International Research Journal of Engineering, IT & Scientific Research
Available online at https://sloap.org/journals/index.php/irjeis/
Vol. 3 No. 6, November 2017, pages: 68~76
ISSN: 2454-2261
https://sloap.org/journals/index.php/irjeis/article/view/11

Resistance Test of Several Varieties and Critical Phase for Cucumis Sativus towards Cucumber Mosaic Virus Infectio

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Article history:

Received: 9 July 2017
Accepted: 18 September 2017
Published: 30 November 2017

Keywords:

critical phase;
cucumber mosaic virus;
cucumber varieties;
cucumber;
resistant varieties;

Abstract

The present study was intended to obtain resistant cucumber varieties and to know the critical phase of cucumber plant towards Cucumber Mosaic Virus infection (CMV) therefore, it could be used as a basis for controlling the mosaic disease spread of CMV-induced at cucumber plants. The research was conducted in Baturiti Village Tabanan Bali within an insect-proof greenhouse. Elisa test was performed using CMV, WMV and PRsV antiserum. The resistance test of several cucumber varieties included Roberto, Harmony, Citra baby and Manggala was conducted by mechanical transmission from CMV inoculum source sap. Testing of cucumber plant critical phase of CMV infection was done by mechanically inoculating sap from CMV infected plant, for healthy cucumber plant as testing plant i.e. inoculation at 2, 3, 4, 5 and 6 weeks after planting and control (plant without CMV inoculation). An observation was done for incubation period variables, disease incidence, disease severity and crop yield. The results and conclusions of the four cucumber varieties tested were not found to be CMV resistant varieties, however, the moderately sensitive Harmony varieties deserve attention for choosing in the cultivation. The cucumber plant that was CMV infected from the age at planting until 4 weeks after planting shows 100% disease incidence and plants could not produce fruits. The critical phase of cucumber plant towards CMV infection occurred from the time at planting until 4 weeks after planting.

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1. Introduction

The one issue on the cucumber cultivation in order to achieve a high production is the diseases caused by a complex mosaic virus. The viruses types that can attack cucumber plants are generally Cucumber Mosaic Virus (CMV), green mottled virus (Cucumber Green Mottle Mosaic Virus = CGMMV), mosaic pumpkin virus (Squash Mosaic Virus = SqMV), cucumber necrosis virus (Cucumber Necrosis Virus = CNV), Zukini virus of yellow mosaic (Zukini Yellow Mosaic Virus = ZYMV), and Watermelon Mosaic Virus (WMV), singly or combined with the virus symptoms above, there is similarity with each other that leaves yellow mosaic, leaf veins such as nets, leaves, and fruits deformed (Yu *et al.,* 2006; Bananej & Vahdat, 2008).

The cucumber plants have infected the virus, the fruit becomes deformed and the size becomes small, and the loss of crops can reach 70% (Muller *et al.,* 2006). The virus-borne disease control is often done through eradication of infected host plants and vector control using pesticides. It is to show that the results are less effective and need to be repeated every time as increasing production costs (Provvidenti, 1986). The difficulties existence in the virus encourages control the effort to get a cucumber plant that resists viruses as problem-solving. Using resistant varieties is the most effective, efficient, easy to apply and can be combined with other control techniques (Melton, 1998; Hadiastomo, 1998).

Based on the research of Balitsa (2006), it is concluded that the loss due to virus attack can range from 10% to 90%, depending on various aspects related to how the cultivation pattern is conducted. The viral disease incidence in the cucumber plants can reach 100%, with varying intensity of attack (Sumpena, 2012). The loss due to infection depends on the infection time and can result in 100% yield loss (Babadoost, 2012). The viral infections incidence at different plant ages, suggesting a different response to the disease severity. The disease severity is strongly influenced by the growth phase and the plant age. The plants are very susceptible to viral infections at young plant life, and have an effect on the high disease incidence, because when the young plants are infected with viruses, the virus incubation period is shorter, and the process of virus distribution and translocation will accelerate (Akhtar *et al.,* 2004; Mandal *et al.,* 2007).

The study was intended to obtain the resistant cucumber varieties and in order to know the critical phase the cucumber plant towards Cucumber Mosaic Virus (CMV) infection, therefore, it can be used as a basis for controlling the CMV spread induced mosaic disease in the cucumber plants.

2. Materials and Methods

The research was conducted in Baturiti Village, Tabanan Bali in an insect-proof greenhouse. Elisa test was performed using CMV, WMV and PRsV antiserum. The positive plant samples were infected with a certain virus if the absorbance result at 405 nm wavelength with *Elisa reader* has a value 2 times greater than negative control value of the healthy plants (Matthews, 2002). The selected isolates detected cucumber plant showed a positive reaction to CMV antiserum, then isolated. The isolation was carried out by mechanical inoculation in Chenopodium amaranticolor and then the isolates obtained were reproduced on the healthy cucumber plants. The inoculum source propagation was carried out mechanically following the standard method of Dijkstra & de Jager (1998). The virus-infected leaf is crushed in a sterile mortar with phosphate buffer (0.01 M; pH 7.0) with a ratio 1 g virus-infected leaves per 5 ml phosphate buffer solution (1: 5 b / v). This juice is immediately inoculated into the healthy plants on the two youngest leaves. The symptomatic plants were used as an inoculum source in the implementation for varietal and subsequent critical stress tests.

The resistance test for several cucumber varieties i.e. Roberto, Harmony, Citra baby and Manggala was done by mechanical transmission from CMV inoculum source sap. The test stages were carried out as follows: the cucumber seeds planted in pots containing sterilized growing media mix (soil and charcoal husk with ratio 1: 2). Each variety was prepared 12 pots, 3 pots as control (without inoculation) and 9 pots inoculated CMV at 3 weeks after planting. The cucumber plant reaction against the virus was evaluated according to the scale proposed by Dolores &Valdez (1988) for the squash on WMV with a slight modification of scoring used:

(0): plants do not show mosaic symptoms;
(1): plants show an exhibit is a very mild mosaic symptoms;
(2): the plants show moderate mosaic symptoms but the leaves do not show transformation;
(3): the plant show exhibits symptoms of medium mosaic and on its leaves shape change or twisting;
(4): plants show heavy mosaic symptoms and on the leaves, many occur leaf shape changes.

The disease severity was calculated on each test crop with the score category. The score measured value was converted to disease severity based on the Townsend & Heüberger (1974) formula in Agrios (2005):

$$KP = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

n = number of plants with i-score
v = value of disease score
N = number of plants observed
Z = highest score

Based on the severity of the disease the plant population, the cucumber plant resistance level to CMV is grouped as follows:

Table 1
Resistance rate of cucumber to CMV

Rate of resistance		Intensity of virus symptom
Immune	(I)	0%
High resistant	(ST)	0 - 10%
Resistant	(T)	10 - 25%
Moderate resistant	(TS)	25 - 40%
Moderate sensitive	(PS)	40 - 50%
Sensitive	(P)	50 - 70%
High sensitive	(SP)	> 70%

The critical phase test for cucumber plant on CMV infection was done by mechanically sap inoculation from CMV infected plant, the healthy cucumber plant as a test plant. The test plants were classified into five age levels at CMV inoculation: inoculation at 2 weeks, inoculation at 3 weeks, inoculation at 4 weeks, inoculation at 5 weeks and inoculation at 6 weeks after planting and control (CMV inoculation without CMV inoculation). The observations were made for incubation period variables, disease incidence, disease severity and crop yield.

3. Results and Discussions

3.1 CMV Inoculum Source

The test result of the serological sample from seven cucumber planting centers was obtained result that one negative sample, two samples showed positive infected with three types of CMV, WMV and PRsV virus; three samples showed double CMV and WMV infection, and eight samples infected CMV. Regarding serology test results showed that 42.85% samples were infected CMV combined WMV and PRsV infection and 57.15% samples infected a single CMV (Table 2). Based on cucumber symptoms and viral detection ELISA, positive plant samples infected CMV showed symptoms of yellowish green mosaic on the young leaves, as well as striped symptoms with blistered or curved leaf surfaces, unlike bowls. The leaves are spiraled and in certain parts, the leaf lamina does not normally grow. The positive samples infected a single CMV was isolated and used as a source of inoculum in the next test (Figure 1).

Table 2
Serological test results cucumber plant samples from several cucumber planting centers

Sample Source From	Specific Antiserum		
	CMV	WMV	PRsV
Br. Batusesa 1	+	_	_
Br. Batusesa 2	+	+	+
Ds. Taman Tandan 1	+	_	_
Ds. Taman Tandan 2	+	+	_
Br. Pacung 1	+	_	_
Br. Pacung 2	+	_	_
Ds. Apit Yeh 1	+	_	_
Ds. Apit Yeh 2	+	+	_
Br. Munduk 1	_	_	_
Br. Munduk 2	+	_	_
Br. Titigalar 1	+	+	+
Br. Titigalar 2	+	_	_
Ds. Pekarangan 1	+	_	_
Ds. Pekarangan 2	+	+	_

Description:
(+) : Positive reaction to specific antiserum
(-) : negative reaction to specific antiserum
Br. : Banjar (village section)
Ds. : Village

Figure 1. Mosaic symptoms on the positive cucumber plant infected CMV

3.2 Variety Resistance

The disease incidence four cucumber varieties tested from ELISA test showed that no varieties were resistant to CMV infection. The varieties tested showed the incidence percentage different diseases, 77.77% in V. Roberto, 77.77% in V. Baby Citra, 66.66% in V. Harmony and 88.88% in V. Manggala. Similarly, the incubation period varies on every variety i.e. 11.00 days on V. Roberto; 10.42 days on V. Baby Citra; 11.83 days on V. Harmony and 11.25 days on V. Manggala. The test results are shown in Table 3.

Table 3
Test results for cucumber varieties with CMV inoculation at 3 mst

No. sample	V. Roberto		V. Baby Citra		V. Harmony		V. Manggala	
	CMV Reaction	Incubation period	CMV Reaction	Incubation period	CMV Reaction	Incubation period	CMV Reaction	Incubation period
1	+	10	+	11	+	12	+	10
2	-		+	10	+	11	+	11
3	+	11	+	10	-		+	10
4	-		+	12	+	13	+	10
5	+	11	+	11	-		+	10
6	+	12	-		-		-	
7	+	11	-		+	11	+	10
8	+	11	+	10	+	11	+	11
9	+	11	+	10	+	13	+	10
Control 1	-		-		-		-	
Control 2	-		-		-		-	
Control 3	-		-		-		-	
Positive reaction CMV (%)	77,77		77,77		66,66		88,88	
Incubation period average (day)	11,00		10,42		11,83		10,25	

Description:
(+) : positive reaction to CMV antiserum
(-) : negative reaction to CMV antiserum

The cucumber varieties were not found to be resistant to CMV infection, but the resistance level for all four varieties tested (Table 4.) was found. Harmony varieties, including moderate sensitivity groups the varieties of Roberto and Baby Citra including the sensitive and Manggala varieties included, are extremely sensitive. However, Harmony varieties need attention to be cultivated by combining other cultivation techniques in mosaic disease control, because according to Melton (1998) and Hadiastomo (1998) using resistant varieties is the most effective, efficient, easy to apply and can be combined with other control techniques.

Table 4
Disease severity and resistance level of cucumber varieties to CMV

Cucumber Varieties	Disease Severity (%)	Resistance Level
Harmony	47,50	Moderate Sensitive
Roberto	65,00	Sensitive
Baby Citra	67,50	Sensitive
Manggala	72,50	High Sensitive

3.3 Critical Phase

CMV infection at age of the different Zukini plants affect is to the incubation period, the incidence and disease severity. If infection occurs at an older age 5 weeks and 6 weeks after planting, the incubation period the virus slows, the disease incidence is low and the disease severity is also low when to be compared with the infection that occurs at a younger age (Table 5).

An infection is occurred in plants at 2 to 4 weeks after planting resulted in an incubation period ranging from 10.80 days to 12.80 days and the disease incidence reached 100 percent. The infections that occur in older plants that are 5 weeks old after planting gives a slower incubation period to the virus is 16.50 days, and resulted in the disease incidence is 60%. The incubation period is closely related to the ability the virus to spread from local infection to other parts plant and then show symptoms. Viruses are able to spread to young plants quickly due to the young plants do not yet have a strong defense system against viral infections (Agrios, 2005).

ZYMV infection on different plant ages, affecting the severity of the disease in plants, i.e. the younger CMV-infected plant is the higher of disease severity. The results of this study indicate that the disease severity is 74.50% in plants when infected 2 weeks after planting and decreases 37% in infected plants 6 weeks after planting (Table 5). These results indicate that if infection occurs earlier then the severity of the disease is higher. Infections from the virus can cause a decrease in the amount of chlorophyll a, chlorophyll b, carotenoids, carbohydrates, proteins, and amino acids, then the percentage decrease is increasing, along with the increasing age for plants that have been infected with the virus (Hemida, 2005). CMV infection in the younger plant age leads to a decrease in chlorophyll earlier than infected plants at older ages, so that in the young plants it affects the decrease in the amount of chlorophyll and resistance system against viral infections, furthermore causing higher disease severity in the young plants due to it does not yet have a strong resistance system, when the infection occurs from the virus (Hull, 2002).

Table 5
Incubation period, disease incidence, disease severity and cucumber plant yield infected CMV

Age at inoculation *	Incubation period **	Disease incidence (%)	Disease severity (%)	Resistance Level	Decrease Results (%)
2	10,80	100	74,50	High sensitive	100
3	11,30	100	69,50	Sensitive	100
4	12,80	100	67,00	Sensitive	100
5	16,50	60	49,50	Moderate Sensitive	44,25
6	17,30	50	37,00	Moderate resistance	15,35

Description:
* : week after planting
** : day after inoculation

CMV infection occurred at the different plant lives affects to plant resistance level and cucumber yield. Viral infections occurred at the age of 2 to 4 weeks after transplanting, show a susceptibility response sensitized to extremely sensitive level. The disease severity is strongly influenced by the growth phase and age of the plant when infected by the virus. Plants are highly susceptible to infection by viruses at young plant life, which has an effect on the high disease incidence, because when the young plants are infected with viruses, the incubation period virus becomes shorter and the process of virus distribution and translocation will accelerate (Akhtar *et al.,* 2004; Mandal *et al.,* 2007). The younger CMV cucumber plant is infected, the more plant is unable to produce fruit, compared with plants infected with the virus at an older age. CMV infection in plants 2 to 4 weeks after planting causes the plant to produce fruit. This result occurs because, CMV infection in the young plants, has an effect on the decrease level on the plant chlorophyll, where the decline of plant chlorophyll occurs earlier so that in the young plants infected with CMV fruit formation process will be disrupted and even no fruit formation process occurs. CMV cucumber plants infected by CMV at 5 to 6 weeks after planting can still produce fruit, although a decrease in yield is 44.25% and 15.35% healthy plants without CMV infection.

4. Conclusion

The results of the present study can be concluded:

1) Among the four cucumber varieties tested are Roberto, Baby Citra, Harmony, and Manggala varieties. CMV resistant varieties are not found, but the moderately sensitive Harmony varieties should receive attention for choosing in cultivation.

2) CMV infected cucumber from age at planting until age 4 weeks after planting shows 100% disease incidence and plants can not bear fruit.
3) From the planting time until the age of 4 weeks after planting, cucumber plants are susceptible to CMV infection, therefore the critical phase cucumber plant towards CMV infection occurs from the time at planting until the age of 4 weeks after planting.

Conflict of interest statement and funding sources
The author(s) declared that (s)he/they have no competing interest. The study was financed by Ministry of Research.

Statement of authorship
The author(s) have a responsibility for the conception and design of the study. The author(s) have approved the final article.

Acknowledgments
The authors would like to extend their gratitude to the Ministry of Research, Technology and Higher Education, the Director General for Research and Development that has provided research funding. Acknowledgments are also conveyed to the Rector of Mahasarawati University (UNMAS) Denpasar who has given permission in conducting the research, as well as to the Dean of the Faculty of Agriculture UNMAS and the chairman of LPPM UNMAS for motivation and assistance in conducting the research.

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