

Response of morphological, anatomical, and agronomic characteristics of soybean genotypes to Cowpea Mild Mottle Virus

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Abstract. Zubaidah S, Mujtahida I, Kuswanto H. 2023. Response of morphological, anatomical and agronomic characteristics of soybean genotypes to Cowpea Mild Mottle Virus. *Biodiversitas* 24: 4017-4026. Cowpea Mild Mottle Virus (CpMMV) is a significant disease in soybeans. This disease can cause plant malformations and reduce seed yields. The study used a randomized block design with three replications. Plant materials were five resistant genotypes (MLGG 0006, MLGG 0106, MLGG 0297, MLGG 0315, and MLGG 0599) and five susceptible genotypes (MLGG 0123, MLGG 0379, MLGG 0603, MLGG 0695, and MLGG 0796). The results showed a significant difference between resistant and susceptible genotypes to CpMMV attack. Resistant soybean genotypes had relatively tall stems, many branches, and larger leaf widths. The number of stomata and trichomes of the resistant genotype was less than that of the susceptible genotype. The number of filled pods, the total number of pods per plant, and the number of seeds of the resistant genotype had more numbers than the susceptible genotype. MLGG 0695 was the most responsive genotype because it had a relatively low stem height, few branches, small leaf size, many stomata and trichomes on the leaf surface, the thickest leaf size, and a small number of pods and seeds. The most resistant soybean genotype was MLGG 0297, having a relatively high stem, many branches, large leaf size, few stomata and trichomes on the leaf surface, thin leaf size, and many more pods and seeds. MLGG 0297 can be used as a gene source to develop a superior variety resistant to CpMMV.

Keywords: Agronomy, anatomy, CpMMV, morphology, soybean

Soybean is an important food crop in Indonesia. In 2019, the soybean consumption level was 6.43 kg per capita per year, while the national demand was about 3.09 million tons per year; consequently, about 2.67 million tons (86.4%) were imported (Triyanti 2020). Therefore, it is necessary to increase soybean productivity. One the reason for low productivity is disease attacks, including cowpea mild mottle virus (CpMMV). This virus primarily attacks soybean plants in Indonesia and disrupts plant vegetative growth (Kulsum et al. 2017). CpMMV infects systemically with obvious symptoms in soybeans and various plants. CpMMV can cause leaf spots with yellow spots, rough mosaic or mosaic, wrinkled, chlorosis, apical necrosis, and leaf malformations, depending on the infected soybean cultivar (da Silva et al. 2020; Nazarov et al. 2020; Sutrawati et al. 2021). However, sometimes there are no symptoms of the disease, but it reduces plant height, the number of pods, and seed size (Barreto d Silva et al. 2020). Further, co-infection with CpMMV and other viruses may cause the symptoms of the disease to worsen (Wei et al. 2021). In addition, the disease incidence of CpMMV on the Anjasmoro variety varies from 32.9 to 81.3% depending on the growing environment (Sutrawati et al. 2021).

CpMMV can be transmitted mechanically by inoculation, semipersistent whitefly vector (*Bemisia tabaci* Genn.), and soybean seeds (Chiquito-Almanza et al. 2021). *B. tabaci* is an effective plant virus vector and a polyphagous bug. Apart from Indonesia, this virus vector

was also detected in India, Australia, Brazil, and China (Gambley et al. 2022; Isnaini et al. 2020; Wei et al. 2021; Zubaidah et al. 2020; de Moraes et al. 2018; Bello et al. 2021; Persley et al. 2020). Whiteflies get food from host plants by sucking leaf fluids, directly damaging plants, and carrying viruses that cause plants to grow stunted. Seed transmission of CpMMV was found in some soybean varieties but not found in other varieties (Sutrawati et al. 2021). CpMMV seed transmission evidence was found in the cultivar Detam 2, Detam 3, Malika, Anjasmoro, and Argomulyo (Sutrawati et al. 2021) and BMX POTNCIA RR (Barreto d Silva et al. 2020). However, the virulence of the virus may decrease due to the introduction of novel variations, and virulence enables the pathogen to adapt quickly to a new host (Zanardo et al., 2021). The resistance range is also reported on other species, French beans (Gambley et al. 2022).

Huge losses due to virus attacks can be prevented through three methods approaches, namely (1) eliminating the source of infection; (2) preventing the spread of the virus; (3) and using virus-resistant varieties. Zhao et al. (2020) also added that genetic improvement is one way to overcome losses due to virus or disease attacks. Zubaidah and Kuswanto (2006) found soybean genotypes resistant and susceptible to CpMMV from 100 genotypes, a genetic constitution the living creature inherits (Vihinen 2022). Knowledge of morphological characteristics (mainly stems and leaves), anatomy (especially leaf anatomy), and

agronomy (especially pods and seeds) can be used to diagnose diseases and their symptoms in plants to develop resistant varieties through plant breeding programs. This study will investigate the anatomy of leaves on trichomes and stomata.

CpMMV infects soybean plants and then utilizes plant cellular performance to interfere with the physiology of soybean plants, reducing their productivity (Gambley et al. 2022; Sutrawati et al. 2021). In addition to disrupting soybean productivity, CpMMV also affects soybean plant morphology. Based on research comparing the morphology of CpMMV-resistant soybean plants, differences appeared in morphological characteristics such as leaf length, leaf petiole length, leaf ratio, and leaf width (Nurrohman et al. 2019; Oliveira et al. 2020; Samiyarsih et al. 2022), and agronomic characteristics (Salihu et al. 2022). Soybean infection is observed seen by local necrotic indicators followed by systemic mosaic, light spotting, and necrosis of the leaves (Nazarov et al. 2020; Sutrawati et al. 2017; Sutrawati et al. 2021). In this study, we investigated the response of soybean genotypes' morphological, anatomical, and agronomic characteristics to CpMMV that others have not conducted. The distinct characteristic is expected to indicate resistance or susceptible soybean genotype.

MATERIALS AND METHOD

Plant materials

The plant materials in this study were five CpMMV-resistant soybean genotypes and five CpMMV-susceptible soybean genotypes. The five soybean genotypes were MLGG 0006, MLGG 0106, MLGG 0297, MLGG 0315, and MLGG 0599, and five soybean genotypes susceptible to CPMMV, namely MLGG 0123, MLGG 0379, MLGG 0603, MLGG 0695, and MLGG 0796.

Planting

The planting media was the soil that has been air-dried and then loosened. The soil was put in a polybag containing 7 kg of soil. Before planting, the ground was sprinkled with Petrofur and then watered to field capacity. Each polybag was planted with four soybean seeds. At the age of seven days, thinning was done so that there were the remaining two plants per polybag.

Infestation of disease vector

Before the insect vector (*B. tabaci*) infestation, vector insects were propagated on soybeans susceptible to CpMMV. The propagation of this vector insect was carried out around the main plantation. When the main crop was ten days after planting, the infestation crops were harvested, so the vector insects switched to the main crop (Zubaidah and Kuswantoro 2016).

Observation of morphological characteristics

The morphology was observed as plant height, number of branches, leaf length, and width. Plant height was measured from the stem above the ground to the growing point. The number of branches that grow from the primary

stem branching was counted. The length and width of the leaf measured was the fifth leaf from the top leaf segment, which was then used for anatomical observations (Kartika et al. 2020).

Observation of anatomical characteristics

Stomata observations were carried out by counting the number of stomata by leaf preparations smeared with corrector fluid and covered with tape. After drying, the tape was removed, attached to the slide, and observed under a light microscope for both upper and lower leaves. Trichome observations were carried out by counting the number of trichomes on the leaf pieces of 0.5 x 0.5 cm, then observed and measured under a stereomicroscope, both for the upper and lower surface leaves. Leaf thickness was observed by preparing fresh leaf cross sections for leaves resistant and susceptible to CpMMV. The leaves the observed under a light microscope with solid magnification, and leaf thickness was measured using a micrometer that had been calibrated first (Amaliah et al. 2019).

Observation of agronomical characteristics

Observation of agronomic characteristics included the number of filled pods and unfilled pods, the total number of pods, and the number of seeds per plant (Nurrohman et al. 2019).

Statistical analysis

Therefore, to compare the characteristics of soybean genotypes that are resistant and susceptible to CPMMV, the data obtained from the observations were analyzed using a contrast orthogonal randomized design performed with the SPSS for Windows program.

RESULTS AND DISCUSSION

Soybean genotype resistant to CpMMV

Morphological characteristics of CpMMV-resistant soybean genotype

Observation of the morphological characteristics of plant height and number of branches in five CpMMV-resistant soybean genotypes showed that the MLGG 0297 genotype had the largest stem size of 64 cm with five branches, followed by MLGG 0315 with a plant height of 61.67 cm and six branches, MLGG 0599 with a plant height of 58.67 cm and four branches, MLGG 0006 with a plant height of 55.33 cm and four branches, and MLGG 0106 had the shortest stem height of 43.67 cm with three branches (Table 1). Based on the exposure to observational data, it can be observed that the tall stems also have many branches. The taller the stem, the more branches are formed. This statement follows Pangestika et al. (2021) that the stem height in a plant can assumed to be the more stem branches formed. The higher the stem, the more branches; each stem can form 3-6 branches. In addition, Zubaidah et al. (2020) report that the interaction between genotype and fertilizer was found in the plant height of soybean plants infected by CpMMV.

This study considers leaf shape as the leaf lamina's shape; the leaf length and width ratio affects leaf shape. The shape of the leaf lamina is determined by its general shape, regardless of the curves, namely forming a line connecting the protrusions or talus. Regarding leaf length, the MLGG 0297 had the most extended leaf size compared to the other four resistant genotypes, which was 8.4 cm, and conversely, the MLGG 0006 was a resistant genotype with the shortest leaf length of 7.2 cm. Kartika et al. (2020) reported the longest leaf length up to 10.08 cm. The lengths of the other three genotypes of MLGG 0106, MLGG 0559, and MLGG 0315 were 8, 7.9, and 7.3 cm, respectively. Similar to leaf width, genotype MLGG 0297 had the widest leaf size compared to the other four resistant genotypes (5.5 cm), while MLGG 0006 had the smallest leaf width (4.4 cm). The leaf width of other genotypes, MLGG 0315; MLGG 0599; MLGG 0106, is 5.3 cm; 5.2 cm; and 5.1 cm (Table 1).

The five resistant soybean genotypes showed variations in leaf length and width. The leaf length of the resistant soybean genotype showed that it was more oversized and out of the range of soybean leaf length in general, namely 3-5 cm, but not in the width of the leaf blade, which still within the soybean leaf width range, in general (2-7.5 cm). The five genotypes had the same comparison value between leaf length and width, namely 1.5: 1, resulting in the same leaf shape, which is an oval shape. The leaf shape includes an oval shape, with the widest part in the middle of the strand at the ratio length: width = 1.5 -2: 1.

Anatomical characteristics of CpMMV-resistant soybean genotype

This study's observation of leaf anatomy characteristics focused on the anatomical structure of leaves, including the number of stomata and trichomes on the leaf surface (epidermis) and leaf thickness of five CpMMV- resistant soybean genotypes. Stomata are small gaps in the epidermis that are used for gas exchange to the atmosphere. This gap is a cavity formed by two distinctive epidermal cells called guard cells. Changes in these cells' size and shape determine the stomata's opening and closing (Nunes et al. 2020). Based on observations, it is known that the MLGG 0297 genotype has an average of 64 stomata on the leaf surface and is the highest number of stomata compared to the other four resistant genotypes. Genotype MLGG 0106 had an average of 47 stomata on the leaf surface and was the least number of stomata compared to the other four genotypes (Table 2). Different cultivars had different epidermis, palisade ratios, stomata length and width, and a number of stomata and trichomes (Husen et al. 2022).

Stomata are small gaps between the leaf epidermis. Stomata on leaves is a tissue needed in the respiration process, which involves CO₂ as the main substance in photosynthesis; this process results in forming pods and seeds. Rasheed et al. (2022) stated that the relationship between the plant body inside and the outside environment could happen with stomata. This phenomenon causes stomata to be important in regulating two critical plant processes, photosynthesis, and transpiration. The more the

number of stomata on the leaf surface (epidermis), the higher the photosynthetic and transpiration activity in the plant. In this case, the result of both processes relates to forming pods and seeds. Simkin et al. (2020); Tamang et al. (2022) also stated that the stomata's size, position, and arrangement influence the photosynthesis rate. It can be analogous that the more the number of stomata, the more the number of pods and seeds produced. In addition, Gahir et al. (2021); Samiyarsih et al. (2020) adds that the structure of the stomata – for example, with tiny openings and large and tall opening guard cells – may also give some varieties resistance to specific pathogens attack.

The number of trichomes can indirectly affect pods and seeds formations. The study showed that genotype MLGG 0106 had an average of 61 leaf surface trichomes, the highest number compared to the other four resistant genotypes. On the other hand, genotype MLGG 0315 had 53 trichomes on the leaf surface and was the least number of trichomes compared to the other four resistant genotypes (Table 2). The number of trichomes in this study is higher than Kartika et al. (2020), which reported a number of trichomes on the lower leaf surface ranging from 21.8 to 40.8. The number of trichomes affects the resistance of soybean plants to disease attacks and even could make a plant resistant to disease attacks, especially diseases carried by insect pests. Therefore, the more trichomes, the smaller the attack of insect pests and vice versa. This phenomenon aligns with Prasifka's (2015) and Rasheed et al. (2022) statement that trichomes protect against external disturbances and reduce evaporation.

Leaf thickness indicates the number and arrangement of the tissues forming the leaf organs. In this study, the tissue makes up the mesophyll and epidermal regions. Mesophyll tissue is located between the upper and lower epidermis and the veins, consisting of thin-walled specialized parenchyma cells, making up most of the leaf inside. Generally, parenchyma cells have two forms: palisade parenchyma or palisade tissue and spongy parenchyma or spongy tissue. Parenchyma tissue always contains chloroplasts. The color of green leaves is due to chlorophyll in the leaf cells in small dams called chloroplasts, which perform photosynthesis functions (Mandal and Dutta 2020).

Table 1. Stem and leaf morphology of CpMMV-resistant soybean genotype

Genotype	Plant height (cm)	Branches	Leaf		
			Length (cm)	Width (cm)	Shape
MLGG 0006	55.33	4	7.2	4.4	Oval
MLGG 0106	43.67	3	8	5.1	Oval
MLGG 0297	64	5	8.4	5.5	Oval
MLGG 0315	61.67	6	7.3	5.3	Oval
MLGG 0599	58.67	4	7.9	5.2	Oval
Average	56.67	3	7.76	5.1	

Table 2. Leaf anatomy of CpMMV-resistant soybean genotype

Genotype	Number of stomata	Number of trichomes	Leaf width (µm)
MLGG 0006	50	56	140.33
MLGG 0106	47	61	125.25
MLGG 0297	64	60	125.75
MLGG 0315	62	53	125.75
MLGG 0599	59	54	125.67
Average	56	57	156.67

Agronomic characteristics of CpMMV-resistant soybean genotype

The number of pods varied in soybean plants with different genotypes. According to Karyawati et al. (2022), this is caused by each soybean plant's different morphological characteristics. Based on the results obtained (Table 3), it was shown that the MLGG 0297 was ranked first as the resistant genotype that produced the most pods per plant compared to the other four resistant genotypes (28 pods), followed by MLGG 0315 (20 pods), MLGG 0559 (19 pods), MLGG 0106 (17 pods), and MLGG 0006 (15 pods). The number of filled pods in this study is lower than Isnaeni et al. (2020), which reached up to 70 pods. Genotype and soil fertility affects the number of filled pods and total pods (Zubaidah et al. 2020). Soil moisture is most needed during germination, early seedling, and pod formation to pod filling. The lack of water at this critical phase will affect yields dramatically. In genotypes that produce pods with tiny seeds, the plants tend to have morphological characteristics that are resistant to dry or low water or low-water (low-water environments) (Mustofa et al. 2020). Sutrino and Kuswanto (2016) reported Korean soybean variety of Daepung had the most resistance to CpMMV but low grain yield, and Daemang-2 was the most tolerant variety with high leaf infection but high seed yield.

The number of seeds in five resistant genotypes in a row from the genotype that produced the most seeds per plant was MLGG 0297 with 56 seeds, MLGG 0599 with 44 seeds, MLGG 0315 with 35 seeds, MLGG 0006 with 30 seeds, MLGG 0106 with 25 seeds (Table 3). Furthermore, environmental factors also influence the number of seeds per plant. The study showed that resistant genotypes that produced large pods would produce many seeds. For example, the MLGG 0297 genotype with the highest number of pods (28 pods) had more seeds than the other four resistant genotypes (56). It can be assumed that the number of seeds per plant is directly proportional to the number of pods per plant, the number of branches, and the plant's height (Pangestika et al. 2021). The average number of seeds and pods produced by soybeans was 1-4 seeds per pod.

CpMMV-susceptible soybean genotype

Morphological characteristics of CpMMV-susceptible soybean genotypes

Observing the morphological characteristics of stem height and number of branches in five soybean genotypes susceptible to CpMMV, it was found that the genotype

MLGG 0695 had the highest stem size of 49.33 cm with one branch, followed by MLGG 0123 with a plant height of 46.67 cm and the number of branches 2, MLGG 0796 with a plant height of 32.67 cm and the number of branches 1, MLGG 0603 with a plant height of 31 cm and three branches, and MLGG 0379 with the shortest plant height of 26.67 cm with one branch. Soybean genotypes with high stems did not necessarily produce more branches (Table 4). Soybean plants classified as susceptible to viruses will experience problems in stem growth, namely stunting, so the stem height will be shorter than normal; consequently, the number of branches also decreases. Zubaidah and Kuswanto (2016) and Logo et al. (2018) stated that one of the characteristics of soybean plants that CpMMV attacked was stunted stems; the number of branches of affected plants generally decreased. However, some genotypes had an increase in the number of branches.

Observations on the length and width and leaf shape of five susceptible genotypes, respectively, from the genotype with the most extended leaves, were MLGG 0379 with a leaf length of 7.7 cm and a width of 4.7 cm, MLGG 0603 with leaves 6.4 cm long and 4.4 cm wide, MLGG 0695 with a leaf length of 6.3 cm and a width of 4.1 cm, MLGG 0123 with leaves 6.2 cm long and 4.4 cm wide, and MLGG 0796 with a leaf length of 6.0 cm and a width of 3.8 cm (Table 4). The comparison value of the length and width of the leaves means that the shape of the leaf blades in the five genotypes is the same, namely the oblong or oval shape (1-1.5: 1). The leaves of the CpMMV-susceptible genotype also showed typical morphological symptoms in the form of yellow spots (necrosis), and the leaf surface was wrinkled or malformed (curling and rolling down or up) (Figure 1). Sutrino and Kuswanto (2016) reported the genetic effect, where the South Korean cultivar Daemang-2 demonstrated a reduced level of leaf malformation than the two Indonesian varieties.

Table 3. Agronomy of CpMMV-resistant soybean genotypes

Genotype	Number of pods			Number of seeds
	Unfilled	Filled	Total	
MLGG 0006	2	13	15	30
MLGG 0106	4	13	17	25
MLGG 0297	5	23	28	56
MLGG 0315	4	16	20	35
MLGG 0599	2	17	19	44
Average	3	16	20	38

Table 4. Plant height and leaf morphology of CpMMV-susceptible soybean genotype

Genotype	Plant height (cm)	Number of branches	Leaf		
			Length (cm)	Width (cm)	Shape
MLGG 0123	46.67	2	6.2	4.4	Oval
MLGG 0379	26.67	1	7.7	4.7	Oval
MLGG 0603	31	3	6.4	4.4	Oval
MLGG 0695	49.33	1	6.3	4.1	Oval
MLGG 0796	32.67	1	6.0	3.8	Oval
Average	37.27	1	6.51	4.28	

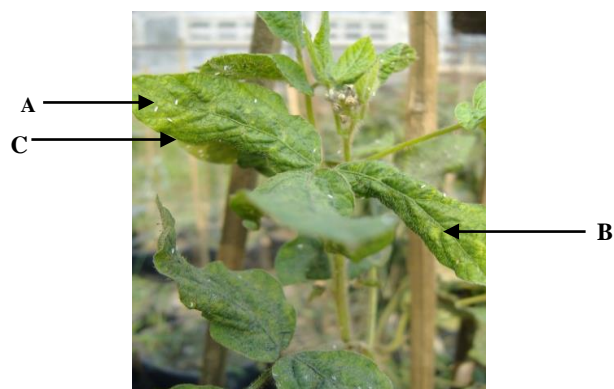


Figure 1. Morphological appearance Of CpMMV-susceptible soybean genotype (MLGG 0695). A. Yellow spots on the surface and leaves appear wrinkled; B. Leaf rolling down; C. Whitefly (*Bemisia tabaci* Genn) insect vector

According to da Silva et al. (2020); Sutrawati et al. (2021), CpMMV can cause leaf spots with yellow spots, rough mosaic or mosaic, wrinkled, chlorosis, apical necrosis, and leaf malformations, depending on the infected soybean cultivar. (Sutrawati et al. 2017; Sutrawati et al. 2021) also mentions that the most common plant symptoms types produced by systemic viral infections are mosaic and ring spots, which are characterized by the appearance of ring-shaped chlorosis or necrosis on leaves and sometimes also on fruit and stems.

Anatomical characteristics of CpMMV-susceptible soybean genotypes

Leaf anatomy observations in this study included the number of stomata and trichomes on the leaf surface (epidermis) and leaf thickness of five soybean genotypes susceptible to CpMMV. Stomata were found almost abundantly on the lower epidermis of soybean leaves which were dorsiventral. In the upper epidermis, stomata were very few or sometimes none. Based on observations, it is known that the MLGG 0796 genotype had 69 stomata, MLGG 0603 had 62 stomata, and MLGG 0695 had 60 stomata on the leaf surface. Genotype MLGG 0379 had 59 stomata, and MLGG 0123 had 41 stomata on the leaf surface (Table 5). Other diseases include *Sclerotium rolfsii*'s inoculation of soybean leaves, reduced stomatal density, and epidermal thickness (Samiyarsih et al. 2022).

Observation of the following anatomical characteristics regards the number of trichomes. The results showed that the genotype MLGG 0379 had 140 trichomes, MLGG 0695 had 106 trichomes, and MLGG 0123 had 90 trichomes on the leaf surface. Genotype MLGG 0796 had 87 trichomes, and MLGG 0603 had 62 trichomes on the leaf surface (Table 5). The formation of trichomes on the leaves on susceptible soybean leaves' upper and lower surfaces showed a greater or more significant number. A response is suspected by forming many trichomes on each genotype susceptible to attack or infection with CpMMV. In

addition, based on observations, the five susceptible genotypes had non-glandular trichomes, mainly reducing evaporation.

Leaf thickness in five CpMMV susceptible genotypes varied. The leaf thickness of genotype MLGG 0695 was 178.42 μm , genotype MLGG 0796 was 161.08 μm , MLGG 0123 genotype was 160.75 μm , MLGG 0603 genotype was 155.83 μm , and the MLGG 0379 genotype was 151.83 μm (Table 5). Leaf thickness in susceptible genotypes was thought to increase in size and number of leaf mesophyll cells as a symptom of infection with CpMMV. According to Nazarov et al. (2020), morphologically and anatomically, the symptoms of plant diseases are divided into eight groups, namely hyperplasia (hypertrophy), hypoplasia (hypotrophy), discoloration, dryness or wilting, necrosis, secretion, fungal growth on the surface and in plant tissues, and damage by insects or other animals.

Agronomic characteristics of CpMMV-susceptible soybean genotypes

Based on the results of the study, the genotype MLGG 0123 produced the most pods per plant compared to the other four susceptible genotypes (15 pods), followed by MLGG 0695 (8 pods), MLGG 0379 (7 pods), MLGG 0603 (6 pods), and MLGG 0796 (3 pods) (Table 6). The number of seeds in five susceptible genotypes in a row from the genotype that produced the most seeds per plant was MLGG 0123 (2 seeds), MLGG 0379 (13 seeds), MLGG 0695 (7 seeds), MLGG 796 (6 seeds), and MLGG 0603 (4 seeds) (Table 6). Like the resistant genotype, the susceptible genotype that produced many pods would also produce more seeds. For example, genotype MLGG 0123, which had the highest number of pods (15 pods), also produces many seeds (27 seeds). This is following Hidayati's (2006) report. The length and diameter of the pods varied significantly between the different soybean varieties (Nurrohman et al. 2019).

Comparison of morphological, anatomical, and agronomic characteristics between CpMMV-resistant soybean genotypes and CpMMV-susceptible soybean genotypes *Comparison of agronomic characteristics*

Morphological characteristics between resistant and susceptible genotypes differed in stem height, number of branches, leaf length, and leaf width. Statistically, the morphological characters between resistant and susceptible genotypes differed significantly. All morphological characters between the two groups were significantly different. The resistant genotypes had higher stem size, leaf size (length and width), and a more significant number of branches than the five susceptible genotypes (Table 7). The susceptible genotypes experienced a quantitative decline in their morphological characters. For example, in leaves, it was observed that the leaf length and leaf width of susceptible genotypes were smaller than those of resistant genotypes (Gahir et al. 2021; Samiyarsih et al. 2020).

Table 5. Leaf anatomy of CpMMV-susceptible soybean genotype

Genotype	Number of stomata	Number of trichomes	Leaf width (µm)
MLGG 0123	41	90	160.75
MLGG 0379	59	140	151.83
MLGG 0603	62	62	155.83
MLGG 0695	60	106	178.42
MLGG 0796	69	87	161.08
	58	97	161.58

Table 6. Agronomy of CpMMV-susceptible soybean genotype

Genotype	Number of pods			Number of seeds
	Unfilled	Filled	Total	
MLGG 0123	4	11	15	27
MLGG 0379	3	4	7	13
MLGG 0603	2	4	6	4
MLGG 0695	5	3	8	7
MLGG 0796	1	2	3	6
Average	3	5	8	11

Table 7. The mean square of morphological characteristics of CpMMV-resistant and CpMMV-susceptible soybean genotypes

SV	df	PH	BRC	LL	LW
Replication	2	23.0583	0.0775	4.0693	0.372
Genotype	9	533.737*	0.7813*	2.2374*	0.9652*
Resistance (G1-G5 >> G6-G10)	1	2822.7*	4.4923*	11.6563*	5.043*
R1 (G-DTF 44 >> G-DTF 45)	1	217.7778	0.0044	1.7921	0.7111
R2 (G-DTF 44 >> G-DTF 45)	1	24.5444	1.7679*	0.4551	0.1068
R145 (G3 >> G5)	1	42.6667*	0.1438*	0.2817*	0.1667
R244 (G6 >> G8)	1	368.1667*	0.104	0.0267	0.0017
R144 (G1, G2, G4)	2	250.1111*	0.2472*	0.6211*	0.7078*
R245 (G7, G9, G10)	2	413.7778*	0.0123	2.3411	0.6211
Error	18	23.8176	0.1778	0.3019	0.1876
Total	29				

Note: *significant at α 0.05, SV: source of variation, df: degree of freedom, R1: resistant, R2: susceptible, G1: MLGG 0006, G2: MLGG 0106, G3: MLGG 0297, G4: MLGG 0315, G5: MLGG 0559, G6: MLGG 0123, G7: MLGG 0379, G8: MLGG 0603, G9: MLGG 0695, G10: MLGG 0796, DTF 44: days to flowering under 45 DAT, DTF 45: days to flowering over 45 DAT, PH: plant height, BRC: branches, LL: leaf length, LW: leaf width

The resistant genotype leaves also experienced changes that were shown as symptoms of viral attack; previous studies stated that the mildest symptoms had looked like healthy leaves and mottle (yellow spots). In contrast, the most severe symptoms (susceptible) had leaves with clear yellow spots, wrinkles, transparent mosaics, necrosis of the lower leaf bone, malformations, leaf shrinkage, and curved downwards or upwards (Figure 2). Some soybean genotypes were severely stunted (da Silva et al. 2020). One or more symptoms can cause a decrease in leaf size in susceptible soybean genotypes. Plant resistance to infections and improved performance under poor conditions may be connected to anatomical changes (Oliviera et al. 2020).

Comparison of anatomical characteristics

The anatomical characteristics between the resistant and susceptible genotypes were also significantly different in the number of stomata, trichomes, and leaf thickness. The susceptible genotype MLGG 0796 had more stomata, trichomes, and leaf thickness than the resistant genotype (Table 8). The study's results on the number of stomata on the leaf surface of five soybean genotypes resistant to CpMMV had a lower average number of stomata than the genotypes of soybeans susceptible (Figure 3). It is suspected that this phenomenon occurred because fewer stomata were formed in resistant genotypes to prevent or minimize the *B. tabaci* as a CpMMV vector. Moreover, suppose soybean plants are resistant to forming many stomata. In that case, the number of viruses that infect the

plant's body through the stomata may also be more significant so that it can affect the growth and development of subsequent plants; It is also possible that the plant has decreased body resistance so that it can be attacked by other micro-organisms that are parasitic. In addition, the resistance may be due to several internal and external factors that play a role in lowering the chance of infection rates. Without the recognition factors in the host, the plant will be resistant to specific pathogens (Gorshkov and Tsers 2022). Therefore, anatomical studies of plants are crucial for illustrating biological control agents' impacts (Oliviera et al. 2020).

The location and number of stomata on the leaf surface are related to the function of stomata on leaves as a means of transpiration. Plant transpiration occurs due to the opening and closing of the two guard cells that form stomata. The opening and closing of stomata depend on the presence of light and are related to photosynthesis. Photosynthesis can occur in guard cells because these cells contain chlorophyll, so glucose formation and water absorption cause the cells to enlarge, opening the stomata. Factors in plants that influence the rate of photosynthesis include chlorophyll content, hydration of the protoplasm, and leaf anatomy. Leaf arrangement that influences the rate of photosynthesis, among others, is the size and distribution of the intercellular spaces in the leaf; the comparison of the distribution of the pole network and the sponge network; the size, position, and arrangement of the stomata; and the size, distribution, and efficiency of the carrier bundle in leaves (Tamang et al. 2022).

Comparing the number of trichomes on the leaf surface showed that the five susceptible genotypes had more trichomes than the five resistant genotypes. The highest number of trichomes was owned by the susceptible genotype MLGG 0603. Trichomata is epidermal derivatives with various forms, structures, and functions, compared to four susceptible genotypes and five other resistant genotypes. The number of trichomes in susceptible genotypes was thought to respond to CpMMV infection to reduce excessive evaporation on the leaf surface. Garcia et al. (2022), Mirnezami et al. (2020), and Nurrohman et al. (2022) stated that the trichomes protect against external disturbances and reduce evaporation; trichomes can be in the form of bubbles (glandular hair). Samiyarsih et al. (2020) showed better anatomical resistance to the leaf rust disease in soybean cultivars with thicker epidermis, higher trichome counts, and lower stomatal densities.



Figure 2. Comparison of leaf size between (A) CpMMV-resistant genotype MLGG 0297 and (B) CpMMV-susceptible genotype MLGG 0695

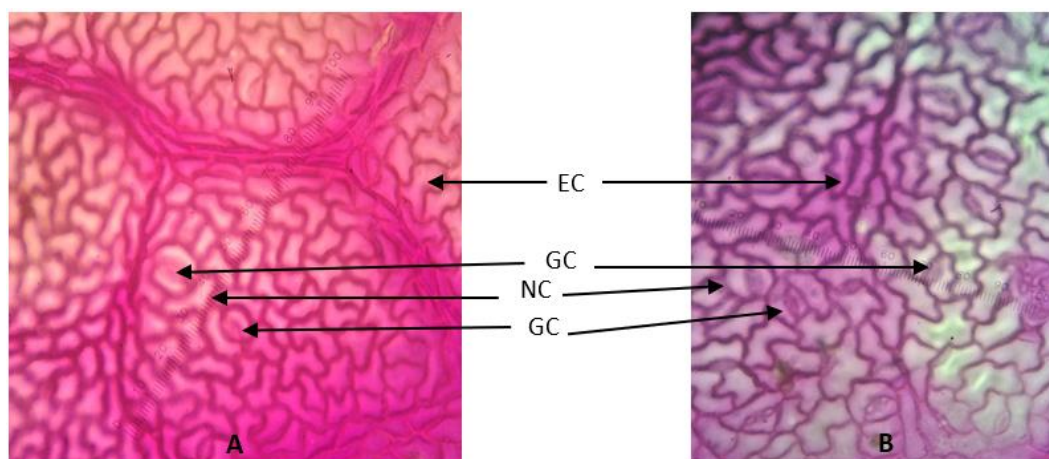


Figure 3. Comparison of the number of stomata on lower surface leaves of the soybean genotype MLGG 0297 (A) and MLGG 0695 (B). GC: gap cell, GC: guard cell, NS: neighbor cell, SE: epidermal cells, EC epidermis cells

Table 8. Comparison of anatomical characteristics of CpMMV-resistant and CpMMV-susceptible soybean genotypes

SV	df	NS	NT	LT
Replication	2	0.1051	0.5153	240.2021
Genotype	9	1.3823*	6.9873*	1124.888*
Resistance (G1-G5 >> G6-G10)	1	5.0487*	36.9741*	6765.0083*
R1 (G-DTF 44 >> G-DTF 45)	1	0.0023	0.0032	341.2507
R2 (G-DTF 44 >> G-DTF 45)	1	2.0331	11.6014*	108.3507
R145 (G3 >> G5)	1	0.5063	0.1955	0.0104
R244 (G6 >> G8)	1	3.4005*	3.893*	36.2604
R144 (G1, G2, G4)	2	0.3337	0.2526*	890.2153
R245 (G7, G9, G10)	2	0.3911	4.8568*	546.3403
Error	18	0.2899	0.1027	177.8039
Total	29			

Note: *significant at α 0.05, SV: source of variation, df: degree of freedom, R1: resistant, R2: susceptible, G1: MLGG 0006, G2: MLGG 0106, G3: MLGG 0297, G4: MLGG 0315, G5: MLGG 0559, G6: MLGG 0123, G7: MLGG 0379, G8: MLGG 0603, G9: MLGG 0695, G10: MLGG 0796, DTF 44: days to flowering under 45 DAT, DTF 45: days to flowering over 45 DAT, NS: number of stomata, NT: number of trichomes, LT: leaf thickness

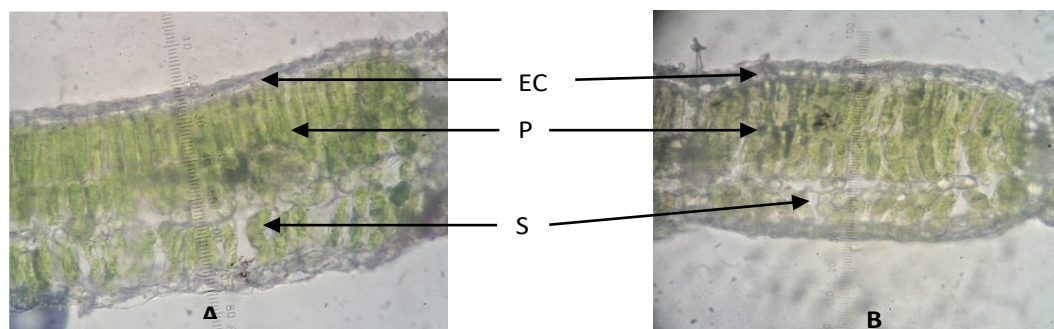


Figure 4. Comparison of leaf thickness on soybean genotype MLGG 0297, (A) and (B) soybean genotype MLGG 0695 (B), EC: epidermal cells, P: palisade, S: spongy tissue

In addition to the higher number of stomata and trichomes, the susceptible soybean genotype had a leaf thickness more significantly than the resistant genotype. It was evident in the susceptible soybean genotypes MLGG 0695, MLGG 0796, MLGG 0123, and MLGG 0379, which had thicker leaves than other genotypes. According to Nazarov et al. (2020), morphologically and anatomically, the symptoms of plant diseases can be divided into eight groups, namely hyperplasia (hypertrophy), hypoplasia (hypotrophy), discoloration, dryness or wilting, necrosis, secretion, fungal growth on the surface and in plant tissues, and damage by insects or other animals. Hyperplasia is an event of changes in the number of cells (an increase in the number of cells), while hypertrophy is an event of cell changes in the form of an increase in cell size (Figure 4). Stomatal density variability can improve productivity (Wakefield et al. 2021).

The genotype soybean plants were susceptible to stretching of the palisade tissue structure, and the chlorophyll pigment was paler than the leaves of the resistant genotype soybean plants. The intervenous strand size of the leaves of the susceptible soybean genotype was narrower than the leaf of the resistant genotype. This is thought to have occurred during the formation of leaf blades due to viral infection. These disturbances can be in the form of disturbances in the meristems that play a role in the formation of leaf blades, besides that it is suspected that in the mesophyll tissue, there is also interference or damage to the palisade tissue and the chloroplasts contained there in so that the chlorophyll pigment contained is reduced. According to Elmore et al. (2022); Nazarov et al. (2020), viruses generally cause a decrease in the photosynthesis rate by reducing the amount of chlorophyll per leaf or chlorophyll efficiency or leaf area per plant (each plant). Viruses also cause a decrease in the number of growth-regulating compounds (hormones) by increasing growth-inhibiting compounds.

Comparison of agronomic characteristics

The agronomic characteristics observed in this study were the number of empty pods, the number of filled pods, the total number of pods, and the number of seeds per

plant. The observations and data analysis showed that the agronomic characteristics between resistant and susceptible genotypes significantly differed in the number of filled pods, the total number of pods, and several seeds per plant, where five resistant genotypes were better than five susceptible genotypes (Table 9). The number of empty pods between resistant and susceptible genotypes showed no significant difference.

Five CpMMV-resistant genotypes produced a relatively higher number of empty pods, filled pods, the total number of pods, and several seeds per plant compared to five CpMMV-susceptible genotypes. It is suspected that resistant genotypes can still produce a lot of pods and seeds because these plants still carry out physiological processes as usual. So that although morphologically resistant soybean plants show symptoms, their physiological processes continue to run normally. On the other hand, susceptible genotypes showed more morphological, anatomical, and physiological processes in the plant, so fewer pods and seeds were produced than resistant genotypes. The results of previous studies showed that the number of empty pods increased in several soybean genotypes. This increase occurred because the CpMMV attack resulted in disturbed pod filling. The decrease in the total number of pods in susceptible genotypes was thought to be due to a decrease in metabolic processes in susceptible soybean plants due to the CpMMV attack (Nurrohman et al. 2019). In many diseases, the ring spot symptom, but not caused by a virus, tends to be invisible after the initial attack and reappears under certain environmental conditions (Sandra et al. 2015).

The decrease in yield caused by the virus was mainly due to a decrease in the physiological activity of soybean plants, such as the high rate of plant transpiration as a result of leaf damage, high respiratory activity, low photosynthetic activity as a result of a decrease in the amount of chlorophyll per leaf area and stomata damage, a decrease in chlorophyll efficiency and a decrease in leaf growth. Many more plant metabolic processes are inhibited due to virus attack (Mishra et al. 2020). Barreto d Silva et al. (2020) reported that yield losses depend on the cultivar ranging from 174 to 638 kg ha⁻¹.

Table 9. Comparison of agronomic characteristics of CpMMV-resistant and CpMMV-susceptible soybean genotypes

SV	df	NUP	NFP	NTP	NSP
Replication	2	0.6687	0.8984	0.3117	8.0207
Genotype	9	0.5393	3.605*	3.5135*	11.1543
Resistance (G1-G5 >> G6-G10)	1	0.1124	24.4587*	21.585*	82.7477*
R1 (G-DTF 44 >> G-DTF 45)	1	0.0441	2.615	1.9091	12.0861*
R2 (G-DTF 44 >> G-DTF 45)	1	0.1145	2.1274	2.2065	0.0846
R145 (G3 >> G5)	1	1.7195	0.5935*	1.3367*	0.9165
R244 (G6 >> G8)	1	0.4472	2.4384*	2.8154*	3.3765
R144 (G1, G2, G4)	2	0.1333	0.0664	0.1626	0.5852
R245 (G7, G9, G10)	2	1.0747	0.0398	0.7218	0.0033
Error	18	0.4056	0.6782	0.7736	4.5709
Total	29				

Note: *significant at α 0.05, SV: source of variation, df: degree of freedom, R1: resistant, R2: susceptible, G1: MLGG 0006, G2: MLGG 0106, G3: MLGG 0297, G4: MLGG 0315, G5: MLGG 0559, G6: MLGG 0123, G7: MLGG 0379, G8: MLGG 0603, G9: MLGG 0695, G10: MLGG 0796, DTF 44: days to flowering under 45 DAT, NUP: number of unfilled pods, NFP: number of unfilled pods, NTP: number of total pods, NSP: number of seeds per plant

Relationship between morphological, anatomical, and agronomic characteristics on CpMMV-resistant and CpMMV-susceptible soybean genotypes

Relationship among characters is crucial in plant breeding to choose an effective selection criterion. The research results on the response of morphological, anatomical, and agronomic characteristics of resistant and susceptible soybean genotypes CpMMV showed a relationship between morphological, anatomical, and agronomic characteristics with resistant and susceptible genotypes. The resistant soybean genotype's morphological, anatomical, and agronomic characteristics were better than the susceptible genotype's. Resistant soybean genotypes have relatively high stems, many branches, and larger leaf surfaces which cause a wider area of light absorption for the photosynthesis process, so they can produce more pods and seeds per plant than susceptible genotypes, although leaf thickness is thinner than susceptible genotypes. The number of stomata and trichomes of the resistant genotype was less than that of the susceptible genotype. This aims to reduce the amount of water released (evaporation) into the air so that the cells or leaf tissue do not lack water for photosynthesis. Amaliah et al. (2019) reported that the width of the stomata and the length of the trichomes, the number of stomata, and the width of the trichomes were positively correlated.

The anatomical structure of plants plays a vital role in the effects of the disease; the effects cannot be well understood without understanding the normal structure of the affected tissue. In addition, counteracting the effects of disease or parasites and even susceptibility to the disease itself can be revealed by changes in the structure of the specific host structure (Hidayat 1995). This change is called a symptom, which reflects particular in certain plants and has typical symptoms. The nature of the symptoms can be divided into local and systemic symptoms. Local symptoms are limited, while systemic symptoms occur throughout the plant body (Sutrawati et al. 2017).

The susceptible soybean genotype has relatively short stems, few branches, a small leaf surface area, and lots of

stomata and leaf trichomes, causing leaves to experience excessive water release and reduced photosynthetic speed. This resulted in the number of pods and seeds per plant being less than the resistant genotypes, although the tissues that made up the leaves experienced an increase in the number and size of cells. In addition, historical selection for high yield is associated with morphological modifications that support increased gop, leaf cooling, and photosynthesis in irrigated rice living in hot, high-light settings (Wu et al. 2020). Positive correlations were found between the traits of the seeds and those of the pods (Ningsih et al. 2019). The association between agronomic traits revealed strong positive correlations between the number of filled pods and the total number of pods, the seed's length with the seed's width, and the seed's thickness (Nurrohman et al. 2019). The number of filled pods, plant height, leaf length, leaf width, and the number of branches through the number of seeds per plant were further indicators of the strong positive indirect effect (Faot et al. 2019).

The morphological characteristics, primarily the stems and leaves of soybean, and the anatomical characteristics of the leaves of the resistant soybean genotype were related to their agronomic characteristics. Tall plant height, many branches, broad leaf surface, and a low number of stomata and trichomes caused the number of pods and seeds to be produced more than the susceptible genotypes. Faot et al. (2019) reported that the number of branches demonstrated a significant positive genotypic association with the seed yield, and 100 seeds weight had a positive phenotypic association with the seed yield. Therefore, the characteristics possessed by resistant genotypes can be used as a basis for obtaining superior varieties in soybean plants. However, Nurrohman et al. (2019) reported that the number of unfilled pods and pod thickness were shown to have a solid negative connection.

In conclusion, the resistant and susceptible genotypes differed significantly from one another. Resistant soybean genotypes had more branches, larger leaves, and relatively tall stems. The resistant genotype had fewer stomata and

trichomes than the susceptible genotype did. The resistant genotype had more complete pods, whole pods produced per plant, and seeds produced per plant than the susceptible genotype. The genotype MLGG 0695 was the most responsive because it had the fewest branches, the smallest leaves, stomata and trichomes on the leaf surface, the thickest leaves, and the fewest pods and seeds. The soybean genotype with the highest resistance was MLGG 0297, which has a somewhat tall stem.

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