International Research Journal of Engineering, IT & Scientific Research
Available online at https://sloap.org/journals/index.php/irjeis/
Vol. 4 No. 6, November 2018, pages: 44~54
ISSN: 2454-2261
https://sloap.org/journals/index.php/irjeis/article/view/340

Exploration and Collection of Cucumber Mosaic Virus Isolates of Horticultural Plants from Bali

Ni Putu Pandawani [a]
Cokorda Javandira [b]
Farida Hanum [c]

Article history:

Received: 20 July 2018
Accepted: 30 September 2018
Published: 7 November 2018

Keywords:

Cucumber mosaic virus;
Cucumis sativus;
Cucurbita moschata;
Lycopersum esculentum;
Vigna sinensis;

Abstract

Information regarding the existence and biological character of CMV isolates from several horticultural plants from Bali is still limited. The results of this research on information are very much needed in the further research and development of science and technology, due to it is known that CMV isolates from different hosts can cause different symptoms of infection or specific symptoms in certain plants. Based on these problems, this research was conducted through exploration, collection, and biological testing of CMV isolates found in several horticultural plants in Bali. Therefore, the existence and biological characteristics of each isolate were known. The results showed that all isolates obtained from the field, as many as 36 isolates, only 18 isolates showed symptoms of *N. glutinousa.* The isolates showed symptoms in *N. glutinosa* plants were isolates from cucumber plants 4 isolates, eggplant 3 isolates, pumpkin 1 isolate, 3 chili isolates, 2 isolates tomatoes, 2 beans isolates, and watermelons 3 isolates. The types of symptoms that appeared varied, *i.e.,* 10 symptomatic mosaic isolates, 4 malformed isolates, and 4 necrotic symptomatic isolates. Detection of I-ELISA using CMV antiserum succeeded in getting 6 positive CMV isolates, *i.e.,* isolates derived from cucumber plants (CMV-MB isolates), eggplant (CMV-TRB isolates) watermelon (CMV-SB isolates), chili (CMV-CB isolates), pumpkin (CMV-LB isolates), and tomatoes (CMV-TB isolates). The results of biological testing of CMV isolates from Bali, mosaic symptoms occurred in the indicator plants *Nicotiana glutinosa*, *Capsicum annuum* (big chili), *Cucumis sativus* (cucumber), *Lycopersum esculentum* (tomato), and *Cucurbita moschata* (pumpkin). Severe mosaic symptoms only occurred in *Cucumis sativus* (cucumber) plants. In *Vigna sinensis* (long bean) infection CMV-Bali isolates only cause symptoms of malformation and even cause no symptoms.

[a] Universitas Mahasaraswati, Denpasar, Indonesia
[b] Universitas Mahasaraswati, Denpasar, Indonesia
[c] Universitas Mahasaraswati, Denpasar, Indonesia

Author correspondence:
Ni Putu Pandawani,
Universitas Mahasaraswati, Denpasar, Indonesia
Ph. +62818558257; Fax: +62361-227019
Email address: pandawaniputu@hotmail.com

1. Introduction

Cucumber Mosaic Virus (CMV) is a species of virus originating from genus *Cucumovirus*, family *Bromoviridae* (Mochizuki and Ohki, 2012). It is round shape with 30 nm diameter, has four types of nucleic acids, which is a single-threaded RNA (Palukaitis *et al.,* 1992; Fauquet *et al.,* 2005). CMV is also one of the mosaic disease-causing viruses found in *Cucurbitaceae* plants. CMV can infect 1200 species in more than 100 families of vegetable and horticulture plants. These characteristics make CMV a wrong plant virus is economically important in commercial plants (Palukaitis *et al.,* 1992). Important economic plants can be infected by CMV are big chili, cayenne pepper, paprika, tomato, cucumber, melon, banana, eggplant, chrysanthemum, lilies, and a number of other ornamental plants (Agrios, 2005).

CMV has a wide host range, its spread in all countries and strains with different biological properties have been reported from various places. CMV has many strains and differences between strains depend on the host type, the symptoms produced, the transmission nature, and other characteristics (Francki *et al.,* 1979; Agrios 2005). The disease symptoms due to CMV infection vary depending on the host species or CMV strain. Therefore, it appears to be a character of the host reaction to CMV infection. Pandawani *et al.*, (2017), in cucumber plants (*Cucumis sativus*) and other *Cucurbitaceae* members, CMV infection causes mosaic and dwarf symptoms and reduces fruit quantity and quality. In tomato plants (*Lycopersicon esculentum*), CMV infections cause mosaic, dwarf, fern leaf reduction (McNab *et al.*, 1983) symptoms. In *Capsicum annuum*, CMV infections cause mosaic symptoms, blistering, fruit malformations, and together with other viruses (TMV, TEV, PVY, and CVMV) cause symptoms of complex viral infections (curly symptoms).

2. Materials and Methods

Exploration of host samples infected viruses

Virus exploration is conducted on several types of symptomatic horticultural plants infected with the virus. Samples were taken from 9 regencies in Bali, included Jembrana, Buleleng, Tabanan, Badung, Gianyar, Bangli, Klungkung, Karangasem, and Denpasar City. Host plants suspected of being infected with CMV were collected and taken at least three samples from one type of plant in each location. To obtain a variety of isolates with more samples collected were samples that showed different symptoms or virus-specific symptoms, namely mosaic, blistering, necrotic leaves/fruit, and leaf malformations.

Propagation of CMV isolates from different hosts

Propagation of isolates is conducted through mechanical inoculation of symptomatic host saplings infected with the virus. Mechanical viral inoculation was conducted 1 gram of CMV inoculum plant leaves were crushed in sterile mortal by adding 10 ml of phosphate/phosphate buffer (1:10 b/v) containing 1% β-mercaptoethanol (0, 01 M, pH 7.0). The *sap yan* is formed immediately inoculated on *N. glutinosa* plant which is on the surface of the first leaf which has been fully opened, the leaves of which have been sprinkled with carborundum 600 mesh first. The plants that have been inoculated are kept in insect vectors that are resistant to symptoms of the disease. The same symptomatic plants on the CMV isolate source plant in the field were then selected, for further purification of CMV isolates.

Purification of CMV isolates

Virus purification was conducted through biological testing to select CMV isolates. The plants needed to obtain pure CMV isolates were *Nicotiana glutinosa, Cucumis sativus,* and *Chenopodium amaranticolor*. The purification

method of CMV isolates was carried out as follows: a sample from the field that had been propagated in N. glutinosa (the result of point 2.2) was infected with *C. sativus* in the cotyledon section, and then infected with *C. amaranticolor* (repeated three times). Furthermore, the virus obtained was re-inoculated into *N. glutinosa* and *C. sativus* plants. Furthermore, the obtained isolates were maintained on *N. glutinosa* and to ensure that CMV isolates were obtained, serological testing of isolates with the *Indirect Enzyme-linked immunosorbent assay* was performed (I-ELISA) using serum anti-CMV (AGDIA, USA).

CMV isolates collection through the biological test on indicator plants

Indicator plants used to study the symptoms of infection by all isolates obtained were plants: *C. annuum* (big chili), *Cucumis sativus* (cucumber), *L. esculentum* (tomato), *Solanum melongena* (green eggplant), *Vigna sinensis* (long beans), and *Cucurbita moschata* (pumpkin). All CMV positive isolates were inoculated on several indicator plants. The differences in isolates are expected to be identified based on differences in symptoms in indicator plants so that CMV isolates collection from several hosts was obtained.

3. Results and Discussions

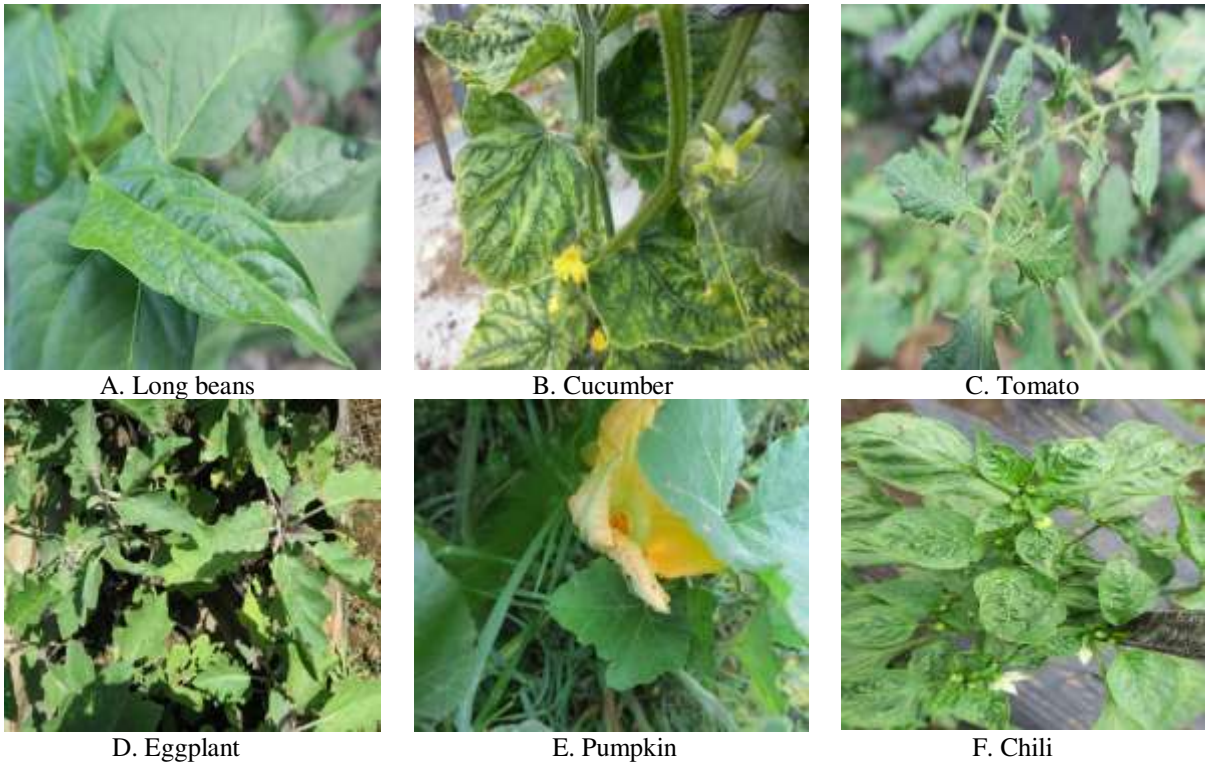
3.1 Collection of plants infected with viruses in the field

Isolates collection, as well as symptoms observations that occur in plants, suspected being infected with CMV have been conducted and data obtained are shown in Table 1. Isolates were obtained from the field as many as 36 isolates namely derived from cucumber plants (5 isolates), big chili (6 isolates), tomatoes (4 isolates), long beans (4 isolates), watermelon (4 isolates), pumpkin (4 isolates) and melons (4 isolates).

Table 1
Isolates collection and host symptoms suspected being infected with CMV

Origin of isolate Regency	Host plants	Symptoms host field	Sample Code
1. Bangli			
	cucumber	mosaic	1 M
	long beans	necrotic	2 K
	eggplant	mosaic	3 Tr
	pumpkin	Malformation	4 L
2. Amlapura			
	watermelon	malformation	5 S
	cucumber	Malformation	6 M
	pumpkin	mosaic	7 L
	melon	necrotic	8 Me
3. Semarapura			
	tomato	malformation	9 T
	chili	mosaic	10 C
	eggplant	necrotic	11 Tr
	watermelon	mosaic	12 S
4. Gianyar			
	cucumber	necrotic	13 M
	pumpkin	mosaic	14 L
	eggplant	Malformation	15 Tr
	tomato	mosaic	16 T
5. Singaraja			
	melon	mosaic	17 Me
	chili	malformation	18 C

	eggplant	mosaic	19 Tr
	long beans	mosaic	20 K
6. Jembrana			
	cucumber	malformation	21 M
	watermelon	necrotic	22 S
	melon	mosaic	23 Me
	chili	necrotic	24 C
7. Tabanan			
	tomato	malformation	25 T
	cucumber	mosaic	26 M
	pumpkin	necrotic	27 L
	chili	mosaic	28 C
8. Badung			
	melon	mosaic	29 Me
	chili	motel	30 C
	long beans	malformation	31 K
	tomato	malformation	32 T
9. Denpasar			
	chili	necrotic	33 C
	watermelon	mosaic	34 S
	eggplant	mosaic	35 Tr
	long beans	motel	36 K



A. Long beans B. Cucumber C. Tomato

D. Eggplant E. Pumpkin F. Chili

Figure 1. Symptoms of plants in the field suspected being infected with CMV

The plants infected with the virus in the field show complex symptoms, which are infected with several viruses simultaneously. The sample obtained it is expected that there is a single infection by CMV. Symptoms appear in the host plants in the field are suspected of being infected with CMV are very varied or different, ranging from mild to

severe mosaic, blistering, and leaf malformations (Figure 1). The different symptoms in the same host plant are expected to be obtained by different CMV strains.

3.2 Purification results of CMV isolates

The plants are from the field suspected being infected with the virus then purified/conducted purification of the virus with biological techniques to separate CMV from other viruses, using specific indicator plants. All isolates obtained from the field were as many as 36 isolates, after purification only 18 isolates showed symptoms in *N. glutinousa* plants, 18 other isolates did not show symptoms. Isolates who showed symptoms were 10 mosaic symptom isolates, 4 isolates from leaf malformations and 4 isolates from necrotic symptoms. The isolates showing symptoms of *N. glutinosa* were isolates from cucumber plants 4 isolates, eggplant 3 isolates, pumpkin 1 isolate, chili 3 isolates, tomatoes 2 isolates, beans 2 isolates, and watermelons 3 isolates. The incubation period in *N. glutinosa* plants ranged from 11 days to 15 days after inoculation (Table 2).

Table 2
Type of symptoms in *N. glutinosa* indicator plants infected with CMV isolates from different hosts

No.	Sample Code	Original host field	Incubation period (hsi)	Symptoms in indicator plants/*N. glutinosa*
1	1 M	cucumber	12	mosaic
2	2 K	long beans	14	necrotic
3	3 Tr	eggplant	13	mosaic
4	5 S	watermelon	11	malformation
5	6 M	cucumber	11	malformation
6	10 C	chili	14	mosaic
7	11 Tr	eggplant	14	necrotic
8	13 M	cucumber	12	necrotic
9	14 L	pumpkin	13	mosaic
10	16 T	tomato	14	mosaic
11	19 Tr	eggplant	13	mosaic
12	20 K	long beans	15	mosaic
13	22 S	watermelon	13	mosaic
14	24 C	chili	15	malformation
15	25 T	tomato	12	malformation
16	26 M	cucumber	11	mosaic
17	33 C	chili	15	necrotic
18	34 S	watermelon	12	mosaic

CMV infects systemically in many plants. Older organ or tissue plants that develop before being infected with the virus are usually not affected by the virus presence, but the young tissue or cells that develop after being infected with the virus are very affected and generally show acute symptoms. Virus symptoms will increase several days after the infection, then decrease to a certain extent or until the plant dies.

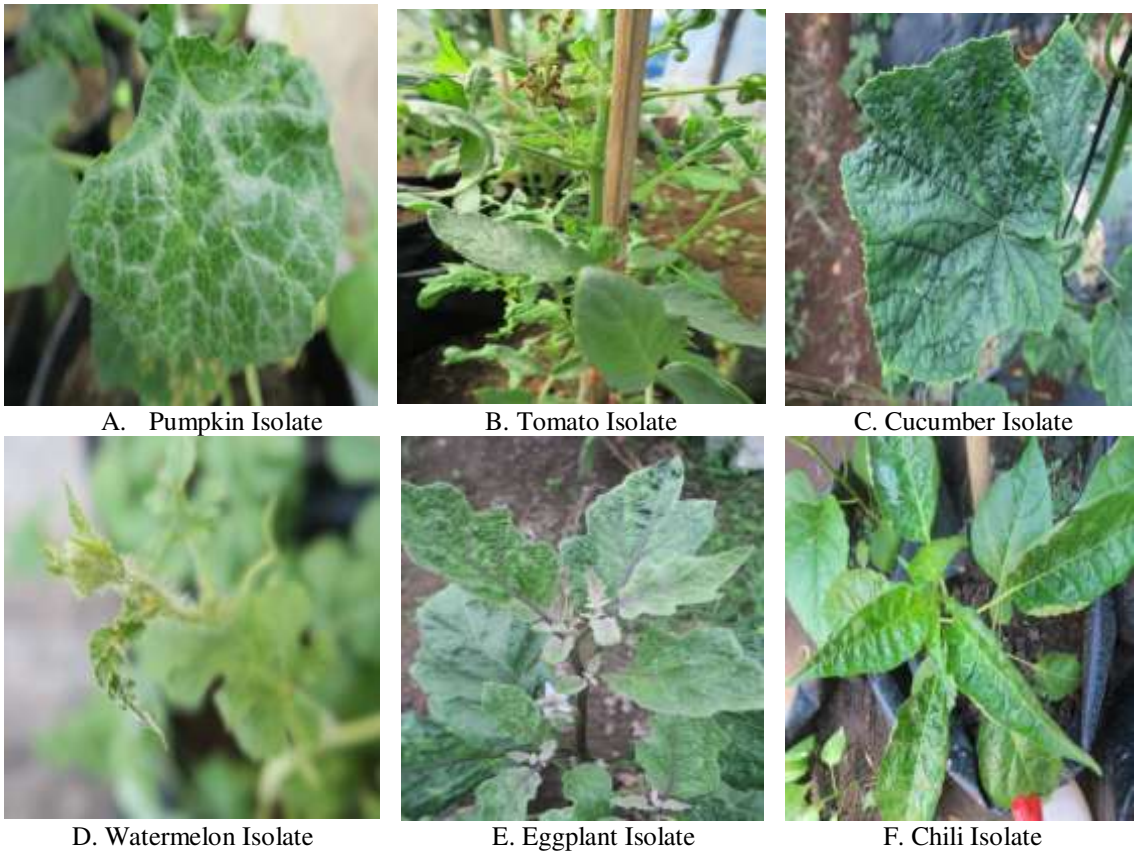
3.3 Serology test results of CMV isolates

The variation in symptoms caused by CMV will be very difficult to identify only based on the symptoms. In addition, it is also difficult to distinguish other CMV from *Cucumovirus* isolates (such as *Alfalfa mosaic virus, Tomato aspermy virus, and Peanut stunt virus*). To further ensure, the virus obtained is CMV, the isolates that have been successfully purified are then tested serologically using I-ELISA test. The isolated sample was expressed positively if the results of the absorbance measurements at 405 nm wavelength with ELISA reader (Table 3) had a value twice as large as the negative control value (Matthews, 2002).

Table 3
I-ELISA absorption value at A 405 nm of CMV isolates

No.	Sample Code	Origin of the host	I-ELISA / Antiserum CMV / absorbance value
1	1 M	cucumber	2.524
2	2 K	long beans	0.522
3	3 Tr	eggplant	2.356
4	5 S	watermelon	2.584
5	6 M	cucumber	0.620
6	10 C	chili	2.422
7	11 Tr	eggplant	0.426
8	13 M	cucumber	0.524
9	14 L	pumpkin	2.482
10	16 T	tomato	0.465
11	19 Tr	eggplant	0.540
12	20 K	long beans	0.582
13	22 S	watermelon	0.488
14	24 C	chili	0.522
15	25 T	tomato	2.528
16	26 M	cucumber	0.642
17	33 C	chili	0.584
18	34 S	watermelon	0.634
	Buffer		0.238
	Positive control		2.634
	Negative control		0.466

Detection by I-ELISA using CMV antiserum succeeded in getting 6 positive CMV isolates (Figure 2) derived from cucumber plants (CMV-MB isolates), eggplant plants (CMV-TRB isolates) watermelon plants (CMV-SB isolates), chili plants (CMV-CB isolates, pumpkin plants (CMV-LB isolates) and tomato plants (CMV-TB isolates) (Table 4). Other isolates also showed symptoms of *N. glutinosa* appeared to be from CMV I-ELISA test result is not positive.



A. Pumpkin Isolate B. Tomato Isolate C. Cucumber Isolate

D. Watermelon Isolate E. Eggplant Isolate F. Chili Isolate

Figure 2. CMV-Bali isolates

Table 4
CMV positive isolates from different hosts

No.	Sample code	Origin of host	Symptom type on indicator plants/*N. glutinosa*	I-ELISA	Isolate name of CMV Bali
1	1 M	Cucumber	Mosaic	+	CMV-MB
2	3 Tr	eggplant	Malformation	+	CMv-TRB
3	5 S	Watermelon	Malformation	+	CMV-SB
4	10 c	Chili	Mosaic	+	CMV-CB
5	14 L	Pumpkin	Mosaic	+	CMV-LB
6	25 T	Tomatoes	Malformation	+	CMV-TB

3.4 Indicators plant symptoms on the biological test of CMV isolates

The symptoms result from infection with six CMV isolates from Bali on several important indicator plants are highly varied and some are asymptomatic. It appears varying from 6 days after inoculation to 13 days after inoculation with severe mosaic, mosaic, local malformations, and necrotic symptoms (Table 5).

Table 5
Symptoms of indicator plants infected with CMV isolates from Bali

Indicator plant	CMV-Bali Isolates					
	CMV-MB	CMV-TRB	CMV-SB	CMV-CB	CMV-LB	CMV-TB
	Symptoms					
Nicotiana glutinosa	mosaic	malformation	mosaic	mosaic	malformation	mosaic
Capsicum annuum (big chili)	mosaic	mosaic	malformation	mosaic	mosaic	malformation
Cucumis sativus (cucumber),	weight mosaic	mosaic	weight mosaic	asymptomatic	mosaic	mosaic
Lycopersum. esculentum (tomato),	malformation	malformation	mosaic	mosaic	malformation	malformation
Vigna sinensis (long beans),	malformation	asymptomatic	asymptomatic	asymptomatic	asymptomatic	malformation
Cucurbita moschata (pumpkin)	mosaic	asymptomatic	malformation	mosaic	mosaic	malformation
Chenopodium amaranticolor	necrotic	necrotic	necrotic	necrotic	necrotic	necrotic

In indicator plants, *Nicotiana glutinosa isolates* CMV-MB, CMV-SB, CMV-CB, and CMV-TB cause mosaic symptoms, whereas other isolates cause symptoms of malformation. In *Capsicum annuum* (big chili) isolates CMV-MB, CMV-TRB, CMV-CB and CMV-LB cause symptomatic mosaics, whereas CMV-SB and CMV-TB isolate cause symptoms of malformation. In *Cucumis sativus* (cucumber) plants, CMV-MB isolates and CMV-SB showed severe mosaic symptoms, CMV-TRB, CMV-LB, and CMV-TB isolates caused mosaic symptoms while CMV-CB isolates showed no symptoms. In *Lycopersum esculentum* plants (tomato), CMV-SB and CMV-CB isolate because of mosaic symptoms while CMV-MB, CMV-TRB, CMV-LB, and CMV-TB isolates cause symptoms of malformation. In indicator plants of *Vigna sinensis* (long bean) isolates CMV-MB and CMV-TB cause symptoms of malformation while CMV-TRB isolates, CMV-SB, CMV-CB, and CMV-LB do not cause symptoms. In the *Cucurbita moschata* plant (pumpkin), CMV-MB, CMV-CB, and CMV-LB isolate caused mosaic symptoms, CMV-SB and CMV-TB isolates caused symptoms of malformation while CMV-TRB isolates showed no symptoms. In *Chenopodium amaranticolor* plants all isolates cause local necrotic symptoms.

Severe mosaic symptoms only occur in *Cucumis sativus* (cucumber) plants due to infection with CMV-MB isolates and CMV-SB. Mosaic symptoms occur in indicator plants *Nicotiana glutinosa* (due to infection with CMV-MB, CMV-SB, CMV-CB, and CMV-TB isolates), in *Capsicum annuum* plants (big chili) (due to infection with CMV-MB isolates, CMV-TRB) , CMV-CB and CMV-LB, in *Cucumis sativus* (cucumber) plants, (due to CMV-TRB, CMV-LB and CMV-TB isolates), in *Lycopersum esculentum* (tomato) plants (due to CMV-SB and CMV isolates) -CB), in *Cucurbita moschata* (pumpkin) plants (due to infection with CMV-MB, CMV-CB and CMV-LB isolates) In *Vigna sinensis* (long bean) infection CMV-Bali isolates did not cause mosaic symptoms, only caused symptoms of malformations (due to infection with CMV-MB and CMV-TB isolates) and even CMV isolates from Bali, namely CMV-TRB isolates, CMV-SB, CMV-CB, and CMV-LB did not cause symptoms.

4. Conclusion

1. Exploration of all isolates from the field as many as 36 isolates, and only 18 isolates showed symptoms in *N. glutinousa* plants. The isolates showed symptoms in *N. glutinosa* plants were isolates from cucumber plants 4 isolates, eggplant 3 isolates, pumpkin 1 isolate, 3 chili isolates, 2 isolate tomatoes, 2 bean isolates, and 3 watermelon isolates. The symptoms types appear vary, 10 of which are symptomatic mosaic, 4 are symptomatic malformations, and 4 necrotic symptomatic isolates.

2. Detection of I-ELISA using CMV antiserum succeeded in getting 6 CMV positive isolates namely isolates derived from cucumber plants (CMV-MB isolates), eggplant (CMV-TRB isolates) watermelon (CMV-SB isolate), chili (CMV-CB isolate), pumpkin (CMV-LB isolate) and tomatoes (CMV-TB isolates).
3. Biological test of CMV isolates from Bali, mosaic symptoms occur in the indicator plant *Nicotiana glutinosa* included *Capsicum annuum* (big chili), *Cucumis sativus* (cucumber), *Lycopersum esculentum* (tomato), and *Cucurbita moschata* (punpkin). Heavy mosaic symptoms only occur on *Cucumis sativus* (cucumber) plant. In *Vigna sinensis* (long bean) plant infection, CMV-Bali isolates only cause symptoms of malformation and do not even cause symptoms.

Conflict of interest statement and funding sources

The authors declared that they have no competing interest. The study was financed by Directorate of Research and Community Service, Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

Statement of authorship

The authors have a responsibility for the conception and design of the study. The authors have approved the final article.

Acknowledgments

This research was supported by the Directorate of Research and Community Service, Ministry of Research, Technology, and Higher Education of the Republic of Indonesia. We also thank our colleague, Dean of the faculty of agriculture, Universitas Mahasaraswati Denpasar, who assisted the research.

References

Agrios, G. N. (2005). Plant pathology 5th Edition: Elsevier Academic Press. *Burlington, Ma. USA*, 79-103.

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., & Ball, L. A. (2005). Virus taxonomy. *Eighth report of the international committee on taxonomy of viruses*, *8*, 455-465.

Francki, R. I. B., Mossop, D. W., & Hatta, T. (1979). Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses, No. 213.

MacNab, A. A., Sherf, A. F., & Springer, J. K. (1983). *Identifying diseases of vegetables* (No. 635.0493/M169). University Park, Pa.: Pennsylvania State University, College of Agriculture.

Matthews, R. C. (2012). *Fundamentals of plant virology*. Academic Press.

Mochizuki, T., & Ohki, S. T. (2012). Cucumber mosaic virus: viral genes as virulence determinants. *Molecular plant pathology*, *13*(3), 217-225.

Palukaitis, P., Roossinck, M. J., Dietzgen, R. G., & Francki, R. I. (1992). Cucumber mosaic virus. In *Advances in virus research* (Vol. 41, pp. 281-348). Academic Press.

Pandawani, N. P., Hanum, F., & Suryani, N. N. (2017). Resistance Test of Several Varieties and Critical Phase for Cucumis Sativus towards Cucumber Mosaic Virus Infectio. *International Research Journal of Engineering, IT and Scientific Research*, *3*(6), 66-73.

Biography of Authors

Ir. Ni Putu Pandawani, M.Si. is a Senior Lecturer of Agrotechnology Studies Program, Faculty of Agriculture, Mahasaraswati University Denpasar. She completed her Bachelor Degree at the Veterinary and Animal Husbandry, Udayana University. She finished her Master Degree at the Biotechnology Faculty of Agriculture, Udayana University. She currently is a Doctoral Student, Doctoral Program in Agricultural Science, and Biological Resource Concentration in Udayana University.
Email: pandawaniputu@hotmail.com

Cokorda Javandira, SP.MP. is a Yunior Lecturer of Agrotechnology Studies Program, Faculty of Agriculture, Mahasaraswati University Denpasar. She completed her Bachelor Degree in the Agricultural Brawijaya University. She finished her Master Degree at the Faculty of Agriculture, Brawijaya University.
Email: javandira11_unmas@yahoo.co.id

Ir. Farida Hanum, M.Si. is a Senior Lecturer of Agrotechnology Studies Program, Faculty of Agriculture, Mahasaraswati University Denpasar. She completed her Bachelor Degree in the Agricultural Technology, Sumatera Utara University. She finished her Master Degree at the Faculty of Dryland Agriculture, Faculty of Agriculture, Udayana University.
Email: farida_ritonga@yahoo.com