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Molecular and morpho-physiological identification of yellow leaf curl disease of cucumber in Salatiga, Indonesia

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Abstract. *Kurnia TD, Purwantoro A, Sulandari S, Basunanda P, Setiawan AB, Fatmawati Y, Andika IP. 2022. Molecular and morpho-physiological identification of yellow leaf curl disease of cucumber in Salatiga, Indonesia. Biodiversitas 23: 1466-1474.* Plant viruses are a limiting factor in Indonesian cucumber production. However, because numerous viruses generate identical symptoms on infected plants across cucumber cultivars, identify the virus discovered in the field. The management of virus diseases on plants is dependent on accurately identifying symptoms associated with certain viruses among cucumber cultivars and evaluating possible insect vectors. Because there have been several reports of infected viruses on cucumbers, generating resistant cultivars, identifying phenotypic and physiological disorders, and probable insect vectors are all important aspects of plant disease management. This study aimed to determine the virus and putative insect vectors of yellow leaf curl disease and its symptoms, including morphology and physiology disorders in cucumber cultivars. Common symptoms found included curling, yellow spots, malformed shapes, mortality from severely infested leaves, yellowing, and malformed cucumbers, with occurrences varying among cucumber cultivars. Curling and yellow spots were found on >70% samples of all tested cultivars. Virus infection affected agronomic features and fruit characteristics differently depending on cucumber cultivars. Molecular detection confirmed that polerovirus infected plants and insects tested also carried the Polerovirus. This study provides initial information on monitoring various infection stages of yellow curling disease and potential insect vectors of this disease that will later be useful to synthesize effective management practice in the future.

Keywords: Agronomy, PCR, plant virus, polerovirus, vector

INTRODUCTION

Cucumber is a crop with high economic value, great market demand, and fairly constant pricing in Indonesia, making it a good crop for farmers (Kusumiyati et al. 2017). However, cucumber production in Indonesia steadily decreased from 2014 to 2019, from 477.971 to 435.975 tons in those respective years (Badan Pusat Statistik Indonesia 2021). Cucumber production is being hampered by plant disease, particularly plant viruses, in addition to a reduction in planting areas (Valarmathi et al. 2016). Numerous viral diseases have been observed to infect cucumbers, surpassing the ability of breeders to develop resistant cultivars, enabling these diseases to spread throughout Indonesian plantation areas.

The transmission of virus infections on cucumber is widespread and originates in several regions of Indonesia, including West Java, East Java, and Yogyakarta (Septariani et al. 2014). Mosaic disease, green mottle disease, yellow mosaic disease, ringspot disease, vein banding disease, and yellow leaf curl disease have been documented in Indonesia (Santoso et al. 2016; Liang et al. 2018; Listihani et al. 2018, 2020; Gadhave et al. 2019). Yellow leaf curl disease in cucumber has been caused by Tomato leaf curl New Delhi virus (Septariani et al. 2014). This virus is also associated with infection of squash mosaic virus (SMV), zucchini yellow mosaic virus (ZYMV), and cucumber mosaic virus (CMV). Laili and Damayanti (2019) reported that cucumber plants from West Java were infected by Papaya ringspot virus, Polerovirus, and Begomovirus. Begomovirus and Crinivirus are viruses transmitted by whiteflies (*Bemisia tabaci*) that are serious agricultural issues worldwide (Tzanetakis et al. 2013). In 2004, the total afflicted area in Indonesia owing to this yellow curl disease was 984.6 hectares, with yield losses ranging from 20 to 100 percent and an economic loss of 7.31 billion IDR (Gunaeni et al. 2008). In addition, Pandawani et al. (2018) revealed that no cucumber varieties were resistant to CMV in Bali, while Wiratama et al. (2015) showed that Begomovirus also induced yellowing symptoms.

Three strategies may be used to control virus disease: appropriately recognizing symptoms based on the infecting viruses, regulating virus vectors within agroecosystems, and employing resistant cultivar. Many viruses exhibit similar symptoms, making a precise visual diagnosis to be difficult; hence, investigations to identify specific symptoms of virus infection are critical. Another aspect of virus disease management is identifying and monitoring potential vectors and their transmission routes. Most plant viruses are transmitted by insects, like whiteflies (Okuda et al. 2013; Tzanetakis et al. 2013; Ghanim 2014; Janssen et al. 2016; Gadhave et al. 2019; Orfanidou et al. 2019), aphid

(Persley and Gambley 2009; Knierim et al. 2010; Kenyon et al. 2014; Amer 2015; Listihani et al. 2020) and thrips (Kenyon et al. 2014; Adachi-Fukunaga et al. 2020). Other disease spreading mechanisms involve seed transmission (Purba et al. 2017) or unhygienic mechanical handling (Abreu et al. 2015). Finally, one of the most effective approaches to managing virus disease is cross-breed resistant cultivars (Zhang et al. 2010). Therefore, collaborations between breeders and plant protection experts should be established in order to develop novel resistant viral cultivars.

The aims of this project were to: (i) identify symptom variations and virus effects on various agronomic aspects caused by yellow curly disease on different cucumber cultivars; (ii) identify disease incidence and intensity of yellow leaf curl disease on cucumber cultivars; and (iii) identify virus and putative insect vectors for yellow leaf curl disease in cucumber. The information obtained from this study will presumably give preliminary data for the development of management plans and future breeding attempts.

MATERIALS AND METHODS

Study location, initial infestation, and experimental settings

The field experiment was conducted in a greenhouse at Salaran, Kopeng Research Station of the Faculty of Agriculture and Business, Universitas Kristen Satya Wacana, Salatiga, Indonesia at an elevation of 750 m above sea level, while molecular identification was carried out in the Genetic Laboratory of the Faculty of Agriculture, University of Gadjah Mada, Yogyakarta, Indonesia. Cucumber plants were cultivated in polybags (35 cm diameter) using a soil: manure growth medium ratio of 1:1. Natural viral inoculation was accomplished by bringing a pre-infested cucumber plant from a yellow curl infected cucumber field into the greenhouse among recognized virus vectors such as whiteflies (*B. tabaci*), thrips (*Trips* sp.), and aphids (*Aphid* sp.). When the cucumber plants in the greenhouse were infested, 14 days after planting.

This study used four cucumbers (*Cucumis sativus* L.) cultivars, namely Ky1, Ky2, Ky3, and Ky4. Two greenhouses were made to separate plants with virus inoculation and control plants. Each greenhouse was divided into 3 blocks consisting of 4 different cucumber cultivars, which contained 10 plants for each cultivar. The morphology of symptoms, disease incidence, and disease intensity was examined on 3, 7, 14, 21, and 28 days after inoculation (DAI) using equations listed in Table 1. The disease severity scoring technique was carried out using Gunaeni et al. (2015)'s method, with slight modifications (Table 2). Then, the scores were used to determine Area Under Disease Progress Curve (AUDPC) and relative resistance level. The plant height and the number of leaves were measured 2-7 weeks after planting (WAP). The leaf area, chlorophyll content, total carotenoid, stomata, and trichome characteristics were examined at 5 WAP. The yield components such as the number of fruits, fruit length, and fruit diameter were observed until physiological maturity stage.

Chlorophyll and carotenoid measurement

Chlorophyll and carotenoid were extracted from fresh leaves ~~at~~ maximum vegetative stages when plants were about 8-weeks-old. In brief, 0.04 g of macerated leaves were put in a ceramic mortar. The mixture was then incubated in a dark room for 48 h with 5 mL dimethyl sulfoxide. The solutions were filtered through filter paper, and absorbances were measured with a spectrophotometer (UV mini-1240 UV VIS Spectrophotometer, Shimadzu) at 649 nm (A665) and 665 nm (A649). Chlorophyll content was determined as $\mu g.mL^{-1}$ using the following formulas:

Chlorophyll a = (12.19 × A665) - (3.45 × A649)

Chlorophyll b = (21.99 × A649) - (5.32 × A665)

Total chlorophyll = (18.54 × A649) + (6.87 × A665)

Stomata and trichome observations were made using a stomatal printing method and then observed under a stereomicroscope to quantify stomata dimension and density (Fauziah et al. 2019).

Table 2. Scoring was used to determine yellow curl disease intensity on cucumber cultivar

Score	Description
0	healthy plant, no symptoms
1	Plants show small number of yellow spots
2	Plants show mosaic symptoms and distinctive yellow spots
3	Plants show mosaic symptoms, distinctive yellow spots, and malformed organs
4	Plants show severe mosaic symptoms, many distinctive yellow spots, many malformed organs, and stunted

Table 1. Formula for calculating disease intensity, disease incidence, area under disease progress curve

Variable	Calculation
Disease intensity (DI)	$DI = \frac{\Sigma(nxv)}{NxV} \times 100\%$
Disease incidence (DInc)	$DInc = \frac{n}{N} \times 100\%$
Area Under Disease Progress Curve (AUDPC)	$AUDPC = \frac{\left(\frac{D1+D2}{2}xT\right)+\left(\frac{D2+D3}{2}xT\right)+\left(\frac{D3+D4}{2}xT\right)+\left(\frac{D4+D5}{2}xT\right)}{n-1}$

Notes: N: total number of plants observed; n: number of plants possessing a designated disease intensity score; V: highest score in scoring scale; v: designated score; D_n: observation period n; h: highest disease score during a period.

Table 3. Primers used for virus molecular identification

Target	Primer	Sequence (5'→3')	References
Polerovirus	Pol-G-F	(5'-GAYTGCTC YGGYTTYGACTGGAG-3')	Knierim et al. (2010)
	Pol-G-R	(5'-GATYT TATAYTCATGGTAGGCCTTGAG-3')	
Crinivirus	Crini Pol F	(5'-GCYCCSAGRGTKAATGA-3')	Ramirez et al. (2008)
	Crini Pol R	(5'-ACCTTGRGAYTTRTCAAA-3')	
Begomovirus	pAL1v1978	(5'-GCATCTGCAGGCCCACATYGTCTTYCCNGT-3')	Septariani et al. (2014)
	pAR1c715	(5'-GATTTCTGCAGTTDATRTTYTCRTCCATCCA-3')	

Sinaga (2003) method was used to determine the resistant levels of each cultivar by comparing AUDPC and DI values (Table 1). Resistance criteria based on DI are 0-25% classified as resistant, >25.1% classified as susceptible, and Resistance criteria based on AUDPC value are 1.0-100.0 classified as resistant, >100.0 classified as susceptible.

DNA and RNA isolation of plant and insect

Total DNA and RNA of plants were extracted from cucumber leaves using Genomic Plant DNA Mini Kit product (Geneaid) and Total Plant RNA Mini Kit (Geneaid) as per manufacturer's instruction. The total RNA of putative insect vectors consisting of whiteflies, trips, and aphids was isolated using Total Tissue RNA Mini Kit (Geneaid) according to the manufacturer's instruction. DNA was extracted to identify Begomovirus and RNA to identify Polerovirus and Crinivirus. For insect samples, DNA extraction was carried out using CTAB according to the method (Kandito et al. 2021). According to the manufacturer's instructions, the cDNA was synthesized from both plant and insect RNA (SensiFAST cDNA Synthesis Kit product Bioline). Total volume used on reverse transcript reaction was 20 µL consisting of 5 µL RNA, 4 µL 5x TransAmp Buffer, 1 µL Reverse Transcriptase and 10 µL DNase/RNase free water. PCR condition: 25°C 10 min (primer annealing), 42°C 15 min (reverse transcription), 48°C 15 min (for highly-structured RNA), 85°C 5 min (inactivation), 4°C hold (or chill on ice).

DNA amplification of a virus and a putative insect vector

The composition of the PCR with a total volume of 12.5 µL consisted of 6.25 µL GoTaq DNA polymerase (Promega), 0.5 µL primer Forward, 0.5 µL primer Reverse, 2.5 µL Nuclease Free Water and 2.75 µL DNA/cDNA. A virus causing yellow leaf curl disease in cucumber was identified using universal primer pairs of Begomovirus, Polerovirus, and Crinivirus (Table 3). PCR protocols used for Begomovirus identification consisted of incubation at 94°C for 5 min, 35 cycles of 94°C for 3 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 5 min. PCR protocols used for polerovirus identification consisted of incubation at 94°C for 3 min, 40 cycles of 94°C for 3 s, 61.5°C for 30 s, and 72°C for 90 s, followed by a final extension at 72°C for 5 min. PCR protocols used for Crinivirus identification consisted of incubation at 94°C for 2 min, 35 cycles of 94°C for 3 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 5 min. Extracted DNA and cDNA were visualized using electrophoresis on 1.5% agarose gel.

Statistical analysis

Cucumber cultivars' agronomic and yield characters were analyzed using a one-way ANOVA using glm in lmer package. Blocks were treated as random factors, and cultivars were set as fixed ones. Data were tested for normality and homogeneity assumptions using a Shapiro-wilk and Levene's test. Data that were not able to meet these assumptions were transformed. Data that were still unable to meet these assumptions were then analyzed using non-parametric tests, such as the Kruskal-Wallis test and a U-Mann Whitney post hoc test. According to the ANOVA test, data that met assumptions and showed significant differences were later tested using a Tukey HSD post hoc test. All statistical analysis was performed in R. 4.1.1 and used $\alpha = 0.05$ (R Core Team 2021).

RESULTS AND DISCUSSION

Symptoms occurrence, disease intensity, disease incidence, and AUDPC

Observations showed that various symptoms and their occurrences varied across cucumber cultivars used in this study (Figure 1 and Table 4). Symptoms consistently occurred in all cultivars were leaf curling, leaf malformation, leaf thickening, leaf, and vein discoloration, and the appearance of yellow or green spots on leaves. Meanwhile, stunting and vein bending only appeared on Ky1 and Ky3 for stunting, while vein bending only occurred on Ky1. Leaf curling and appearance of yellow or green spots were also symptoms that appeared the most across plant samples, appearing in >70% of samples across all cultivars, while leaf malformation, thickening, discoloration and vein discoloration only appeared on <30% of samples across all cultivars. Disease incidence and intensity observation showed that incidence and intensity stably increased over time, with Ky1, Ky2, and Ky3 showing higher disease incidence, intensity, and AUDPC values compared to Ky4 (Figure 2 and Table 5). Based on their AUDPC value, all cultivars were considered to be susceptible. However, Ky4 showed the lowest AUDPC indicating that Ky4 was able to resist virus infection and was supported by the low disease intensity. Although all cultivars were considered susceptible against virus infection, Ky4 still showed to be less susceptible among the others.

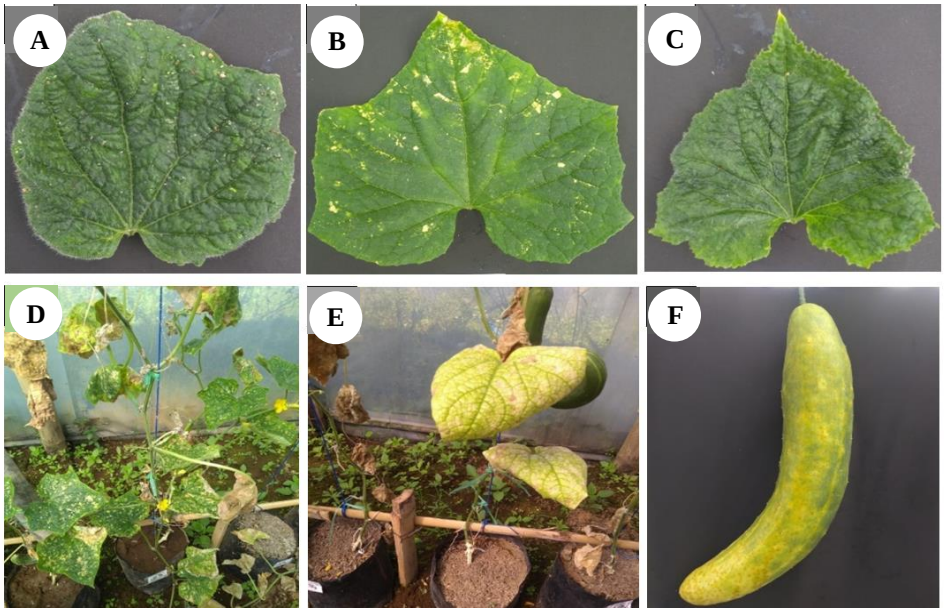

Figure 1. Symptom variations of yellow mosaic disease were observed on cucumber leaves infected by polerovirus. A. Curl; B. Yellow spots; C. Malformed shapes; D. Mortality from severely infested leaves; E. Yellowing; F. Yellowing and malformed cucumbers

This study's symptoms were similar to those caused by virus on cucumber plants reported in previous research. Chlorosis, yellowing, curly and wrinkled leaves were common symptoms found on cucumber plants infected with Begomovirus (Sanchez-Chavez et al. 2020). Cucumber plants infected with polerovirus also showed similar symptoms, including chlorosis, yellowing and vein banding (Listihani et al. 2020), while cucumber plants infected with Crinivirus showed yellowing, chlorosis and stunted growth (Orfanidou et al. 2019). Research by Kumari et al. (2021) tested plant samples showed several symptoms, such as mosaic, yellowing, mottling with chlorotic spots, leaf distortion, puckering, yellowing, vein clearing, upward leaf curling, necrosis, and stunted plant growth with reduction in leaf size for Begomovirus, Potyvirus, Tobamovirus, Cucumovirus, Polerovirus and Crinivirus infection, implying that these symptoms were closely related to viral infection. Based on symptoms of these cultivars and the literature of virus found in Indonesia, cucumber cultivars may be infected by Begomovirus, Polerovirus and Crinivirus; thus, further molecular identification using PCR was required to precisely identify the infecting viruses.

Molecular identification of yellow leaf curl disease

Molecular identification results using PCR is shown in Table 6, where 75 bp bands appeared on Polerovirus primer while Begomovirus and Crinivirus primer did not result in any bands. This indicated that samples tested were positively infected by polerovirus without double infection by Begomovirus or Crinivirus. Molecular identification of viruses from insect showed that all insects tested carried Polerovirus, Begomovirus, and Crinivirus. Unfortunately, amplification results from insect samples alone cannot precisely determine the vectors of each virus genus. To confirm whether or not an insect species is a virus vector, an exact bioassay is required where suspected insect species confirmed to be clean from tested virus are placed on infected plants and then transferred to uninfected plants as shown in research by Mituti et al. (2018). However, it does provide information on possible insect species and insights on the interaction between insects, viruses, and host plants (Alexander et al. 2014). To this date, research has stated that multiple viruses may infect plants or be carried within insects, but the virus genus that dominates later may change among ecosystems (Gutiérrez et al. 2010; Gil-Salas et al. 2012). Insects may provide selection on viruses that are transmitted to plants and are able to multiply within their bodies (Webster et al. 2018; Tian et al. 2019). Meanwhile, competition between viruses may also occur within plants that result in different dominating virus genus in infected plants and will later affect virus genus to be transmitted by insect vectors (Syller 2012). These results provide initial information for basic knowledge on insect-virus-plant interaction, which later is used in applied condition to protect plants from virus infection and further studies.

Table 4. Symptoms caused by yellow mosaic disease on four different cucumber cultivars

Cultivar	Symptom (%)							
	C	S	Mal	LT	VB	Dis	VD	YS
Ky 1	0.90	0.17	0.10	0.10	0.03	0.07	0.30	0.90
Ky 2	0.87	0.00	0.23	0.07	0.00	0.10	0.10	0.73
Ky 3	0.83	0.07	0.20	0.03	0.00	0.13	0.23	0.93
Ky 4	0.73	0.00	0.13	0.10	0.00	0.10	0.07	0.93

Notes: C: Curl; S: Stunt; Mal: Malformation; LT: Leaf Thickening; VB: Vein Banding; Dis: Discoloration; VD: Vein Discoloration; YS: Yellow Spots.

Table 5. Resistance level of different cucumber cultivar based on disease intensity (DI) and AUDPC

Cultivar	DI on 28 DAI	Resistant ability based on (DI)	AUDPC	Resistant ability based on AUDPC
Ky 1	76.67	Susceptible	302.92	Susceptible
Ky 2	51.67	Susceptible	285.07	Susceptible
Ky 3	78.33	Susceptible	313.26	Susceptible
Ky 4	27.50	Susceptible	208.99	Susceptible

Table 6. Electrophoresis results for testing Crinivirus, Polerovirus, and Begovirus from different cucumber cultivar and insect vectors

Test subject	Crinivirus		Polerovirus		Begomovirus		Ef-1alpha	
	+RT	-RT	+RT	-RT	+RT	-RT	+RT	-RT
Plant								
Ky1	-	-	✓	-	-	-	✓	-
Ky2	-	-	✓	-	-	-	✓	-
Ky3	-	-	✓	-	-	-	✓	-
Ky4	-	-	✓	-	-	-	✓	-
Insect								
Aphid	-	-	✓	-	-	-	✓	-
White-fly	-	-	✓	-	-	-	✓	-
Thrips	-	-	✓	-	-	-	✓	-

Molecular diagnostics indicated that symptoms found on cucumber plants were caused by virus infection. Decrease of photosynthesis activity has been reported to be due to down-regulations of several genes after viral infection on cucumber (Bengyella et al. 2015; Xia et al. 2017). Decrease of photosynthesis activity will later affect plant metabolic process and eventually result in stunting, especially on susceptible plants. Other symptoms, such as curling, can be caused by damaged palisade and mesophyll tissue where tissues are damaged possibly by insect feeding or shrinkage due to virus using plant nutrients for multiplying. During these conditions, plant cell organelle, such as chloroplasts and mitochondria; experience leakage, different sizes between cells, causing surface to be uneven and appearing to be curled or malformed (Khalil et al. 2014). This leakage leads to chlorophyll degradations, decreased photosynthetic activity, and virus infection may cause yellow spot symptoms to occur on leaves. Stunting did not occur on Ky 2 and Ky 4 cultivar implying that these cultivars were able to maintain plant growth compared to Ky 1 and Ky 3 cultivar. Results indicate that cultivar responded differently to virus infection.

Plant and fruit character response to virus infection across different cucumber cultivars

Infected cucumber plants will experience alteration of their morphological and physiological characters and results of this study showed this on certain measured characters. Cucumbers infected with polerovirus have been reported to experience yield decrease due to lower numbers of female flowers and inhibition of shoot regeneration which suppresses the increase in plant height (Vafaei and Mahmoodi 2017; Menzel et al. 2020). Plant height continually increased across all cultivars with Ky4 being the tallest cultivar (Figure 3) implying that it had better chances to produce higher yield than other cultivars because their ability to store photosynthetic products during vegetative stage for fruit filling. Ky4 showed significantly higher fresh weight, leaf area, and measured fruit features compared to other cultivars while other agronomic characteristics were not significantly different among cultivars. Although not having the densest stomas, stoma density of Ky4 showed to be not significantly different compared to other cultivars. Besides that, the number of leaves of infected plants was lower compared to healthy ones across all cultivars (Figure 3), which may decrease psychosynthesis produce and later yield eventually.

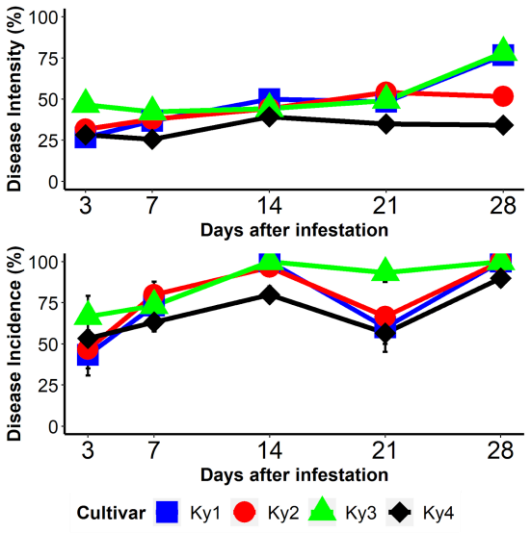

Figure 2. Incidence and intensity of yellow curly disease on four cucumber cultivars across different observation days

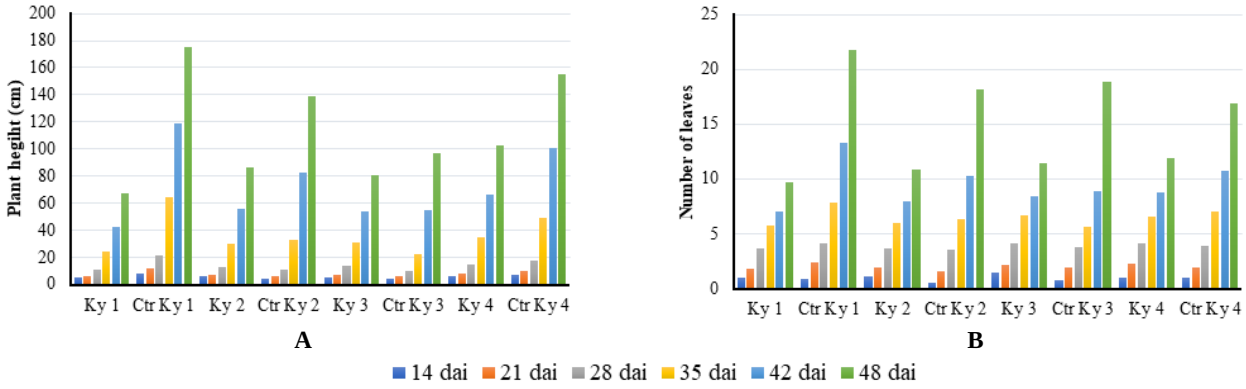

Figure 3. A. Plant height and B. number of leaves of different infected and uninfected cucumber cultivars at different observation periods

Number of leaves is a widely used parameter to determine productivity of cucumber cultivars due to its important tissues and roles for photosynthesis, plant growth, and fruit development. Cucumber cultivars with higher number of leaves will likely produce higher yield compared to cultivars with less leaves (Eifediyi and Remison 2010). At 48 DAI, number of leaves Ky1 to Ky4 were respectively 55.5%; 40.47%; 39.33%; 29.19% lower compared to the control plants. Ky4 had the lowest reduction of leaf numbers between the infected and uninfected plants. This result indicated that Ky4 was able to produce better vegetative growth compared to other cultivars. These results were consistent with research by Hamed Derbalah and Elsharkawy (2019) that demonstrated cucumber resistance against CMV can be increased by the application of nickel oxide nanostructures, which experience higher leaf numbers and plant dry weight. Resistant cultivars will have better growth due to higher photosynthetic rates. To our knowledge, this study is the first to examine plant height, number of leaves, chlorophyll and carotenoid content in different cucumber cultivars after virus infection and may be further indication of gene regulation in plants after virus infection.

Infested status and cultivar type responded differently depending on the measured characters (Table 7). In general, infested plants had significantly lower total fresh weight, leaf area, chlorophyll and carotenoid content compared to healthy plants among all cucumber cultivars while trichome length and diameter were only significantly lower on infested Ky1 and Ky2 cultivars. Stoma density was also only significantly lower on Ky2, Ky3, and Ky4 cucumber cultivars. Leaf area decreased by 69.63%, 59.86, 52.34%, 54.66%, respectively from Ky1 to Ky4 as plants were infected. These results indicated that insects and viral infected plants can inhibit growth by feeding on photosynthetic products or multiplying on the same substances (Gao et al. 2015). In general, plant mechanism in responding virus infection are similar, but the plant genes related to resistance may differ between virus species. Plants also respond to virus infections by showing hypersensitive response (HR), systemic acquired resistance (SAR) or alteration in gene expressions (Mandadi and Scholthof 2013). Response will later alter metabolic processes, such as formation of jasmonic acid (JA), salicylic acid (SA), which will increase plant defence against viruses. Therefore, specific alterations to this process require further examination. A study done by Wang et al. (2015), on *Nicotiana glutinosa* demonstrated that the RP01 gene had a role in Polerovirus resistance on this plant species indicating a further gene target to study in cucumbers.

Trichome density was not significantly different among all cultivars, but significant increases were found on Ky1 after virus infection. This shows that in general, virus infection does not affect trichome density of cucumber and is more correlated to infection of insect vectors although previous research has shown different results depending on focal crops. Research on cotton demonstrated that dense trichome was higher oviposition of white flies occurred (Grover et al. 2016). Meanwhile, soybean (*Glycine max*) leaves with lower trichome density experienced higher whiteflies infestation compared to leaves of cultivars with higher trichome density (Sulistyo 2016). Another example is beans (*Vigna unguiculata* ssp. *sesquipedalis*) used as trap plant of *Liriomyza trifolii* imagoes and resulted in lower feeding rates, walking and walking oviposition on leaves (Xing et al. 2017). However, less work has been done to correlate virus infection with trichome density.

Results from this study showed that trichome length of all cucumber cultivars tested, except Ky3, was significantly shorter when plants were infected with virus although no significant differences among cultivars were found. Trichome diameters were also no different among cultivars, but Ky1, Ky2, Ky3 possessed significantly smaller trichome diameters when plants were infected with viruses. Trichome developed from cells with the function to defend plants against biotic and abiotic stressors (Zhang et al. 2021). Gibberellin, cytokinin, salicylic acid, jasmonic acid, brassinolide have been reported to be related to the development of plant trichome and plant resistance against stressors (Wang et al. 2021). Results imply that stated hormones usually used for trichome development were allocated for plant defenses against virus infection.

Table 7. Mean ± SE of various plant and fruit characteristics from 4 infected cucumber cultivars

Variable	Treatment	Cultivar			
		Ky1	Ky2	Ky3	Ky4
Fresh weight (g)	Infested	2.68 ± 0.22aA	2.96 ± 0.16aA	3.25 ± 0.19aA	3.53 ± 0.15A
	Control	8.89 ± 0.55B	7.50 ± 0.45B	6.55 ± 0.31B	8.40 ± 0.59B
Leaf area (cm^2)	Infested	114.73 ± 11.34aA	129.51 ± 8.21aA	134.26 ± 7.61abA	161.96 ± 7.34bA
	Control	377.78 ± 25.92B	322.63 ± 17.26B	281.69 ± 8.95B	357.21 ± 23.91B
Chlorophyll A ($\mu g.ml^{-1}$)	Infested	1.12 ± 0.05A	1.21 ± 0.06A	1.03 ± 0.07	1.18 ± 0.04
	Control	0.63 ± 0.08B	0.67 ± 0.06B	0.68 ± 0.10	1.18 ± 0.05
Chlorophyll B ($\mu g.ml^{-1}$)	Infested	0.51 ± 0.04A	0.62 ± 0.06	0.51 ± 0.04	0.59 ± 0.05
	Control	1.13 ± 0.08B	0.35 ± 0.03	0.24 ± 0.04	0.57 ± 0.09
Total carotenoid ($\mu g.ml^{-1}$)	Infested	0.23 ± 0.01A	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.02
	Control	0.11 ± 0.03B	0.14 ± 0.02	0.19 ± 0.01	0.26 ± 0.03
Stoma density (mm^2)	Infested	148.35 ± 2.43a	138.35 ± 2.13bA	147.69 ± 3.05abA	143.21 ± 1.64abA
	Control	154.89 ± 1.63	159.62 ± 2.34B	161.05 ± 2.22 B	158.34 ± 1.74B
Stoma length (m)	Infested	32.25 ± 0.91	33.81 ± 0.95	33.19 ± 0.83	34.24 ± 0.71
	Control	35.74 ± 1.39	37.03 ± 1.55	33.88 ± 1.06	37.16 ± 2.19
Stoma diameter (µm)	Infested	9.41 ± 0.43A	9.33 ± 0.46A	9.71 ± 0.46A	9.47 ± 0.47A
	Control	7.21 ± 0.39B	7.34 ± 0.49B	8.2 ± 0.51B	8.89 ± 0.42B
Trichome density (mm^2)	Infested	2.46 ± 0.19A	2.13 ± 0.17	2.11 ± 0.13	2.58 ± 0.16
	Control	1.88 ± 0.20B	2.33 ± 0.24	2.26 ± 0.32	2.41 ± 0.27
Trichome length (µm)	Infested	280.02 ± 17.13A	278.03 ± 25.87A	300.73 ± 17.36	296.04 ± 20.52A
	Control	449.41 ± 44.83B	389.12 ± 46.04B	375.83 ± 49.07	417.21 ± 46B
Trichome diameter (µm)	Infested	37.57 ± 2.59A	45.61 ± 8.75A	39.37 ± 2.14A	39.54 ± 2.82
	Control	52.42 ± 5.51B	52.55 ± 4.91B	54.76 ± 5.24B	45.99 ± 5.32
Number of fruits	Infested	1.4 ± 0.16aA	2.07 ± 0.31abA	1.3 ± 0.13aA	2.07 ± 0.19bA
	Control	3.5 ± 0.48B	5.90 ± 0.55B	4.50 ± 0.48B	3.90 ± 0.31B
Fruit length (cm)	Infested	28.79 ± 1.28abA	28.73 ± 0.79abA	25.77 ± 1.00aA	30.18 ± 0.81bA
	Control	42.2 ± 0.69B	42.42 ± 2.02B	43.14 ± 1.43B	47.00 ± 0.63B
Fruit diameter (mm)	Infested	41.47 ± 1.46abA	44.77 ± 0.84aA	39.47 ± 1.40bA	44.20 ± 0.87abA
	Control	55.10 ± 1.07B	61.34 ± 2.79B	54.94 ± 1.86B	57.56 ± 1.29B

Note: mean ± SE followed by lowercase letters in the same row were significantly different based on Tukey HSD at α=0.05 while ones followed by uppercase letters in the same column and plant characteristics were different based on t-Test at α=0.05.

Implication for future plant breeding and plant protection

The results from this study demonstrated the different susceptibility levels of several cucumber cultivars against virus infection based on different agronomic aspects, the possible virus causing yellow curly disease, and possible insect vector of this disease. It also provides comparison of symptoms found between cucumber cultivars in the field and the virus causing these symptoms was confirmed using molecular techniques. Monitoring is an essential step in virus disease management and together with severity levels, determines management actions to take. These results help to provide practical identification knowledge of commonly found symptoms and possible insect vectors to monitor in the field. For virus disease transmitted by insects, management and monitoring insect populations are key actions as symptoms start to appear.

The results also provide suggestions for further breeding efforts. In general, Ky4 demonstrated to be less affected by virus infection based on severity levels and agronomic characteristics. This implies that Ky4 may have certain characteristics related to resistance against virus infections, requiring further studies to reveal these mechanisms, such as observing JA and SA content after virus infections.

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