000 m2	500	125	25 %	1090

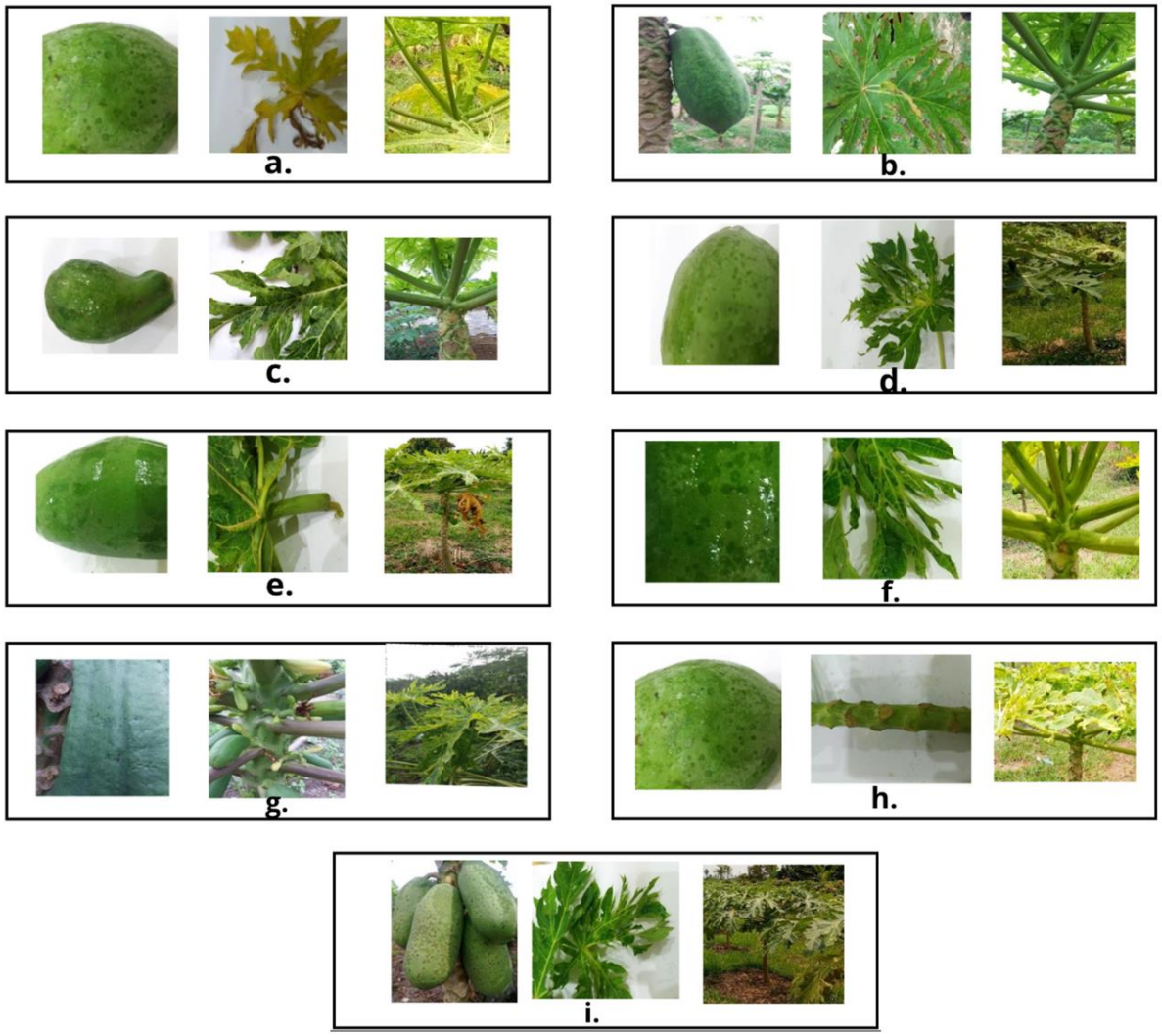

Figure 1. Symptoms and Incidence of PRSV-P. a. Hamparan Perak Village, Hamparan Perak Subdistrict (Deli Serdang Regency); b. Saentis Village, Percut Sei Tuan Subdistrict (Deli Serdang Regency); c. Patumbak I Village, Patumbak Subdistrict (Deli Serdang Regency); d. Lau Njuhar Village, Tanah Pinem Subdistrict (Dairi Regency); e. Lae Parira Village, Lae Parira Subdistrict (Dairi Regency); f. Sitinjo 1 Village, Sitinjo Subdistrict (Dairi Regency); g. Boang Manalu Village, Salak Subdistrict (Pakpak Bharat Regency); h. Siempat Rube I Village, Siempat Rube Subdistrict (Pakpak Bharat Regency); i. Kutadame Village, Kerajaan Subdistrict (Pakpak Bharat Regency).

Figure 1 and Table 2 revealed that the entire surveyed papaya plants in Deli Serdang Regency and Dairi Regency displayed PRSV-P symptoms, with every location sampled showing 100% infection rates, including ring blotches on fruits and mosaic patterns on leaves. This aligns with global reports of rampant PRSV-P outbreaks, in which the virus can rapidly permeate regions, ultimately causing total crop failure without proper control measures in place.

The highest infection rates were observed in lowland areas (below 50 m a.s.l.), where all surveyed plantations showed 100% incidence. In contrast, highland areas above 900 m a.s.l. showed significantly lower incidence (25–50%), such as 40% in Boang Manalu Village and 25% in Kutadame Village. These variances suggest that altitude may play a role in mitigating the spread and impact of PRSV-P, despite further investigation being necessary to validate this observation.

The complete devastation of crops in areas like Lau Njuhar Village and Lae Parira Village, where massive commercial farms experienced infection rates of one hundred percent, underscores the urgent need for improved strategies for disease management. The lack of commercially available antiviral treatments for PRSV-P exacerbates this issue, leaving farmers to resort solely to the costly and labor-intensive removal of contaminated plants to prevent further dissemination. Moreover, the prospect of seed transmission of PRSV-P, as evidenced through RT-PCR outcomes in this analysis, raises concerns regarding the efficacy of current standards of quarantine and sanitation. The profound genetic similarity between PRSV-P strains in North Sumatra and those elsewhere in Southeast Asia, such as Bali and Thailand, emphasizes the regional connectivity related to the distribution of this virus.

These discoveries emphasize the necessity for an integrated approach to managing PRSV-P, encompassing the employment of virus-free seed stock, consistent field surveillance, and the advancement of papaya varieties resistant to PRSV. Without such measures, the virus will persist in jeopardizing papaya production in North Sumatra and beyond, resulting in significant financial losses for local farmers and the regional agricultural sector.

3.2. Detection Results of PRSV-P Using DAS ELISA Test

Detection of PRSV-P was performed using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method. The ELISA method is one technique; serology is the most popular because it is not only sensitive but also easy to carry out, accurate, simple, and does not require high costs (Ma et al., 2011). Test results DAS-ELISA can also be measured in a quantitative manner using an ELISA reader with a spectrophotometer at a wavelength of 405 nm (Table 3).

Table 3. Results of the watershed test ELISA on papaya plants.

No.	Location	Leaf					Rind					Seed				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1.	Hamparan Perak Village, Hamparan Perak District, Deli Serdang Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
2.	Kolam Village, Percut Sei Tuan District, Deli Serdang Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
3.	Patumbak Village Two Patumbak District Deli Serdang Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
4.	Lau Njuhar Village, Tanah Pinem District, Dairi Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
5.	Lau Baleng Village, Lau Baleng District, Dairi Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
6.	Village: Sitinjo 1, Sitinjo District, Dairi Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
7.	Boang Manalu Village, Salak District, Pakpak Bharat Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
8.	Siempat Rube Village, Siempat Rube I District, Pakpak Bharat Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
9.	Kutadame Village, Royal District, Pakpak Bharat Regency	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Seed samples at all research locations showed negative results for PRSV-P (Table 3). Based on research conducted by Bayot et al. (1990) in the Philippines, it showed that only 2 out of 1,335 seeds (0.15%) of Cavite papaya grown from seeds infected with PRSV-P exhibited symptoms similar to PRSV-P. In another study, no seed-borne viruses were

detected in papaya (Joy, Manoranjitham, Karthiba, Meenakshisundaram, & Suganthy, 2022). The virus will spread easily because these fleas can fly and move from place to place easily. In addition, its light body will easily follow the direction of the wind. Therefore, it is not surprising that the attack power and spread of this virus are very fast. If one plant has been attacked by a virus, its transmission to other plants will not take long, even though the distance between the plants is quite far (Sá Antunes et al., 2020).

3.3. Detection of PRSV-P in Papaya Leaves Using RT-PCR and Nucleotide Sequence Analysis

Leaf samples from Deli Serdang, Dairi, and Pakpak Bharat showed symptoms of malformation, yellowish mosaic, and dark green mosaic. RT-PCR detection successfully amplified a 475 bp DNA band using the primer pair PRSV-326: 5'-TCG TGC CAC TCA ATC ACA AT-3' and PRSV-800: 5'-GTT ACT GAC ACT GCC GTC CA-3'. The visualization of the DNA amplification is shown in Figure 2 and Table 4.

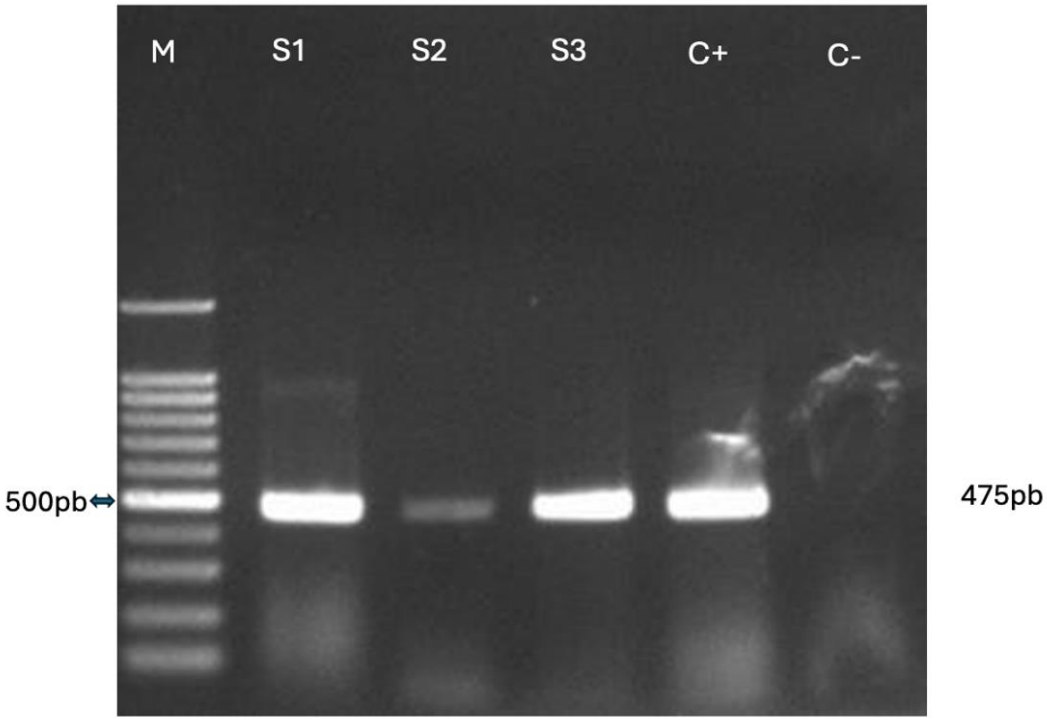

Figure 2. DNA amplification of papaya leaves samples using RT-PCR: S1: Deli Serdang Regency; S2: Dairi Regency; S3: Pakpak Bharat Regency; C+: Positive control; C-: Negative control.

Table 4. RT-PCR testing data of papaya leaf samples.

No.	Papaya leaf sample	Target/Method	Testing results
1.	S1 leaf	PRSV-P/RT-PCR	Positive
2.	S2 leaf	PRSV-P/RT-PCR	Positive
3.	S3 leaf	PRSV-P/RT-PCR	Positive

Note: S1: Deli Serdang regency; S2: Dairi regency; S3: Pakpak Bharat regency.

The RT-PCR analysis successfully detected Papaya ringspot virus type P (PRSV-P) in leaf samples from Deli Serdang, Dairi, and Pakpak Bharat regencies, as indicated by the amplification of a 475 bp fragment. This result confirms the presence of PRSV-P in these regions, correlating with the typical symptoms of malformation, yellowish mosaic, and dark green mosaic observed in infected papaya leaves. The positive control (C+) showed a distinct band at 475 bp, confirming the specificity of the primers used, while the negative control (C−) did not show any amplification, verifying the accuracy of the results. This finding is consistent with previous studies, such as Mesta et al. (2023), which also reported successful PRSV-P detection using RT-PCR in infected papaya plants, amplifying similar fragment sizes. The slight size variations may result from differences in viral strains or primers. Early detection like this is critical for disease management strategies in papaya cultivation. Both studies underscore the effectiveness of RT-PCR in the early and accurate detection of PRSV-P in papaya plants, which is crucial for disease management and control strategies.

3.4. Detection of PRSV-P in Papaya Seeds

The RT-PCR results in Figure 3 and Table 5 confirmed the presence of PRSV-P in all tested samples (S1: Deli Serdang, S2: Dairi, and S3: Pakpak Bharat), as indicated by the 475 bp DNA bands. These results contrast with the negative outcomes from DAS-ELISA serology tests, suggesting that RT-PCR is a more sensitive method for detecting PRSV-P in seeds. The amplification pattern is consistent with prior findings in leaf samples, supporting RT-PCR as a reliable tool for early detection of PRSV-P in both seeds and leaves.

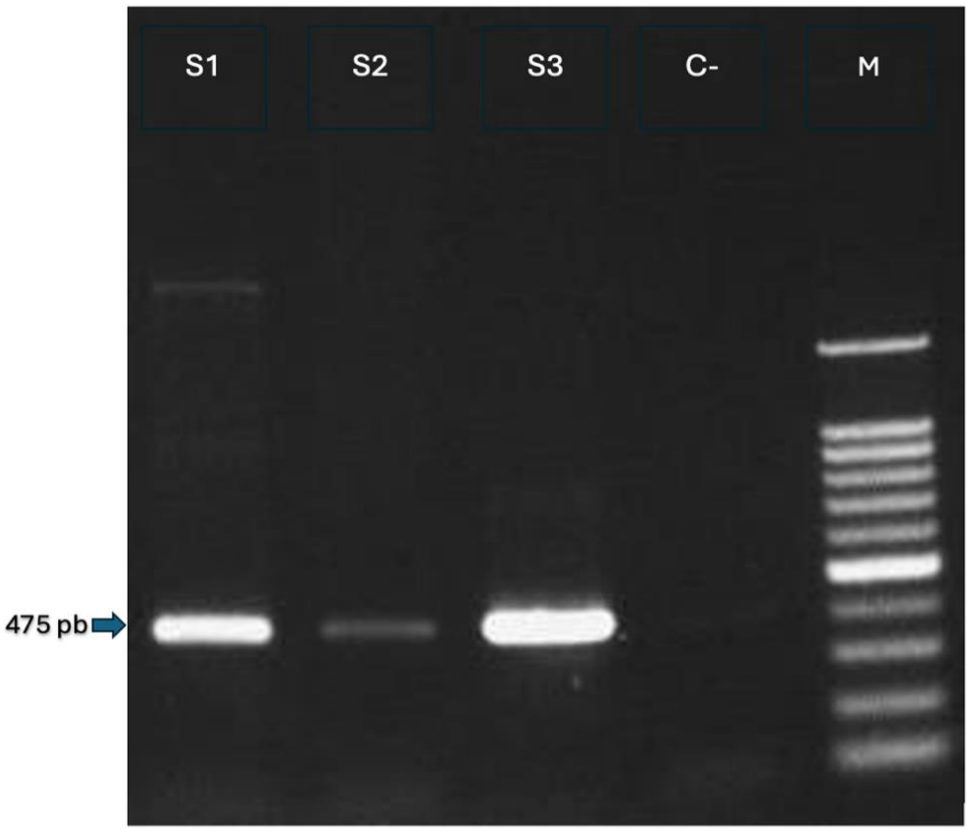

Figure 3. DNA amplification of papaya seed samples using RT-PCR, S1: Deli Serdang Regency; S2: Dairi Regency; S3: Pakpak Bharat Regency; C+: positive control; C-: negative control.

Table 5. RT-PCR Testing Data of Papaya Seed Samples.

No.	Papaya Seed Sample	Target/Method	Testing Results
1.	S1 Seed	PRSV-P/RT-PCR	Positive
2.	S2 Seed	PRSV-P/RT-PCR	Positive
3.	S3 Seed	PRSV-P/RT-PCR	Positive

Note: S1: Deli Serdang Regency; S2: Dairi Regency; S3: Pakpak Bharat Regency.

3.5. Nucleotide Sequence Analysis

Nucleotide sequence analysis revealed high similarity between PRSV-P isolates from North Sumatra and other regions, indicating potential common origins. The sequences were highly homologous (98-99%) with isolates from Bali, KPS, THP-14, Chiang Mai, and Ratchaburi, as shown in Tables 6-7.

Table 6. Similarity level of nucleotide cycles of coat protein gene partial CDS PRSV-P isolate on leaf samples.

Sample	Accession No.	Origin of Isolate	Similarity rate (%)
S1	LC223115.1	Bali	98
	AF374862.1	KPS	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98
	AY010721.1	Ratchaburi	98
S2	LC223115.1	Bali	98
	AF374862.1	KPS	98
	U14743.1	Thailand	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98
S3	LC223115.1	Bali	99
	AF374862.1	KPS	99
	U14743.1	Thailand	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98

Table 7 presents the nucleotide sequence similarity of PRSV-P isolates derived from papaya seeds in North Sumatra in comparison to reference isolates from Bali, Thailand, and Chiang Mai. The findings indicate that the seed isolates exhibit 98–99% sequence identity with the regional strains, reflecting a significant degree of genetic homology. The observed similarity indicates that the viral populations in North Sumatra are closely related to those in other Southeast Asian areas, thereby reinforcing the hypothesis of regional connectivity and potential seed-mediated dissemination.

Table 7. Level of similarity of nucleotide cycles of coat protein gene partial cds PRSV-P isolate on seed samples.

Samples	Accession no.	Origin of isolate	Similarity rate (%)
S1	LC223115.1	Bali	99
	AF374862.1	KPS	99
	U14743.1	Thailand	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98
S2	LC223115.1	Bali	99
	AF374862.1	KPS	99
	U14743.1	Thailand	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98
S3	LC223115.1	Bali	99
	AF374862.1	KPS	99
	U14743.1	Thailand	98
	AF506898.1	THP-14	98
	PQ85856.1	Chiangmai	98

The nucleotide sequence analysis using Clustal W revealed a high level of homology between PRSV-P isolates from North Sumatra and those from other countries such as Thailand, Vietnam, Japan, Philippines, India, Brazil, Bali, Nganjuk, Jatim, Brebes, Subang, Chiangmai, and Ratchaburi. This high similarity, ranging from 98% to 99%, suggests a potential common origin or recent divergence among these isolates. The detection of PRSV-P in both papaya leaves and seeds from Deli Serdang, Dairi, and Pakpak Bharat indicates that the virus is widespread in these regions. The study's finding that isolates from various locations shared a high degree of nucleotide similarity supports the hypothesis that regional transmission and related viral lineages account for the distribution. The RT-PCR method employing specific primers for PRSV-P has proven to be a crucial technique for early identification and management of infections, effectively detecting even low concentrations of the virus. As highlighted by the research conducted by Babu, Sreenivasulu, and Nayak (2012), this molecular detection process relies on conserved regions within the envelope protein gene, aligning with the outcomes of the current analysis. Interestingly, whereas previous work by Tripathi et al. in 2008 suggested that PRSV-P does not transmit through seeds, our results, along with those from Laney et al. in 2012 and Mederos et al. in 2020, indicate that the pathogen can indeed be conveyed via seeds. This discrepancy may stem from differences in detection techniques or strains of the virus. The extensive nucleotide sequence homology between isolates from North Sumatra and other regions underscores the need for coordinated management policies across geographical boundaries. As demonstrated in this study, molecular characterization and phylogenetic examination are essential tools for understanding the spread and development of PRSV-P.

The detection of PRSV-P in papaya seeds from Deli Serdang, Dairi, and Pakpak Bharat presents compelling evidence for the potential seed transmission of PRSV-P. The nearly identical nucleotide sequence at 99% with isolates from Bali and KPS strongly indicates that these seeds likely harbor the same viral strain prevalent in those areas. Contradicting earlier assumptions that PRSV-P is not seed-borne, this finding aligns with more recent research supporting the possibility of seed transmission. The implications of seed transmission are profoundly consequential for controlling and managing PRSV-P. Seeds potentially serving as carriers could aid the virus's dissemination over vast distances, rendering quarantine and sanitary measures increasingly difficult. This underscores the necessity of exclusively employing certified virus-free seeds for cultivation and rigorously screening seed producers through stringent testing protocols.

Furthermore, the high similarity rates between 98-99% for papaya seed isolates and leaf isolates from an identical region suggest local seed stocks may act as a reservoir perpetuating the virus's presence in the area. This highlights the need for integrated pest management strategies encompassing regular testing of seeds, plants, and soil to identify and remove sources of infection.

Future studies should focus on exploring the mechanisms of seed transmission as well as developing resistant papaya cultivars. Advanced biotechnological approaches, comprising genetic engineering and selective breeding, could potentially offer long-term solutions for managing PRSV-P infections.

4. CONCLUSION

This study presents the inaugural molecular evidence of PRSV-P detection in papaya seeds in North Sumatra, thereby affirming seed transmission as a new mode of dissemination. Field surveys indicated a significant prevalence in lowland areas, whereas molecular analysis demonstrated 98–99% similarity with isolates from Bali and Thailand,

suggesting regional interconnectivity. The results underscore the necessity for verified virus-free seeds, molecular screening, stringent quarantine measures, and integrated management techniques. Future studies must elucidate seed transmission mechanisms, enhance molecular epidemiology, and cultivate resistant varieties to ensure the sustainability of papaya production in Indonesia.

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Institutional Review Board Statement: The Research Ethics Committee of Universitas Sumatera Utara, Indonesia, has granted approval for this study on 28 January 2019 (Ref. No. 45/KE/KT.210/K.9.A/01/19).
Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.
Disclosure of AI Use: The author used OpenAI's ChatGPT (GPT-4) to edit and refine the wording of the Introduction and Literature Review. All outputs were thoroughly reviewed and verified by the author.

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