

Research Article

Presence and Effects of Papaya Ringspot Virus (PRSV) on the Leaves of *Carica papaya* Linn.

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ABSTRACT

Papaya (*Carica papaya* Linn.) is an economically important tropical fruit crop and a major fruit export commodity in Malaysia. However, its production is severely threatened by Papaya ringspot virus (PRSV), which causes significant yield losses in papaya cultivation. Therefore, this study aimed to identify the presence of PRSV and to characterise leaf anatomy and the anatomical adaptations that occur as a result of PRSV infection. Virus identification was performed using reverse-transcription polymerase chain reaction (RT-PCR) targeting the PRSV coat protein plus partial nuclear inclusion b (Nib) region. Anatomical analyses were conducted under a light microscope, including cross-sections of the lamina, midrib, petiole, and leaf margin, as well as epidermal peeling of the adaxial and abaxial surfaces. Micromorphological studies involved examination of the abaxial and adaxial leaf epidermis using a scanning electron microscope. The results showed that PRSV infection induced several anatomical adaptations, including a reduction in chlorophyll content, an increase in the thickness of chlorenchyma and collenchyma cell layers, alterations in vascular tissues, changes in the external morphology of the leaf margin and midrib, and an increase in crystal density. Moreover, changes were observed in the presence and type of epicuticular wax, cuticular ornamentation, anticline wall patterns of the adaxial epidermis, as well as a reduction in stomatal index and stomatal size. These findings demonstrate that PRSV infection causes significant modifications in leaf anatomical characteristics as part of the plant's response to viral attack. The anatomical indicators identified in this study may serve as valuable references for early detection and management of viral infections in plants.

Key words: Leaf adaptation, papaya leaves, plant defence mechanisms, plant pathogens

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INTRODUCTION

Carica papaya Linn. or papaya is a species classified in the family Caricaceae that is native to the lowlands of Central America, between southern Mexico and Nicaragua (Storey, 1969). This species is either dioecious (male and female flowers on separate trees), ginodioecious (some individuals have only female flowers (pistillate), while others have bisexual flowers (hermaphrodite), coexisting in the same population, allowing female plants to rely on bisexual plants for pollination), or monoecious (both male and female flowers on the same tree). It can grow up to 10 m tall with palmate leaves that are 40-60 cm wide, have 7-9 lobes, relatively long petioles, and are hollow, pale green or purplish green in colour (Chan &

Sim, 2019). The unripe green fruit contains abundant white latex, while the ripe fruit turns light to deep yellow-orange and becomes thick and fleshy with orange to red juicy pulp and a sweet aroma (Koul, 2022). Papaya fruits vary in shape from oval to nearly round, elongated, club-shaped, or pear-shaped and may reach weights of up to 9 kg (Morton, 1987).

Papaya is an economically important and nutritionally rich tropical fruit crop and one of Malaysia's major fruit export commodities, with a planting area of 3,218 hectares in 2017 and an annual production value reaching RM120 million (Ganisan & Muhammad Roff, 2021). Papaya is rich in vitamins A and C and is commonly consumed fresh when ripe, while immature fruits are often processed

into pickles. The latex extracted from young fruits and leaves contains the proteolytic enzyme papain, which is widely used in the pharmaceutical industry for meat tenderisation and the treatment of gangrenous wounds and digestive disorders. In addition, papain is utilised in cosmetic products such as soaps and creams due to its exfoliating properties (Chan & Sim, 2019). However, the papaya industry often faces serious threats from viral pathogens, especially Papaya ringspot virus (PRSV) (Koul *et al.*, 2022). PRSV is a linear positive-sense RNA virus from the Potyviridae family and the Potyvirus genus (Mohsin Mohsen, 2024). This virus is rod-shaped and filamentous without a wrapper (envelope) and has a genome size of approximately 10 kilobases. Several aphid species act as natural vectors that spread PRSV (Medina-Salguero, 2021). Based on the host range, PRSV is divided into two strains: (1) PRSV-W (Watermelon strain), which infects cucurbit plants but cannot infect papaya trees, and (2) PRSV-P (Papaya strain), which can infect both cucurbit plants and papaya trees (Gonsalves, 2010).

Papaya ringspot virus (PRSV) infection was first detected in Malaysia in June 1991 and has since spread to the southern regions of the country, causing a significant reduction in papaya production (Wahab, 1991; Mohamad Roff, 2005). The disease has been declared a serious threat under the Malaysian Plant Quarantine Act 1976, following the massive destruction of papaya production among smallholders and commercial plantations in Johor (Zubir & Hassan, 2021). According to Sósol-Reyes *et al.* (2020), infected plants (PRSV) will show several characteristic symptoms such as chlorosis (yellowing) of young leaves, mosaic patterns, and yellow-green streaks on the leaf lamina, prominent leaf veins, abnormal leaf shape, shrinkage, and oily spots on the petioles, stems, and fruits.

Therefore, this study was conducted to analyse in detail the presence and changes in the anatomical and micromorphological structures of PRSV-infected papaya leaves, as scientific information on the effects of viral infection on the morphological and anatomical characteristics of papaya leaves remains limited. Such information is valuable for the development of effective management, treatment, and prevention strategies against diseases caused by Papaya ringspot virus (PRSV).

MATERIALS AND METHODS

Materials

Healthy papaya leaves, used as the control samples, were collected in residential areas at Jalan 3/4 Section 3, Bandar Baru Bangi, Selangor (Figure 1). Leaves with symptoms of Papaya ringspot virus (PRSV) infection were obtained from the Azman Hashim Sports Arena area, Universiti Kebangsaan Malaysia, Bangi, Selangor (Figure 1). Infected leaves had symptoms of yellowing, curling, and wrinkling.

Methods

Total RNA from symptomatic leaf samples was extracted using the commercial QIAGEN RNeasy Plant Mini Kit according to the manufacturer's protocol. RNA quality and concentration were assessed using a NanoDrop, and cDNA was synthesized using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit according to the manufacturer's procedures. Conventional polymerase chain reaction (PCR) using primers PRS8617F and PRSPATR was carried out based on the previous study by Usharani *et al.* (2013) to confirm the presence of PRSV in symptomatic papaya leaves.

The anatomical study methods were carried out according to Johansen (1940), Sass (1958), and Noraini *et al.* (2022). For cross-sections of the lamina, midrib, petiole, and leaf margin, leaves were cut at 1 cm² size (Figure 1e & 1f) sliced using a Leica SM2000R sliding microtome with a Leica 818 disposable knife at a thickness of 20 – 30 µm, Clorox clearing, distilled water rinsing, Safranin and Alcian Blue staining, 50%, 70%, 95%, 100% alcohol series dehydra-

tion, xylene solution immersion, and mounting using Euparal on glass slides. The epidermis preparation method was performed by scraping the abaxial epidermis layer to obtain the adaxial epidermis layer and vice versa. Staining was performed using Safranin and Alcian blue, distilled water rinsing, 95% and 100% alcohol series dehydration, mounting on glass slides with Euparal, and oven-dried at 50°C for two weeks. Leaf chlorophyll content was measured using a SPAD-502 chlorophyll meter on healthy and PRSV-infected leaves. Observations were performed under an Olympus microscope connected to an Olympus DP72 digital camera. The micromorphological method was according to Manching *et al.* (2018). Leaf samples of 1 cm² size were fixed with 4% Glutaraldehyde (12 – 24 hr) at 4°C, washed with 0.1 M Phosphate Buffer for 10 min (3x), dehydrated with a series of 30%, 50%, 70%, 80%, 90%, and 100% alcohol, critical drying process (30 min), gold plating in a Sputter Coater and observed using a Thermoscientific QuattroS scanning electron microscope (FESEM). Analysis of microscopic anatomical features of PRSV-infected and healthy leaves was performed to identify structural differences that occurred due to virus infection.

RESULTS AND DISCUSSION

Determination of the presence of Papaya ringspot virus (PRSV)

In the present investigation, using primers specific to PRSV as described by Usharani *et al.* (2013), we tested symptomatic papaya leaf samples for virus presence. Conventional RT-PCR produced the expected 1.4 kb amplicon in PRSV-infected papaya leaf samples, whereas no PCR products were detected in healthy plant samples.

Anatomical and microscopic characteristics of leaves

A summary of the comparison of anatomical features of healthy leaves and leaves infected with Papaya ringspot virus (PRSV) is given in Table 1. A total of 12 anatomical features show adaptation to the effects of infection.

Chlorophyll content changes

The reduction in chloroplast density in PRSV-infected leaf lamina signifies chronic disruption that impacts the photosynthetic process. The chlorophyll content in healthy leaves is 57.6 nmol mg⁻¹, but PRSV-infected leaves showed a substantial reduction to 24.6 nmol mg⁻¹ (Figure 2). The findings of this study align with those of Ananthu and Umamaheswaran (2019), which revealed that healthy plants possess a higher chlorophyll content than virus-infected plants.

The accumulation of chloroplasts on the abaxial leaf surface is believed to be associated with the presence of palisade cells in that region (which are absent in healthy leaves), as the plant attempts to enhance light absorption to compensate for the photosynthetic deficiency of chloroplasts on the adaxial epidermis surface. Caldwell and James (1938) also reported that stripe disease in daffodils causes a significant deficiency of chloroplasts and the occurrence of hyperplasia and hypertrophy of palisade cells. Chloroplasts serve as the target for viral infection and also the site of viral replication (Zhao *et al.*, 2016). Therefore, viruses can affect the structure and function of chloroplasts responsible for the photosynthesis process, leading to symptoms such as leaf chlorosis resulting from alterations in pigmentation and chloroplast structure (Bradamante *et al.*, 2021; Taufik *et al.*, 2024).

Cell structures

The results of the study showed that 1 layer of palisade cells and 6 – 7 layers of spongy mesophyll cells were observed in healthy leaves (Figure 2a & 2b). On the other hand, PRSV-infected leaves showed significant changes, with a decrease of up to 1 – 3 layers of palisade cells in the adaxial part of the lamina and 1 – 2 layers of palisade cells in the abaxial part of the lamina (Figure 2c & 2d). Meanwhile, the spongy mesophyll was reduced to 5 – 6 layers in the central part of the lamina (Figure 2c & 2d).

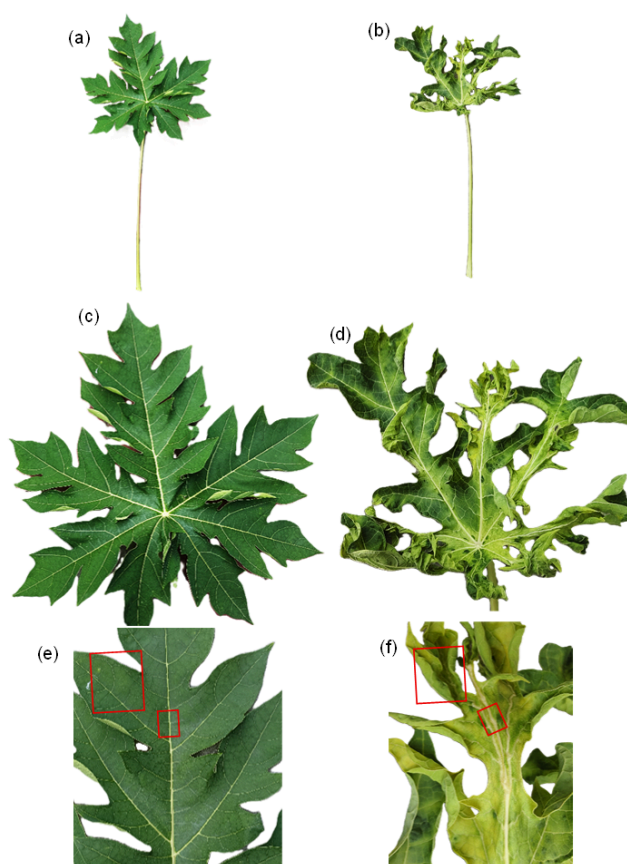


Fig. 1. Leaf samples of *Carica papaya*. (a & c) Healthy leaves (control). (b & d) PRSV-infected leaves. (e) Area used from the healthy leaf (red box). (f) Area used from the PRSV-infected leaf (red box).

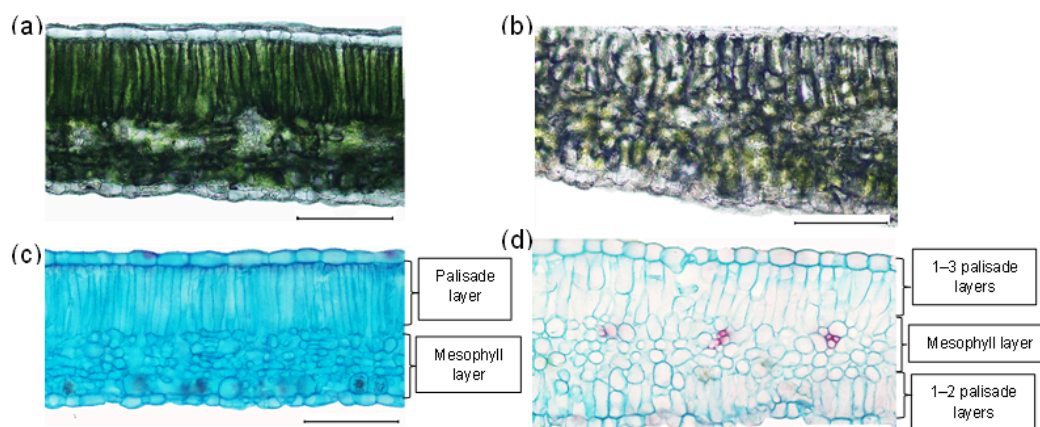


Fig. 2. Leaf cross-sections. (a & c) Healthy leaf lamina. (b & d) PRSV-infected leaf lamina. Scale bar: 100 μ m.

The results of this study are consistent with the findings of Taufik *et al.* (2024), which demonstrated that palisade tissue consists of two layers of cells that shorten, and some are arranged in an irregular manner when infected. According to Gamboa-Tec *et al.* (2018), infection by *Euphorbia* mosaic virus Yucatan Peninsula (EuMV-YP) in *Nicotiana benthamiana* mesophyll tissue causes three main effects, namely increased uncontrolled mitotic activity, significant changes in cell morphology, and alterations in the regulation of genes related to the cell cycle. These consequences lead to the development of severe disease symptoms.

The reduction in the thickness of the spongy mesophyll on infected leaves could harm the plant because the spongy mesophyll area plays a role in ensuring that the physiological functions of the

plant run smoothly, especially as a site of gas exchange between the stomata and the external environment (Xiao *et al.*, 2016). The thinning of this spongy mesophyll layer is expected to result in a reduction in the photosynthesis rate of the plant (Su, 2018). This will affect the overall growth and productivity of the plant.

The results of the study further showed the presence of a continuous palisade cell layer almost to the tip of the leaf margin on the adaxial surface of healthy leaves. Contrary to this, in PRSV-infected leaves, the palisade cell layer was discovered solely in the abaxial region of the lamina between the vascular bundles up to the leaf margin.

In PRSV-infected leaves, hyperplasia of parenchyma cells at the margin is very clearly visible, causing significant swelling in that area (Figure 3). The increase in the number of cells in virus-infected

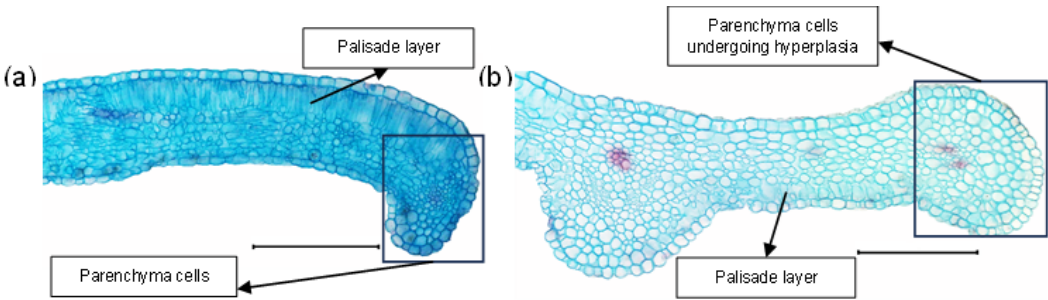


Fig. 3. Leaf cross-sections. (a) Healthy leaf margin. (b) PRSV-infected leaf margin. Scale bar: 200 μm .

leaves results in swelling at the margin. In addition to being different in shape, their size is also larger than that of healthy leaves. The phenomenon of increased cell number due to virus infection is in line with the general mechanism of plant tissue response to virus infection, as in the study by Taufik *et al.* (2024), who reported that citrus leaves infected with *Citrus Leprosis Virus* become thicker due to significant hypertrophy and intensive hyperplasia activity in parenchyma cells.

Collenchyma cells play an important role as a supporting tissue in herbaceous stems to continue to grow, expand, and develop.

Collenchyma cell walls contain high pectin and water content with thickened sections consisting of alternating layers of cellulose and non-cellulosic pectic materials (Dickison, 2000). Collenchyma cells in PRSV-infected leaves displayed hypertrophy, with an increase in cell diameter ranging from 6.09 μm to 44.52 μm , compared with healthy leaves, which showed cell diameters ranging from 5.48 μm to 23.48 μm (Table 1). This hypertrophic response caused the adaxial surface of the midrib to become more convex, forming an inverted U-shape, whereas healthy leaves exhibited a convex adaxial surface with an inverted V-shape (Figure 4a & 4b). These

Table 1. Comparison of anatomical characteristics of healthy papaya leaves and leaves infected with PRSV.

No.	Anatomical features	Healthy leaves (control leaves)	Leaves infected with PRSV virus
1.	Chlorophyll content	57.6 nmol mg^{-1}	24.6 nmol mg^{-1}
2.	Chlorenchyma cells	Palisade cells and spongy mesophyll can be distinguished. 1 layer of palisade cells and 6-7 layers of spongy mesophyll cells. Palisade cells fill $\frac{1}{2}$ of the lamina thickness; spongy mesophyll fills the remainder.	Palisade cells and spongy mesophyll are difficult to distinguish. 1-3 layers of palisade cells on the adaxial side of the lamina; 1-2 layers on the abaxial side; 5-6 layers of spongy mesophyll. Palisade cells fill $\frac{2}{3}$ of the lamina thickness; spongy mesophyll fills the remainder.
3.	Vascular tissue	Normal Midrib: Number of metaxylem: ± 50 ; Number of phloem: ± 26 Petiole: Number of xylem: ± 24 ; Number of phloem: ± 125	Hyperplasia Midrib: Number of metaxylem: ± 55 ; Number of phloem: ± 29 Petiole: Number of xylem: ± 28 ; Number of phloem: ± 148
4.	The outer shape of the margin	Rounded-tapered tip, palisade cell layer present almost to the leaf margin.	Rounded-swollen tip, palisade cell layer present abaxially between vascular bundles up to the leaf margin.
5.	External shape of the midrib	The adaxial surface is convex and inverted V-shaped.	The adaxial surface is convex, inverted U-shaped (swollen).
6.	Collenchyma cells	Diameter 5.48 μm – 23.48 μm	Diameter 6.09 μm – 44.52 μm
7.	Drusen crystals	Less densely present on the petiole	Present densely on the petiole
8.	Waxes	Adaxial epidermis: verrucate wax Abaxial epidermis: buttressed/spongy wax	Adaxial epidermis: crustose wax Abaxial epidermis: buttressed/spongy wax
9.	Epidermal cuticle ornamentation	Adaxial epidermis: relatively thick cuticle layer; anticlinal wall raised Abaxial epidermis: relatively thick cuticle layer; anticlinal wall raised	Adaxial epidermis: relatively thin cuticle layer; anticlinal wall sunken Abaxial epidermis: relatively thin cuticle layer; anticlinal wall sunken
10.	Stomatal index	23% / Dense	10% / Less dense
11.	Stomata size	Length: $\pm 8.71 \mu\text{m}$ / Width: $\pm 14.07 \mu\text{m}$	Length: $\pm 6.11 \mu\text{m}$ / Width: $\pm 12.86 \mu\text{m}$
12.	Adaxial epidermal anticlinal wall pattern	Curve to wavy	Straight to curve

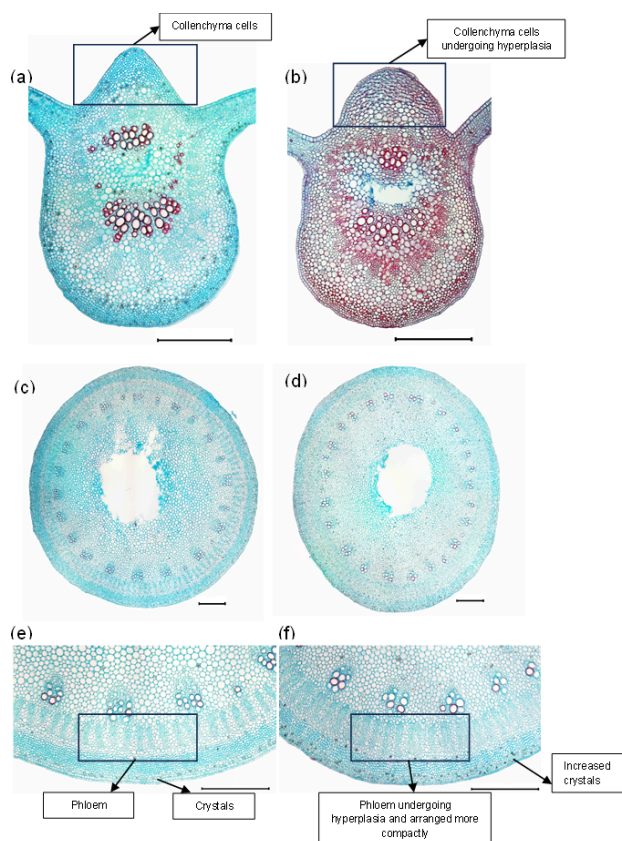


Fig. 4. Leaf cross-sections. (a) Healthy midrib. (b) PRSV-infected midrib. (c & e) Healthy petiole. (d & f) PRSV-infected petiole. Scale bar: 500 µm.

anatomical changes are likely related to the plant's reaction to pathogen infection, which can increase transpiration rates due to damage to the cuticle and leaf epidermis (Sharma, 2004). Increased transpiration has been identified as one of the factors influencing the development and transformation of collenchyma tissues in plants (Venning, 1949; Noraini *et al.*, 2019). Collenchyma cells also serve as sites for the accumulation of defence compounds such as hydroxyproline-rich glycoproteins (HRGPs) that help strengthen cell walls and restrict pathogen movement (Leach *et al.*, 1982).

Viral infections of leaves can usually result in tissue swelling due to hyperplasia and cell hypertrophy. However, the extent of the infection depends on the type of virus, as some viruses could cause leaf swelling while others can trigger more extreme morphological changes, such as tumor formation and enation on the leaf surface. For example, a study by Wu *et al.* (2025) reported that parenchyma and vascular parenchyma cells in rice plants showed tumor formation as a result of the proliferation and differentiation of vascular parenchyma cells when infected with Rice Black Streaked Dwarf virus (RBSDV). Furthermore, Anikina *et al.* (2023) found that studies on the histological structure of plants revealed the presence of hypertrophy and hyperplasia, which eventually led to tumor formation and enation, which is an abnormal growth on the leaf surface.

Vascular tissue structure

Xylem and phloem are the main transport networks in plants. Xylem is a complex tissue consisting of various types of specialized cells that play a major role in water flow, while phloem is the main transport system to distribute the products of photosynthesis to all parts of the plant (Esau, 1977). These two tissues also indirectly help provide support to the plant. According to Gilbertson and Lucas (1996), the way viruses move is also the same, either through movement from cell to cell through plasmodesmata or through phloem. Phloem is the main target of various plant pathogens, including viruses, because the phloem structure provides a systematic route

for the movement and spread of pathogens within the host (El-Banna *et al.*, 2014).

The results of this study found that hyperplasia also occurred in the xylem and phloem of the leaf axil and petiole. In healthy leaves, the midrib showed ± 50 metaxylem cells and ± 26 phloem cells, while the petiole had ± 24 metaxylem cells and ± 125 phloem cells (Figure 4c). In contrast, PRSV-infected leaves showed an increase in cell number, with ± 55 metaxylem cells and ± 29 phloem cells in the midrib, while ± 28 metaxylem cells and ± 148 phloem cells were found in the petiole (Figure 4d).

The phloem arrangement was also closer to the petiole in PRSV-infected leaves, indicating the plant's response to infection (Figure 4f). This increase indicates that PRSV infection affects the development of vascular tissue in the leaves. In a previous study by Noraini *et al.* (2024), hyperplasia was also observed in vascular tissue in the midrib and petiole, as well as in collenchyma cells in *Sauropus androgynus* leaves infected with Sauropus yellowing virus (SaYV). Reoviruses such as Rice black streaked dwarf virus (RBSDV) and Rice gall dwarf virus (RGDV) also cause hyperplasia of cells in the phloem, resulting in tumor formation or swelling of leaf veins (Lv *et al.*, 2017). Xylem hyperplasia can also facilitate the spread of pathogens in plants. The development of pathogens in the xylem will disrupt the transport of water and minerals, increasing stress on the plant and leading to a more severe decline in the health of infected trees (Kashyap *et al.*, 2021; Malinowski *et al.*, 2024).

Crystals

Druses crystals are located in idioblast cells and serve as a plant defence mechanism (Noraini *et al.*, 2019). The formation of calcium oxalate crystals in plants functions to regulate calcium levels efficiently and to provide physical protection against herbivores through the development of sharp crystalline structures (Franceschi & Nakata, 2005). Numerous studies indicate that the crystal content increases in virus-infected leaves compared to healthy leaves (Yu-

sop *et al.*, 2023; Noraini *et al.*, 2024; Taufik *et al.*, 2024). Studies have shown that calcium oxalate crystals are densely distributed in the petiole of PRSV-infected leaves (Figure 4f). The comparison of crystal abundance in this study was conducted solely on a relative basis, as drusen crystals exhibit uneven distribution and are challenging to quantify precisely. Similarly, Singh and Shukla (2012) reported an increased density of calcium oxalate crystals in the stems of PRSV-infected plants. Consistent with these findings, the present study also observed a very dense accumulation of crystals in the petiole region of virus-infected leaves.

According to Noraini *et al.* (2024), the presence and increase in the presence of druses indicate an effective defence mechanism against pathogens in two main ways. First, the sharp structure of druses is able to physically damage the pathogen cell wall to facilitate the action of other defence mechanisms. Second, drusen act as a physical barrier that prevents the penetration of pathogens into plant tissues. In this study, the density of crystals on the petiole is likely because the plant may respond to infection by producing more crystals as an adaptation and defence mechanism to prevent the movement of viruses to other parts of the plant.

Wax and cuticle on leaf epidermis

Analysis of the micromorphological characteristics of the leaf surface showed significant differences in the cuticular ornamentation between healthy and PRSV-infected leaves (Figure 6). Healthy leaves showed a relatively thick cuticle layer with a raised anticline wall and a sunken periclinal wall (Figure 6c & 6g). Meanwhile, infected leaves had a thin and eroded cuticle layer with a sunken anticline wall and a raised periclinal wall (Figure 6d & 6h). The cuticle pattern and thickness showed structural diversity between plant species but generally was organized as a composite material consisting of cutin (a type of polyester) partially coated and interspersed with plant waxes, including epicuticular and intracuticular waxes (Serrano *et al.*, 2014).

According to Ziv *et al.* (2018), the cuticle functions to prevent excessive water loss, regulate biotic and abiotic stresses, and also resist pathogen attack. Normally, a thick cuticle layer may also act as additional mechanical support in plants (Cutler *et al.*, 2008). Therefore, thinning and erosion of the cuticle layer are likely caused by disruption of cutin synthesis and can weaken the important functions of the cuticle to the plant.

Healthy leaves show the presence of a verrucous wax layer on the adaxial epidermal surface and a spongy wax layer on the abaxial leaf surface (Figure 6c & 6g). Meanwhile, a crustous wax layer can

be observed on the adaxial leaf surface and a spongy wax layer on the abaxial surface of the leaf (Figure 6d & 6h). Wax is the outer layer that covers the plant cuticle and consists of various shapes, types, and chemical contents, and is important in the interaction between the plant and the environment (Noraini *et al.*, 2019). Wax forms as a deposit on the plant surface and can be relatively thin or thick (Linsbauer, 1930).

Stomata

Stomata are specialized structures in the plant epidermis that consist of a pair of guard cells that control the opening of the stoma (Esau, 1960; Noraini *et al.*, 2019). Stomata play an important role in regulating the exchange of major gases such as carbon dioxide (CO₂) and water vapor with their environment to carry out photosynthesis, transpiration, and metabolism (Meidner & Mansfield, 1968). Viral infections can change the density and stomatal index (SI) because stomata are also a route of entry for pathogens (Noraini *et al.*, 2024). The density of stomata is determined by the stomatal index as follows:

$$SI = \frac{S}{S + E} \times 100$$

Equation 1

Where SI is the stomatal index (%), S is the number of stomata, and E is the number of epidermal cells.

The results of the study showed that symptomatic leaves experienced a significant reduction in the size and stomatal index compared to healthy leaves. Healthy leaves showed a stomatal index of 23%, while PRSV-infected leaves decreased to 10%. The stomatal size was also larger in healthy leaves (8.71 µm long, 14.07 µm high) compared to PRSV-infected leaves (6.11 µm long, 12.86 µm high) (Figure 5). According to Mishra *et al.* (2018), the reduction in stomatal density in leaves infected with Papaya Leaf Curl Virus (PaLCuV) and the rate of gas exchange and transpiration through leaf stomata depended on the density, size, and degree of stomatal opening. For example, *Nicotiana tabacum* infected with Tobacco Mosaic Virus (TMV) and *Arabidopsis thaliana* infected with Turnip Vein-Clearing Virus (TVCV) showed a reduction in stomatal index of 9.8% and 12.3%, respectively (Murray *et al.*, 2016). TMV infection of *N. tabacum* also caused a significant reduction in transpiration rate. In *Arabidopsis*, Turnip Mosaic Virus (TuMV) infection reduces stomatal opening, gas exchange rate, and daily water use, indicating the influence of the virus on water uptake, circulation, and transport in plants (Dutton *et al.*, 2019).

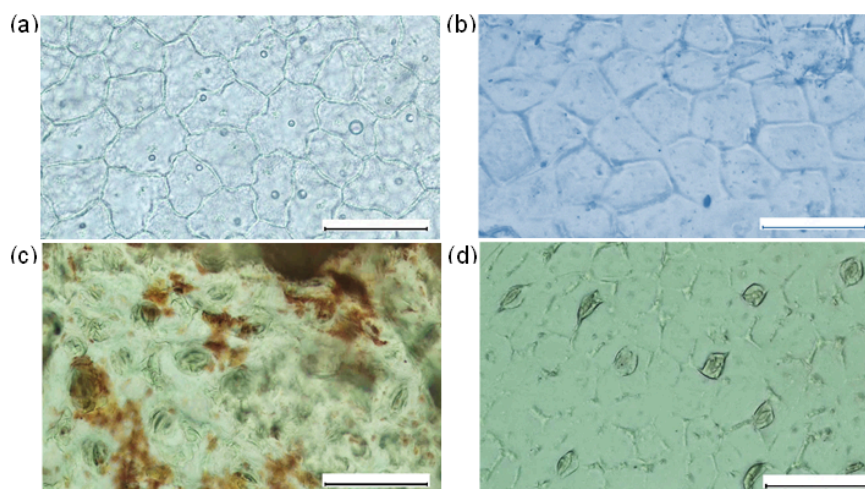


Fig. 5. Leaf epidermal surface. (a) Adaxial epidermis of healthy leaf. (b) Abaxial epidermis of healthy leaf. (c) Adaxial epidermis of PRSV-infected leaf. (d) Abaxial epidermis of PRSV-infected leaf. Scale bar: 50 µm.

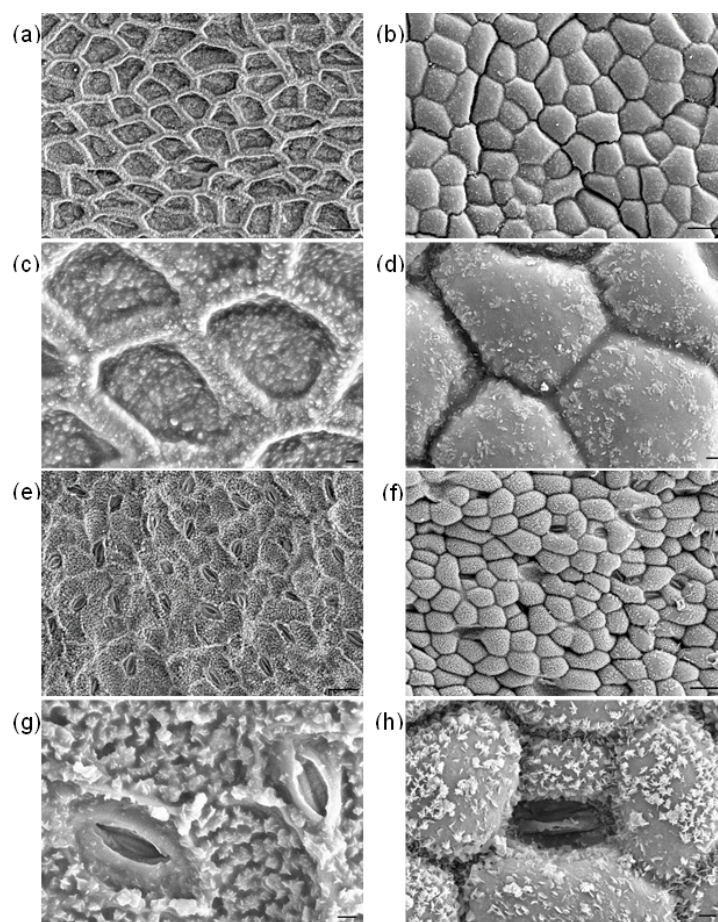


Fig. 6. Leaf epidermal surface. (a & c) Adaxial epidermis of a healthy leaf. (b & d) Adaxial epidermis of a PRSV-infected leaf. (e & g) Abaxial epidermis of a healthy leaf. (f & h) Abaxial epidermis of PRSV-infected leaf. Scale bars: (a), (b), (e) & (f) = 20 µm; (c), (d), (g) & (h) = 2 µm.

Anticlinal wall pattern

Dicotyledonous plants usually have polygonal or nearly polygonal epidermal cells, and the anticlinal wall pattern of epidermal cells has significant value in plant systematics due to its wide variation (Noraini *et al.*, 2019). In this study, healthy leaves showed a different anticlinal wall pattern from PRSV-infected leaves (Table 1 & Figure 5). Although this pattern is interesting to be associated with plant responses to viral infection, there is still no specific study that discusses the mechanism of anticlinal wall pattern changes in leaf epidermal cells. Further studies are recommended to understand the relationship between anticlinal wall changes and viral infection.

CONCLUSION

This study demonstrates that infection by Papaya Ringspot Virus (PRSV) induces several modifications in the anatomical features of papaya leaves as part of the plant's response to viral invasion. PRSV infection reduces chlorophyll content in leaves; however, this reduction appears to be partially compensated by the development of palisade cell layers on both epidermal surfaces, which may help maintain photosynthetic efficiency. Hyperplasia of vascular tissues may enhance cellular or tissue transport efficiency, but it may also facilitate the systemic spread of pathogens within the plant. Hyperplasia of parenchyma cells, together with a reduction in stomatal index and stomatal size, reflects general anatomical responses of plant tissues to viral infection. In addition, hypertrophy of collenchyma cells and an increase in crystal density may function as structural defence mechanisms by strengthening cell walls and limiting pathogen spread. Nevertheless, the observed thinning of the cuticle layer and alterations in the anticlinal and periclinal wall patterns of epidermal cells require further investigation to clarify their functional significance in relation to PRSV infection. Overall,

the anatomical characteristics identified in this study may serve as potential early indicators of PRSV infection and provide useful references for developing disease management strategies in papaya cultivation, particularly for early detection and control of viral diseases.

Overall, the anatomical characteristics identified in this study may serve as potential early indicators of PRSV infection; however, their reliability as early biomarkers requires further validation. Future studies should quantitatively correlate anatomical changes with PRSV disease severity, infection progression, and physiological performance, including photosynthetic and gas-exchange parameters. Longitudinal studies involving different stages of PRSV infection would also help determine which anatomical characteristics appear at the earliest stages of infection and whether they are consistently associated with disease progression. In addition, comparative evaluation of PRSV-resistant, PRSV-tolerant and PRSV-susceptible papaya genotypes is recommended to determine whether specific anatomical traits can be used as reliable screening criteria for resistant germplasm. Such studies could contribute to the development of anatomy-based screening approaches and support papaya breeding and disease management programmes.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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