

RESEARCH PAPER

## The changes of chili leaf structure by Geminivirus infection

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### ABSTRACT

The Geminivirus in chili plants is a significant problem in chili cultivation. Symptoms of Geminivirus infection are quite easy to find in chili plants because the symptoms are quite typical: mosaic, yellowing, small leaves, leaf lamina malformation, and stunting. The visual changes in leaf morphology are due to the leaf tissue structure, such as the upper and lower epidermis and the mesophyll tissue, including the palisade and sponge. There is little information about changes in the structure of chili plants infected with the Geminivirus. Therefore, this study aims to add information about changes in the leaf tissue structure of chili plants infected with the Geminivirus. The structural changes observed were palisades. Long palisades were less preferred by whiteflies, so the incidence of geminivirus disease was lower. This information is a reference for assembling chili plants with better structural resistance. The method used was a cross-section of chili leaves, double staining, and observation with a light microscope. Observations on mild symptoms showed epidermal cells shrinking. Some parts of palisade leaves were composed of two layers with shortened cells and tend to be oval. The symptom is that the upper epidermal cells were shrunken, and the palisade also tends to shorten. Severe symptoms of the epidermis on the leaves were curly, shriveled, thinned, and even dying; the palisade is shortened and sometimes looks irregular/tight. Changes in the leaf tissue of chili plants infected with the Geminivirus cause changes at the ultra-structural, cell, or tissue level, depending on the type of virus attack and the attack level.

**Key words:** Geminivirus, leaf structure, symptoms, mosaic

### INTRODUCTION

Chili (*Capsicum* spp.) is a horticultural crop with high economic value in Indonesia. Apart from being consumed as a food ingredient, chilies are also used as raw materials for industry, pharmaceuticals, and ornamental plants. Chilies contain nutrients beneficial to the human body, especially carbohydrates, phosphorus, alkaloid compounds, and vitamins A and C (Wahyuni et al., 2013). Sanati et al. (2018) explained that capsaicin, the main ingredient in chilies, provides a spicy sensation and is useful in various treatments. Red pepper has beneficial effects on metabolic syndrome and can reduce the risk of mortality due to cardiovascular

diseases. Chili consumption is predominantly in the household sector, which has increased over the last three years. The consumption of red chilies in 2019, 2020, and 2021 was 406.77, 446.46, and 490.83 thousand tons, respectively (BPS, 2021).

The increase in chili consumption has not been followed by chili production and productivity. For instance, chili production in Southeast Sulawesi has been decreasing, from 12,754 tons in 2016 to 5,443 tons in 2017, 5,591 tons in 2018, 5,077 tons in 2019, and 5,764 tons in 2020. On the other hand, the planted area has tended to increase, from 1,603 ha in 2016 to 1,859 ha in 2018 and 1,715 ha in 2020. Chili productivity in Southeast Sulawesi is 3.13 tons/ha, still far below the target potential chili production in Indonesia, which is around 10–20 tons/ha, with an average national chili production of about 8.77 tons/ha (BPS, 2021; Dirjen Hortikultura Kementan, 2019).

The *Geminiviridae* family has become the largest family of plant viruses, with 320 species. The family *Geminiviridae* consists of nine genera: *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Mastrevirus*, *Topocuvirus*, and *Turncurtovirus*. The Genus *Begomovirus* is the largest genus within the family *Geminiviridae*,

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classified based on genome organization, host range, and the type of insect vector-*Bemisia tabaci* (Brown et al., 2015; Arif et al., 2022). Whiteflies are invasive and polyphagous with a wide host range, including chili plants. However, the type of host plant determines several characteristics of the whitefly, including life cycle, personality, sex ratio, and variations in puparium morphology (Hidayat et al. 2020; Rahayuwati et al. 2020; Arif et al. 2022). Begomoviruses have a genome of circular single-stranded DNA (ssDNA), which consists of single molecules 2.7–2.8 kb (monopartite) and mostly double molecules, DNA-A and DNA-B (bipartite) (Wilisiani et al., 2014). Proteins encoded by DNA-A are associated with viral DNA replication, vector transmission, and encapsidation, while those encoded by DNA-B are required for the intercellular and intracellular movement of viral particles (Arif et al., 2022).

Geminivirus infection is one of the limiting factors for the optimal production of chili peppers. As a cause of jaundice, Geminivirus can inhibit plant growth vegetatively and even lead to chili crop failure. It has been reported that yield loss due to Geminivirus infection can reach 70–100% (Sulandari et al., 2001). The spread of Geminivirus in various regions in Indonesia is occurring faster than efforts to prevent its spread. Taufik et al. (2018) reported the presence of Geminivirus in Southeast Sulawesi for the first time. It was reported that the Geminivirus has spread to the mainland (Kolaka, North Kolaka, Bombana, Konawe, and South Konawe) and two large islands, namely Muna and Buton islands, in Southeast Sulawesi (Widodo et al., 2023; Taufik et al., 2023).

Diseases caused by the Geminivirus in chili plants can result significant losses. However, farmers may not be aware of their presence because infected chili plants do not die immediately and continue to produce yields, albeit with decreased quantity and quality. Chili leaves infected with the Geminivirus generally show symptoms of mosaicism, leaf malformation, leaf curling up or down, shrinking of the leaf lamina, and early infection cause stunting and prevent the plant from bearing fruit (Sulandari et al., 2001; Hidayat, 2003; Santoso et al., 2008; Kesumawati et al., 2019; Selangga et al., 2021; Selangga et al., 2019; Selangga & Listihani, 2021). It is believed that these changes due to virus infection affect photosynthetic efficiency, along with reductions in net CO<sub>2</sub> assimilation and chlorophyll a/b content (Gonçalves et al., 2005). Very high viral activity is thought to impact metabolic processes, thereby reducing primary metabolites and plant growth. Central to this process is photosynthesis, which is

associated with chlorophyll pigment (Gonçalves et al., 2005). It is suspected that virus infection will inhibit growth during both the vegetative and generative phases. Several studies have shown that viral infections can reduce plant growth, including ultrastructural changes in organs related to photosynthesis, such as palisade and spongy tissues. Therefore, this study aimed to determine the effect of changes in the leaf tissue of chili plants infected with Geminivirus. The structural changes observed in the palisades suggest that longer palisades are less preferred by whiteflies, resulting in a lower incidence of Geminivirus disease. This information serves as a reference for developing chili plants with better structural resistance. It can be used to breed chili varieties that are less attractive or resistant to the Geminivirus vector insect in the field and could be part of an integrated control strategy against Geminivirus in the future.

## MATERIALS AND METHODS

**Research Site.** The research was carried out in chili plantations in Konawe, East Kolaka, Kolaka, and North Kolaka Regencies from August to October 2022. Observations were made on chili plants that showed typical symptoms of the Geminivirus in cayenne pepper (CP), Big red chili pepper (BCP), and Curly red chili (CRC).

### Field Survey and Collection of Chili Leaf Samples.

The survey was conducted in chili plantations in Konawe (4°01'12.9"S 122°00'27.2" E), East Kolaka (4°01'37.7"S 121°52'52.9"E), Kolaka (4°02'41.4"S 121°53'34.6"E), and North Kolaka (3°41'45.9"S 121°02'52.2"E) Regencies. At each survey location, the incidence of jaundice was observed by taking a sample of 10% of the chili plant population. The survey research employed a non-probability sampling method, specifically purposive sampling, to determine the observation sample based on the criteria for typical begomovirus symptoms, including mild, moderate, or severe symptoms. The position of the sample leaves taken was consistent for each individual plant, namely the third petiole. The types of symptoms were grouped into mild, moderate, and severe (Table 1) (Sudiono et al., 2005; Sulandari et al., 2006; Kesumawati et al., 2019). The collected leaf samples were wrapped in tissue, then placed in a ziplock plastic bag and moistened with 70% ethanol to prevent the samples from drying out during storage. The collected samples were placed in a cold box to be sent to the Plant Anatomy Laboratory-National Research and Innovation Agency (BRIN),

Cibinong, Bogor.

**Chili Leaf Tissue Analysis.** Cross-sections of chili leaves were obtained using the paraffin method (Sass, 1951). In this method, chili leaves were fixed using an FAA solution (formaldehyde: acetic acid: alcohol) and then dehydrated with a series of ethanol: tert-butanol concentrations. Double staining with safranin and fast green was used, then sections were covered with Entellan mounting media for microscopy. Observations were conducted using a Nikon Eclipse 80i light microscope (Japan) and photos were taken with an XCAM Indomicro HDMI camera 1080 PHB with a magnification of  $2.4 \times 2.4$  megapixels (Indomicro, Indonesia) using Betaview software. Observations of healthy and infected leaves included tissue structure and involved 30 measurements of the upper and lower epidermis, mesophyll (palisade and spongy), and leaf thickness. Data were analyzed to calculate mean and standard deviation.

## RESULTS AND DISCUSSION

The examined chili leaves include cayenne pepper, big red chili pepper, and curly red chili. These three chili varieties exhibit different symptoms and are affected by anatomical characteristics (Table 2). The anatomy of cayenne pepper leaves (upper epidermis, lower epidermis, palisade, and sponge) planted in Konawe tends to become thinner as the severity of the symptoms increases. However, the plants found in Tamborasi exhibit the opposite tendency. Meanwhile, only cayenne peppers planted in Ponggia and Tojabi were found to be healthy and had mild symptoms. Healthy cayenne peppers grown in Ponggia have thicker tissue (upper epidermis, lower epidermis, palisade, and sponge) compared to the leaves of chili plants with mild symptoms. Conversely, cayenne pepper grown in Tojabi showed the opposite results.

Healthy big red chili pepper leaves generally have thinner tissue than those with severe symptoms (Table 2). For example, leaf tissue on large chilies (upper and lower epidermis of leaves, palisade, and sponge) planted in Ulu Wolo (Kolaka) showed that leaves with mild symptoms had the thinnest leaves compared

to healthy leaves, with severe symptoms having an average thickness of 13.45  $\mu\text{m}$ , 10.72  $\mu\text{m}$ , 55.95  $\mu\text{m}$ , and 101.00  $\mu\text{m}$ , respectively. The other variety, exhibited healthy conditions with an average thickness range of 13.60-17.13  $\mu\text{m}$ , 11.98-13.08  $\mu\text{m}$ , 33.02-79.31  $\mu\text{m}$ , and 57.18-105.38 for the upper epidermis, lower epidermis, palisade, and sponge, respectively.

Healthy and symptomatic chili plants generally have the same anatomical structure. In general, leaves of three chili variations have a single layer of epidermis on the upper and lower surfaces of the leaves. Stomata are present on both sides of the leaf surface. This section also shows two types of trichomes. Non-glandular trichomes are uniseriate and multicellular, while glandular trichomes have stalks and head cells that are club-shaped and round (multicellular). Mesophyll tissue consists of a palisade on the top of the leaf and spongy (spongy) underneath. The palisade is cylindrical and comprises 1–2 layers of tissue, with several layers of spongy tissue below (Figure 1). In the mesophyll, there are druse-type  $\text{CaCO}_3$  crystals.  $\text{CaCO}_3$  crystals are known to have several important roles, such as calcium regulation and sodium and maintaining sodium and potassium balance. The presence of these crystal is closely related to plant protection against herbivore attacks. According to Rodrigues et al. (2009), the increase  $\text{CaCO}_3$  in latex is evidence of viral infections in papaya.

Chili leaves with mild symptoms exhibit shrinkage in epidermal cells in some parts of the leaf (Figure 2a). In addition, some portions of the palisade layer consist of 2 layers with shortened cells that tend to be oval (Figure 2a). Meanwhile, leaves with similar symptoms from Ponggia have cylindrical palisade layers that are more loosely arranged (Figure 2b). Glandular trichomes with round heads appear to be more numerous compared to healthy leaves. These symptoms resemble those of tomato plants attacked by tomato yellow leaf curl geminivirus. Khalil et al. (2014) demonstrated that infected leaves have smaller, more compact upper and lower epidermises and exhibit shortened, non-cylindrical palisade cells. Additionally, their study indicated that mesophyll cells were differentiated into several layers.

Chili leaves with moderate symptoms were

Table 1. Symptoms of virus infections in chili plants

| Types of symptoms | Indications                                                              |
|-------------------|--------------------------------------------------------------------------|
| Mild              | Mosaic symptoms                                                          |
| Moderate          | Leaf malformations                                                       |
| Severe            | Mosaic symptoms, leaf malformations, and leaves rolling up and shrinking |

Table 2. Measurement of the character of chili leaves

| No.                                          | Location                 | Symptoms               | Upper Epidermis ( $\mu\text{m}$ ) | Palisade ( $\mu\text{m}$ ) | Sponge ( $\mu\text{m}$ ) | Lower Epidermis ( $\mu\text{m}$ ) |
|----------------------------------------------|--------------------------|------------------------|-----------------------------------|----------------------------|--------------------------|-----------------------------------|
| <b>A. Cayenne pepper (CP) variety</b>        |                          |                        |                                   |                            |                          |                                   |
| 1.                                           | Unaha (Konawe)           | Mild CR1               | 13.99 $\pm$ 3.39                  | 41.34 $\pm$ 7.94           | 80.09 $\pm$ 22.88        | 12.13 $\pm$ 2.86                  |
|                                              |                          | Moderate CR2           | 12.12 $\pm$ 1.76                  | 42.64 $\pm$ 7.11           | 73.11 $\pm$ 19.43        | 10.84 $\pm$ 2.27                  |
|                                              |                          | Severe CR3             | 11.69 $\pm$ 2.49                  | 41.56 $\pm$ 7.55           | 67.65 $\pm$ 15.53        | 10.63 $\pm$ 2.36                  |
| 2.                                           | Tamborasi (Kolaka)       | Mild CR8               | 11.98 $\pm$ 4.38                  | 43.49 $\pm$ 8.47           | 60.81 $\pm$ 9.19         | 10.27 $\pm$ 2.62                  |
|                                              |                          | Moderate CR9           | 12.11 $\pm$ 2.24                  | 40.88 $\pm$ 9.54           | 64.41 $\pm$ 11.23        | 10.96 $\pm$ 1.56                  |
|                                              |                          | Severe CR 10           | 12.18 $\pm$ 3.03                  | 43.32 $\pm$ 12.32          | 75.54 $\pm$ 16.97        | 11.02 $\pm$ 2.63                  |
| 3.                                           | Ponggia (Kolaka Utara)   | Healthy CR11           | 11.92 $\pm$ 3.76                  | 72.84 $\pm$ 9.28           | 95.19 $\pm$ 17.07        | 9.43 $\pm$ 2.14                   |
|                                              |                          | Mild CR12              | 9.65 $\pm$ 3.15                   | 49.84 $\pm$ 8.69           | 71.88 $\pm$ 16.36        | 9.39 $\pm$ 2.59                   |
| 4.                                           | Tojabi (Kolaka Utara)    | Healthy CR6            | 9.43 $\pm$ 1.75                   | 28.13 $\pm$ 4.74           | 51.65 $\pm$ 10.97        | 9.97 $\pm$ 2.16                   |
|                                              |                          | Mild CR7               | 12.08 $\pm$ 3.35                  | 36.67 $\pm$ 7.88           | 66.88 $\pm$ 11.82        | 10.42 $\pm$ 2.13                  |
| 5.                                           | Latowe                   | No Symptoms CR4        | 11.23 $\pm$ 1.91                  | 51.88 $\pm$ 8.75           | 82.96 $\pm$ 16.10        | 11.90 $\pm$ 2.11                  |
|                                              |                          | Wawo (Kolaka Utara)    | Healthy CR5                       | 9.90 $\pm$ 2.01            | 47.85 $\pm$ 7.07         | 80.14 $\pm$ 12.18                 |
| <b>B. Big red chili pepper (BCP) variety</b> |                          |                        |                                   |                            |                          |                                   |
| 1.                                           | Ulu Wolo (Kolaka)        | Healthy CB1            | 14.44 $\pm$ 4.87                  | 69.58 $\pm$ 12.35          | 150.78 $\pm$ 26.34       | 13.53 $\pm$ 5.15                  |
|                                              |                          | Mild CB2               | 13.45 $\pm$ 2.44                  | 55.95 $\pm$ 13.37          | 101.00 $\pm$ 19.52       | 10.72 $\pm$ 2.60                  |
|                                              |                          | Severe CB3             | 16.49 $\pm$ 4.40                  | 71.52 $\pm$ 23.91          | 153.43 $\pm$ 43.55       | 12.78 $\pm$ 3.04                  |
| 2.                                           | Totalang (Kolaka Utara)  | Helathy CB4            | 12.99 $\pm$ 2.28                  | 59.91 $\pm$ 8.49           | 110.25 $\pm$ 15.40       | 12.09 $\pm$ 2.61                  |
|                                              |                          | Kalongi (Kolaka Timur) | Severe CB5                        | 14.44 $\pm$ 5.14           | 77.22 $\pm$ 25.45        | 125.48 $\pm$ 40.56                |
| <b>C. Curly red chili (CRC) variety</b>      |                          |                        |                                   |                            |                          |                                   |
| 1.                                           | Lalosingi (Konawe)       | Helathy CK1            | 14.98 $\pm$ 2.59                  | 79.31 $\pm$ 11.50          | 105.38 $\pm$ 15.90       | 13.08 $\pm$ 3.17                  |
|                                              |                          | Helathy CK2            | 16.13 $\pm$ 4.39                  | 64.53 $\pm$ 14.65          | 89.17 $\pm$ 21.96        | 12.08 $\pm$ 2.86                  |
| 2.                                           | Rate-Rate (Kolaka Timur) | CK3                    | 17.13 $\pm$ 6.00                  | 49.67 $\pm$ 13.25          | 97.59 $\pm$ 22.37        | 12.04 $\pm$ 3.11                  |
|                                              |                          | Sample 21              | 13.60 $\pm$ 3.99                  | 33.02 $\pm$ 8.15           | 57.18 $\pm$ 7.52         | 11.98 $\pm$ 2.91                  |

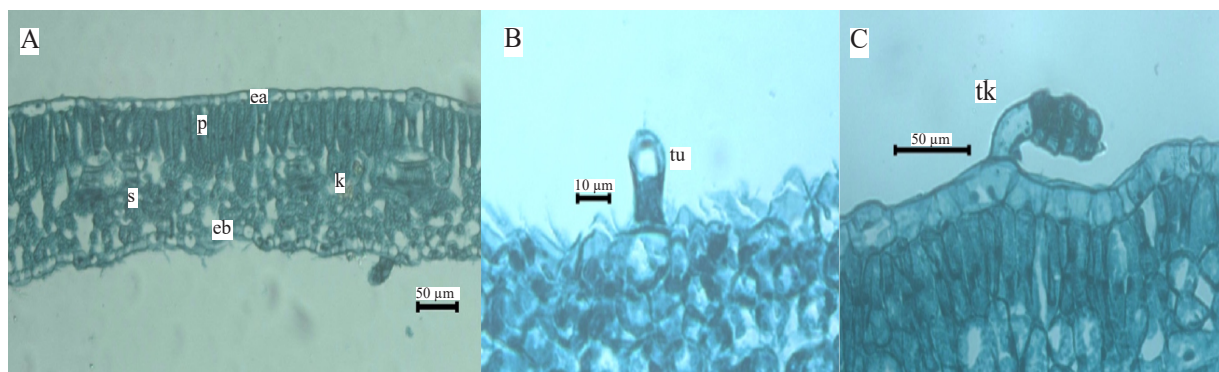


Figure 1. Healthy pepper leaves. A. Anatomical structure of pepper leaves (CR4; scale bar: 50  $\mu\text{m}$ ); B. Uniseriate trichomes (CR9; scale bar: 10  $\mu\text{m}$ ); C. Glandular trichomes (CB3; scale bar: 50  $\mu\text{m}$ ). ea: upper epidermis; p: palisade parenchyma; s: sponge parenchyma; k: Calcium oxalate ( $\text{CaCO}_3$ ) crystals; eb: lower epidermis; tk: glandular trichomes; tu: uniseriate trichomes.

only found in cayenne pepper plants. The palisade and sponge were densely packed conspicuously. In some parts of the leaf, notched leaves were observed. Anatomically, the anticlinal walls of the epidermal cells were higher, and the cell size was larger than the control leaf (Figure 3a). On the other hand, the upper epidermal cells shriveled (Figure 3b) and palisade tend to shorten. It was reported that chili genotypes with long and dense palisades can reduce whitefly nymph colonization to below ten nymphs per plant. In contrast, genotypes with short and less dense palisades are colonized by 86 whitefly nymphs/plants (Kamaliah et al., 2022). In several studies, plants infected by viruses have shown changes in anatomy at the ultrastructural, cellular, or tissue level, depending on the type of virus attack and the level of attack. Research by Otulak-Kozielec et al. (2020) on *C. annuum* leaves infected with *Bell pepper endornavirus* (BPEV) virus showed that the tissue structure of infected and uninfected leaves did not show any significant difference by light microscopy observation, but TEM microscopy revealed changes in the mitochondria, chloroplasts, and nucleus. These ultrastructural changes can also extend to cell walls, cell membranes, and endoplasmic reticulum, as seen in leaves affected by *Tomato ringspot virus* (El-Banna et al., 2014).

Plant tissue in curly leaves with severe symptoms showed a shrinking epidermis, thin, and even died (Figure 4a). While on a curved leaf, the epidermal walls appear elevated (Figure 4b) and tend to be oval. The other indications were shortened, funnel palisade, and sometimes arranged irregularly/tight. Furthermore, in some parts, palisades occur iso-diametrically dense in layers (Figure 4c). This study agreed with Esau (1948), who mentioned that parenchyma in the virus-infected banana leaf has shortened and smaller cells. Meanwhile, in other parts of the leaf, the mesophyll cells are still dividing, resulting in a kind of still-dividing protrusion that looks like leaf gall. This structure had been seen in some uneven parts. Some parts expand, and other parts shrink (Figure 4d). In addition, glandular trichomes appear more abundant than healthy leaves. Virus-infected plant leaves experienced a reduction in cell size in both the epidermal, palisade, and spongy tissues.

The data indicated a tendency for the structure of infected chili leaves to change. According to Hamida & Suhara (2013), after the tobacco plants' leaves are infected with the virus, there are changes in the morphology, plant anatomy, and chlorophyll content of the tobacco leaves. The results showed a significant difference between the leaves of healthy plants and those infected with CMV. However, the leaf structure

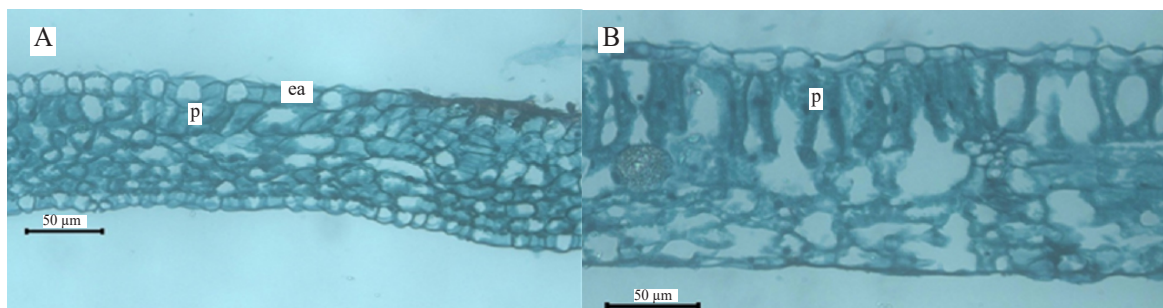


Figure 2. Leaf anatomy of mild symptoms. A. Epidermal cells shrink and palisade shortens (CR1, Unaha), and B. Palisade leaves are cylindrical and tenuous (CR12, Ponggia). a: upper epidermis; p: palisades. Scale bar: 50 µm.

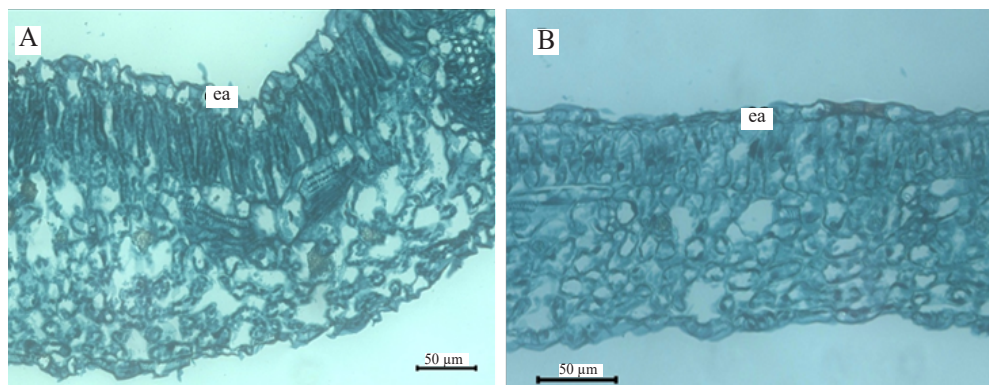


Figure 3. Moderate symptoms leaf. A. Epidermal cell walls are elevated (CR2, Unaha); B. Upper epidermal cells shrivel (CR2, Unaha). Ea: upper epidermis. Scale bar: 50 µm.

of chili plants infected with the Geminivirus differs from that of healthy plants. However, there was some damage to the leaf cell structure, as indicated by mosaic symptoms, malformations, and leaf shrinkage (Figure 4 d). Damage to plant leaf structure is thought to occur due to damage to plant physiology due to the Geminivirus. Physiological damage due to viral infection includes photosynthesis, respiration, and transpiration (Hull, 2013). A reduction in plant size or hypoplasia is the most common symptom due to viral infection.

The interaction between viruses and plants causes an increase in protein content and photosynthetic rate by 100% after the virus infects the plants. The rate of photosynthesis decreases by 60–70% as the replication rate decreases (Funayama-Noguchi & Terashima, 2006). The interaction between viruses and chloroplasts is important because viruses can damage the structure and function of chloroplasts. Chloroplasts are important in the viral infection cycle, and the role of chloroplasts is to fight viral infections. Viruses can affect the structure and function of chloroplasts, which are responsible for photosynthesis (Bradamante et al., 2021). This can lead to symptoms such as leaf chlorosis (yellowing of leaves) due to altered pigmentation and structural changes in chloroplasts. Therefore, chloroplasts are the target of viral infection and a site for replication (Zhao et al., 2016).

Based on the results of this study, changes in the ultrastructure of chili leaf tissue, such as epidermal, palisade, and spongy cells. Changes that occur, such as epidermal cells dying and the palisade shrinks or

shrinking and becoming thinner. It also appears that there are protrusions or indentations in the epidermal cells. Therefore, the chili leaves appear to have malformations. The growth of plant cells is also inhibited, allegedly due to energy diversion by viruses, which causes diseased plant cells to be smaller in size than healthy plants.

Viruses replicate themselves to form viral proteins in the plant cell (viruses cannot replicate independently and are highly dependent on the host's metabolic machinery; DNA virus genomes replicate in the nucleus. The viral RNA is in the cell cytoplasm, which then comes into contact with ribosomes for protein synthesis. The proteins produced are proteins the virus needs, such as protein coats, not those the host plant needs. Viral replication begins in infected cells for the first time, both in the epidermis and mesophyll tissue of leaves. Then, move to the vascular tissue of plants. Plant viruses actively and passively move from cell to cell through plasmodesmata (Hull, 2013).

Replication and movement of the virus systemically cause chili leaves to change shape morphologically and ultra-structurally. These shape changes can be seen in chili plant leaves' mesophyll and epidermal tissues. The cells shorten, are arranged irregularly, in layers, and are more tightly arranged. Geminivirus infection also causes discoloration of chili plant leaves. A viral infection of the mesophyll, palisade, and spongy tissues causes leaf discoloration. Leaf chlorophyll is present in these organs, so organ damage will impact changing the color and shape of the chili

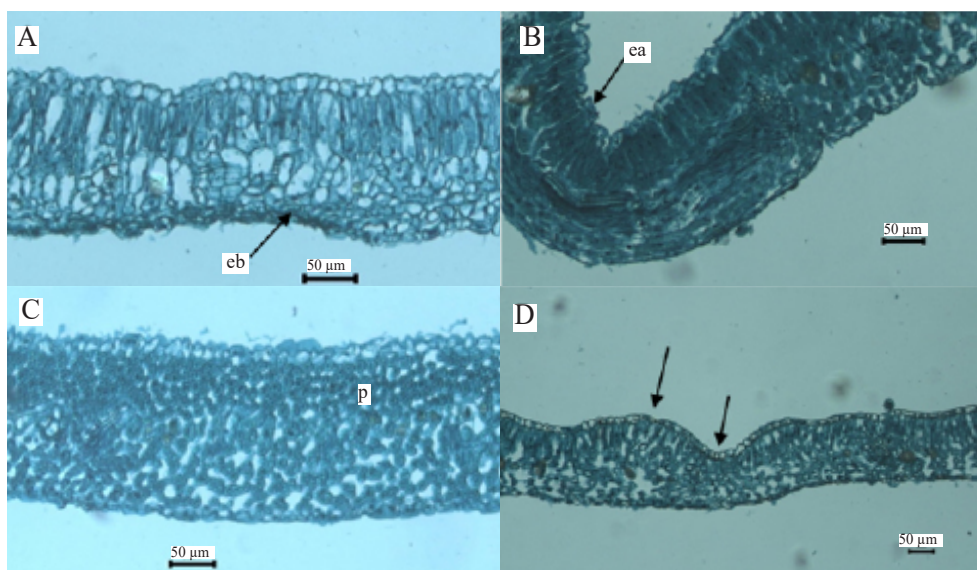


Figure 4. The severe symptoms. A. The lower epidermis is wrinkled (CB5, Kalongi); B. The upper epidermis is elevated at the indentation (CR10, Tamborasi); C. The palisade is isodiametric (CR10, Tamborasi); D. leaf protrusions and indentations (arrows) with severe symptoms (CR10, Tamborasi). Ea: upper epidermis; eb: lower epidermis; p: palisades. Scale Bars: 50 µm.

plant leaves. This is consistent with what was reported: healthy chloroplasts are attached to the mesophyll, palisade, and spongy cell walls with elongated and dense cell structures (A'yuningsih, 2017). As a result, the formation of chlorophyll is inhibited due to virus infection, so the rate of chlorophyll formation is slower than the degradation of leaf chlorophyll (Funayama-Noguchi & Terashima, 2006).

The tissues most affected by this viral infection are the mesophyll, xylem, phloem, meristem, and lead structure (Otulak-Kozieł et al., 2020; El-Banna et al., 2014; Hamida & Suhara, 2013; Bradamante et al., 2021). Marques et al. (2010) showed that the anatomical structure of leaves that were attacked by a virus until the lesions formed showed many changes. In citrus leaves infected with the *Citrus leprosis virus*, it consists of three parts: the edges, the intermediate part in the form of a "halo" lesion, and the central part of the lesion, on the edge of the mesophyll seen undergoing plasmolysis. In the "halo" of the lesion, a more prominent leaf structure will be seen, resulting in thicker leaves due to intense hypertrophy and hyperplastic activity of the parenchyma cells, especially in part associated with the transport bundles. Meanwhile, in the center of the lesion (necrotic center), palisade parenchyma cells do not appear to contain chloroplasts. The necrotic center is mainly composed of mesophyll (mainly spongy parenchyma) and vascular cells, which accumulate lipid compounds. The efficiency of sunlight absorption and plants' photosynthesis process is disrupted due to the abnormal condition of the leaves (Hamida & Suhara, 2013). Leaves with yellow leaf curl disease symptoms have smaller and denser cell sizes, especially the epidermis and mesophyll tissues. The symptomatic leaf epidermis is smaller than that of healthy plants.

## CONCLUSION

Geminivirus infection, which showed mild symptoms, causes the epidermal cells to shrink; the palisade is composed of two layers; the cells shorten and tend to be oval. Moderate symptoms: the upper epidermal cells are shriveled, and the palisade tends to shorten. Severe symptoms of the epidermis on the leaves were curly, shriveled (shrink); the epidermis was thinned and dead; the palisade was shortened and sometimes looks irregular. Changes in the leaf tissue of chili plants infected with the Geminivirus caused changes at the ultra-structural, cell, or tissue level, depending on the type of virus attack and the attack level. Accumulated changes in the structure of chili leaves, such as thinning of the epidermis and

shortening of the palisade and other parts, will cause photosynthesis organs to be disrupted so that in the next stage, it would significantly affect the growth and production of chili plants.

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## AUTHORS' CONTRIBUTIONS

MT considered and planned the experiment; performed PCR-based Begomovirus and DNA-betasatellite detection; wrote and reviewed the manuscript. MZF, GHS, and VIV reviewed the manuscript. SY and MB conducted surveys and took plant samples for virus detection. AH considered and planned the experiment; recorded and processed plant images; analyze data; wrote and reviewed manuscripts. EFT and TYIW observed histology-based changes in chili plant leaf tissue with the help of a microscope.

## COMPETING INTEREST

No competing interest.

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