AGRITROPICA
Journal of Agricultural Sciences

Content available at:
https://ejournal.unib.ac.id/index.php/jagritropica/index

ISSN (PRINT) 2621-217X ISSN (ONLINE) 2621-699X

Response of Cucumber (*Cucumis sativus* L.) Varieties Against TMV (*Tobacco mosaic virus*) Infection

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ABSTRACT: The tobacco mosaic virus (TMV) is a Tobamovirus that can infect cucumber plants. There is limited information about the response of cucumber varieties against TMV infection. Research was conducted to evaluate response of cucumber varieties to TMV infection using TMV isolates from Bogor. The evaluation of resistance to TMV isolates was conducted by sap transmission method using TMV isolates fom Bogor that propagated on tobacco as the source of inoculum. Variable observations covered the period of incubation (day after inoculation/DAI), the symptoms of the disease, disease incidence and disease severity. The presence of viral infection was observed through the accumulation of starch and inclusions bodies. Based on the results of the research, the TMV isolate was able to infect systematically all test plant with symptoms of which appear in the form of mosaic light to heavy with the incidence of the disease reached 100%. Response of cucumbers varieties to TMV isolate can be categorized into resistant (Bandana F1, Neptun, and Pandu), tolerant (Ethan F1), and susceptible (Vario F1).

Keywords: accumulation of starch, inclusions bodies, mosaic, sap transmission,

Reference to this paper should be made as follows :
Utomo, W. M., M. Sutrawati, and E. Supriyono. 2021. Endurance Test Five Varieties of Cucumber (*Cucumis sativus* L.) To Infection Virus TMV (*Tobacco mosaic virus*). *Agritropica: Journal of Agricultural Science.* 4 (2): 129-136. Doi: https://doi.org/10.31186/Jagritropica.4.2.129-136

INTRODUCTION

Cucumber plants (*Cucumis sativus* L.) are one of the fruit vegetables that are popular and widely consumed fresh by the people of Indonesia (Elsya, 2003). According to BPS (2015), cucumber production in Indonesia has fluctuated from 2009 until 2014, consisting of 583.139 tons, 547.141 tons, 521.535 tons, 511.485 491.636, and 477.976 tons. The decrease in cucumber production can be caused by a decrease in cucumber planting area from 2009-2014, which decreased from 58,578 ha to 56,009 ha, and the impact of pests and diseases.

Cucumber varieties have different resistance to pests and plant diseases (Sing *et al.*, 2005). Some viruses infect cucumber plants, including *Cucurbit aphid borne yellows virus* (CABYV), *Papaya ringspot virus* (PRSV), *Tobacco mosaic virus* (TMV), and Tomato leaf curl *New Delhi virus* (ToLCNDV) (Septariani *et al.*, 2014; Listihani *et al.*, 2018). TMV is a virus that is stable with heat inactivation achieves 93ºC. TMV can also infect healthy plants even though it has done dilutions up to 1:1,000,000 (Agrios, 2005). The transmission of TMV can occur through seed inoculation mechanical and the contact between plants but can not be transmitted via a vector (Alishiri *et al.*, 2013).

TMV was first reported to infect the plant cucumbers in Iran, with the Incidence of the disease amounted to 8.1% (Alishiri *et al.*, 2013). In Indonesia, TMV infection on cucumber was first reported in

Java (Listihani *et al*., 2018). The symptoms caused by TMV infection are mosaic bright green to dark, malformation of leaves, chlorosis, wrinkles, and vein banding (Rifqi, 2019). The development of disease symptoms on the plants is significantly affected by the vulnerability of the plant host, the virus strain, the environment, and humans (Agrios, 2005).

Until now, this has not been found virucidal to suppress the virus's spread in the field. The necessary treatment in prevention so that the plants are not infected with the virus is by using resistant varieties. There have not been many reports of cucumber varieties resistant to TMV infection, so it is necessary to research to evaluate the resistance response of several cucumber varieties to TMV infection and the effect of TMV infection on cucumber plant growth.

MATERIALS AND METHODS

This research was conducted from October to December 2020 in the Laboratory of Plant Protection, Faculty of Agriculture, University of Bengkulu, using Split Plot Design, inoculation treatment as the main plot, and varieties as a subplot. Treatment consists of P0 = Inoculation with buffer and P1 = inoculation with TMV isolates Bogor. The varieties consist of V1 = Bandana F1, V2 = Neptun, V3 = Pandu, V4 = Ethana F1, V5 = Vario F1.

As a source of TMV inoculum, tobacco leaves were ground in a sterile mortar and then mixed with phosphate buffer 50 mL. Inoculation on plant tests on the cotyledons of plants dusted with carborundum 600 mesh. Sap plant spread right on surface of the cotyledons as much as three times with the direction of the leaf from the leaf base to the tip of the leaf after it is watered with distilled water. Then the plants are kept in the room light-tight.

The observation of loss of inclusion is performed by making an incision in thin sections of bone were observed using a light microscope (100×10). While the starch accumulation test was carried out by taking the cotyledons 7 days after inoculation (DAI) and then putting them in a glass cup containing 70% ethanol, heated using a hotplate at 80°C until the chlorophyll in the cotyledons dissolved. Then the cotyledons were taken using tweezers to drop the tryphan blue solution and incubated for 15 minutes. Furthermore, the cotyledons were washed using distilled water and observed for starch accumulation.

Observation in the form of high plants in 7-14 days after inoculation (DAI), the number of leaves on 7-14 DAI, the level of green leaves on a 7 to 14 DAI, the Incidence of the disease, and the disease severity. Disease incidence using the following formula:

$$\text{Disease Incidence} = \frac{n}{N} \times 100\%$$

Description: DI = Incidence of disease; n = the number of symptomatic plant; N = Number of plants observed. As well as carried out the calculation of the severity of the disease based on the scoring (Fraser *et a*l., 1980)

$$Disease\ Severity = \frac{\Sigma(\mathrm{n} \times \mathrm{z})}{\mathrm{N} \times \mathrm{Z}} \times 100\%$$

Description: DS = severity of the disease, n = the number of plants on the score to the-i, z = the Value of the score of the disease, N = Number of plants observed, Z = highest score.

Scoring	Criteria
0	No symptoms
1	Symptoms of a mosaic of light (= 30% of leaf area)
2	mosaic Symptoms with spots of light green/ dark green on the young leaves (from 30.5 up to 60% of leaf

	area)
3	mosaic Symptoms weight with freckles clearly, the dwarf and the distortion on the top of the plant (>61% of the leaf area)

The observations in the form of inclusion bodies, incubation symptoms, starch accumulation, disease incidence, and disease severity were analyzed descriptively. While data on plant height and the level of greenish leaves carried out statistical analysis using the F test at level 5% if different real continued with the test least significant difference at the level of 5%.

RESULTS AND DISCUSSION

The Symptoms of the Disease

The incubation period is the time required for the pathogen to cause symptoms in plants. The average incubation period of five varieties of cucumbers is 7,3 - 9,6 day after inoculation (DAI). The incubation period for Vario F1 and Ethana F1 cucumber varieties was shorter than Bandana F1 and Neptum varieties. The difference in incubation period for each variety is closely related to the response of plants to viral infection and the success of the virus is multiplying in plant tissues.

Cucumber plants show typical symptoms of TMV infection such as dark green and bright green mosaic, and chlorosis on the vein or vein clearing and vein banding on Bandana F1, Neptun, Ethana F1, and Vario F1. Symptoms appear only mild mosaic and vein banding on Pandu varieties (Figure 1). According to (Agrios, 2005) the development of disease symptoms in plants is strongly influenced by the host's susceptibility, virus strain, environment, and humans in the selection of planted varieties.

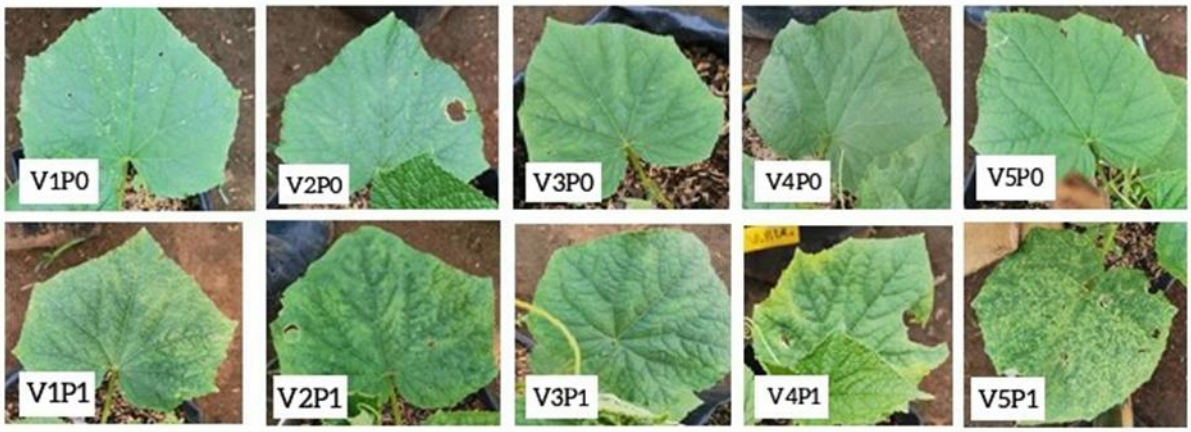

Figure 1. The symptoms on cucumbers varieties are not inoculated with TMV (V1P0, V2P0, V3P0, V4P0, V5P0) and inoculated with TMV (V1P1, V2P1, V3P1, V4P1, V5P1) 14 DAI. V1=Bandana F1; V2=Neptun; V3=Pandu; V4=Ethana F1; V5=Vario F1

The Inclusion Bodies of TMV on Tobacco

Observation of inclusion bodies was carried out on tobacco plants as sources of TMV inoculum. In tobacco leaves infected with TMV, inclusion bodies were formed in the cytoplasm consisting of virus particles. Light microscopy can observe these intracellular inclusions even though they are not stained. This observation found crystal inclusions at 10 x 40 from cucumber leaves, proving that the TMV isolate used contained TMV (figure 2).

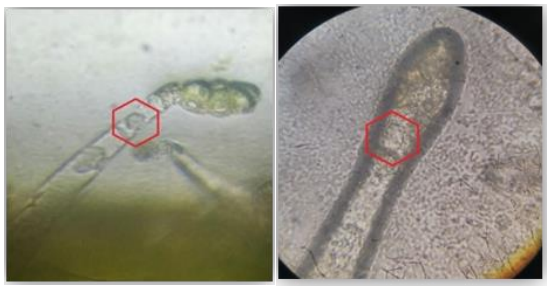

Figure 2. The inclusion bodies of TMV on tobacco leaves using a light microscope at a magnification of 10 x 40 (400x)

Starch Accumulation on Cotyledon

Based on the observation of cucumber cotyledons, the highest starch accumulation was observed on the cotyledons of Bandana F1, Neptun, Ethana F1 varieties. While the least starch accumulation in Vario F1. Starch lesion became evident under the light microscope seven days after inoculation. In viral diseases, starch accumulation in TMV-infected cucumber cotyledons, where no necrosis occurs, may indicate that changes associated with the chloroplast and not cell necrosis are involved in starch accumulation (Cohen & Loebenstein, 1974).

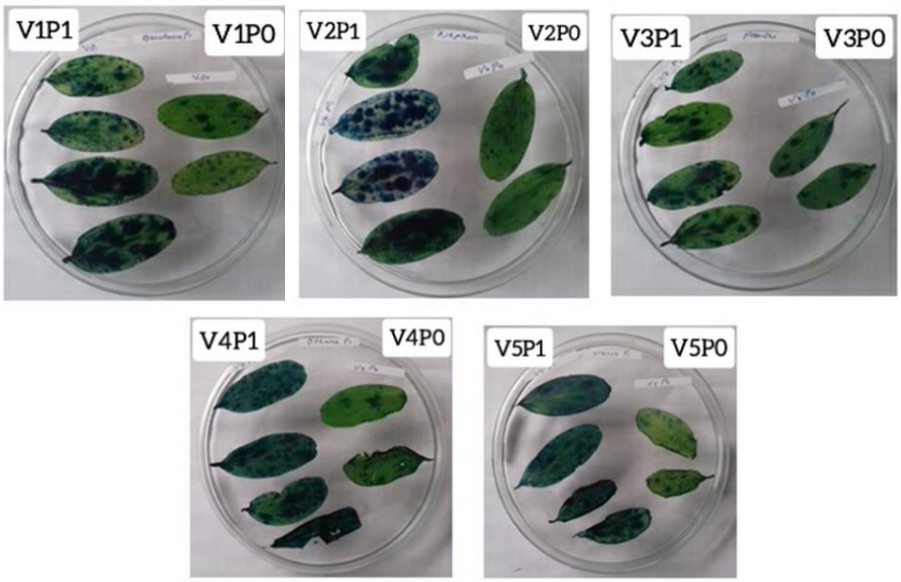

Figure 1. Starch accumulation on cotyledons of cucumber; not inoculated with TMV (V1P0, V2P0, V3P0, V4P0, V5P0) and inoculated with TMV (V1P1, V2P1, V3P1, V4P1, V5P1); V1=Bandana F1; V2=Neptun; V3=Pandu; V4=Ethana F1; V5=Vario F1

Plant Growth

The results of data analysis showed that the Inoculation of TMV significant effect on plant height. Plants inoculated with TMV showed lower plant height than plants not inoculated with TMV. According to (Agrios, 2005 the activity of the pathogen will disrupt the hormonal system of plants, which cause a growth response that is not normal. Nurhayati (1996) reported the infection of the virus could inhibit the growth of plants.

Table 1. The Results of The Analysis of Inoculation on Plant Height

Treatment	average plant height (cm)
Non inoculated	33,19 b
Inoculation with TMV	26,32 a

Description: the numbers followed by the same letter are influential, not real on the least significant difference level of 5%

The average height of plants in the treatment of inoculation buffer that 33,19 cm, while the average size of plants

inoculated TMV that 26,32 cm with a resistance value of 20,45%. According to (Sastrahidayat, 1990), the virus can cause a decrease in the amount of the compound regulator of the plant by increasing the compounds inhibiting growth.

Leaf Greenness Level

The results of data analysis showed that the virus inoculation had a significant effect on the greenness of the leaves. The greenness of plants inoculated with TMV leaves was lower than that of plants not inoculated with TMV.

Table 2. Leaf greenness in TMV-infected and healthy plants

Treatment	Leaf greenness level (%)
Inoculation with buffer (negative control)	46,46 b
Inoculation with TMV	39,14 a

Description: the numbers followed by the same letter do not affect real on the BNT level of 5%.

Goodman et al. (1986) reported that in addition to causing a decrease in leaf width, viral infection, in general, will reduce the amount of chlorophyll and malformation of the chloroplast shape, which disrupts the photosynthesis process in plants. The analysis results showed that the diversity of varieties significantly affected the level of the greenness of the leaves.

Table 3. The results of the analysis of the influence of varieties on the level of greenish leaves

Varieties	Average of leaf greenness level (%)
Bandana F1	46,38 b
Neptun	47,5 b
Pandu	42,13 a
Ethana F1	41,05 a
Vario F1	36,96 a

Description: the numbers followed by the same letter do not affect real on the BNT level of 5%.

Varieties of Neptun has the average level of greenish leaves of the highest, was not different with varieties Bandana F1 but different with varieties of Pandu, Ethana F1 and Vario F1. Agrios (2005) generally states viruses cause a decrease in photosynthesis by decreasing the amount of chlorophyll.

Disease Incidence dan Disease Severity of TMV Infection

All varieties of cucumber plants mechanically inoculated with TMV showed viral infection with disease incidence up to 100%. The presence of symptoms in plants that were not mechanically inoculated with TMV was thought to be due to infection with other viruses by vectors in the screen house.

Table 4. Disease incidence of TMV infection on cucumber varieties

Treatment	Disease Incidence (%)				
	Bandana F1	Neptun	Pandu	Ethana F1	Vario F1
Inoculation with buffer (negative control)	0	0	33,33	66.66	66.66
Inoculation with TMV	100	100	100	100	100

Description: the greater the percentage of the Incidence of the disease, the more susceptible to viral infections

In the treatment of inoculation of TMV, the Incidence of the disease in five varieties of cucumbers amounted to 100%, which indicates that the five varieties of cucumbers are tested relatively susceptible to TMV infection. Hadiastono (2001) stated

that the virus's movement and spread in the plant would occur if compatibility between the virus and its host. The success of the infecting depends on the virus in the host plant that can move from one cell to another cell and reproduce themselves in most or all cells that passed to give rise to symptoms. The symptoms of the disease appear if the strain of the virus that attack is virulent, the plants are attacked vulnerable and environmental conditions favour the development of disease (Akin, 2006).

Table 5. Disease severity of TMV infection on cucumber varieties

Inoculation	Disease Severity (%)				
	Bandana F1	Neptun	Pandu	Ethana F1	Vario F1
Inoculation buffer	0	0	22,22	44,44	55,55
Inoculation of TMV	66.66	55,55	of 66.66	of 66.66	88,88

Description: the greater the percentage of the severity of the disease, the more susceptible to viral infections

In the treatment of inoculation of TMV on the severity of the disease, five varieties of cucumber, cucumber varieties Vario F1 have the severity of the disease is the highest in 88,88% while the cucumber varieties Neptun has a severity level of disease low that 55,55%.

Control of viral diseases is difficult so that control can be done to do prevention happened tohis viral infections by using resistant varieties. Plant varieties susceptible to the virus TMV are allegedly due to genetic factors such as plants. The plant metabolism is disturbed and cause damage to the cucumber plants. Resistance of a variety of plants is affected by the properties of each variety, the environment, and the virus's ability in infecting the plant (Bos, 1990).

CONCLUSION

Cucumber plants show typical TMV infection symptoms such as dark green and bright green mosaic, vein banding, and chlorosis. Plants inoculated with TMV showed lower plant height than plants not inoculated with TMV. Cucumber varieties Bandana F1, Neptun, Ethana F1, Pandu and Vario F1 are susceptible to TMV infection with disease incidence up to 100%. The highest starch accumulation was observed on the cotyledons of Bandana F1, Neptun, Ethana F1 varieties. TMV infection reduced leaf greenness level on cucumber leaves.

REFERENCE

Agrios, G.N. 2005. Plant Pathology. 5th edition.): Elsevier Academic Press, New York (US).

Akin, H. M. (2006). Plant Virology. Kanisius, Yogyakarta.

Alishiri A, Rakhshandehroo F, Zamanizadeh HR, Palukaitis P. (2013). Prevalence of *Tobacco mosaic virus* in Iran and evolutionary analyses of the coat protein gene. *The Plant Pathology Journal*, 29:260-273.

The Central Bureau Of Statistics. (2015). The production of vegetable crops in Indonesia tahun 2010-2014. The Central Bureau Of Statistics Of The Republic Of Indonesia, Jakarta.

Cohen, J., G. Loebenstein. (1974). An Electron Microscope Study of Starch Lesions in Cucumber Cotyledons Infected with

Tobacco Mosaic Virus. Phytopathology 65: 32-39.

Goodman, R. N., Z. Kiraly, K. R. Wood. (1986). The Biochemistryand Physiology of PlantDisease. University of Missouri Press, Columbia.

Hadiastono, T. (2001). Virology Growan Basic. Universitas Brawijaya, Malang.

Listihani, S H Hidayat, W Wiyono, and T A Damayanti. (2018). First Report of Tobacco Mosaic Virus on Cucumber [(*Cucumis sativus (*L.)] in Java Indonesia. Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Indonesia.