

Biological Character of *Cucumber Mosaic Virus* (CMV) Isolates from Bali

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Abstract

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This research aims at biologically characterizing the CMV isolates from Bali so that they can add the collection and knowledge about the biological character of CMV isolates from Bali. Characterization of the biological properties of CMV isolates performed through the following steps: 1) Propagation of CMV isolates from different host plants (cucumber, melon, watermelon, tomato, pumpkin, chilli, green eggplant and long bean) on *N. glutinosa* plant, 2) Purification of CMV isolates through inoculation mechanics of *C. sativus* and *C. amaranticolor* plants, 3) CMV isolates and 4) serological detection with I-ELISA. The results showed that total isolates obtained from the field are 36 isolates, only 18 isolates showed symptoms on *N. glutinosa* plants, while 18 other isolates did not show symptoms. Isolates showed symptoms of *N. glutinosa* plants were 4 isolates from cucumber plant, 3 isolates from eggplant, 1 isolate from pumpkin, 3 from isolates chili, 2 isolates from tomatoes, 2 isolates from long beans and 3 isolates from watermelon. The types of symptoms appeared were varied, e.g, 10 mosaic symptomatic isolates, 4 malformation symptomatic isolates, and 4 necrotic symptomatic isolates. I-ELISA detection using CMV antiserum was able to obtain 6 positive CMV isolates from cucumber (CMV-MB isolate), watermelon (CMV-TRB isolate) watermelon (CMV-SB isolate), chili (CMV-CB isolate), pumpkin (CMV-LB isolate) and tomato (CMV-TB isolate). The result of biological isolate test of CMV from Bali, mosaic symptoms occurred on *Nicotiana glutinosa* plant, *Capsicum annuum* (big chili), *Cucumis sativus* (cucumber), *Lycopersum esculentum* (tomato) and *Cucurbita moschata* (pumpkin). Severe mosaic symptoms occurred only in the *Cucumis sativus* (cucumber) plant. *Vigna sinensis* (long bean) infections of CMV isolate -Bali only caused symptoms of malformations and even no symptoms.

Keywords : character, biology, isolate, CMV , Bali

1. Introduction

Cucumber Mosaic Virus (CMV) is a species of virus originally from the genus *Cucumovirus* , family of Bromoviridae (Mochizuki and Ohki, 2012). It has round form with a diameter about 30 millimeters and has four types of nucleic acids, each of which is a single stranded RNA (Palukaitis *et al* ., 1992; Fauquet *et al.*, 2005). CMV is also one of the most

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common mosaic virus-causing viruses on the *Cucurbitaceae* plant . CMV can infect 1200 species in more than 100 families of vegetable and horticultural crops. These characteristics make CMV the most important plant virus that is economically important in commercial plants (Palukaitis *et al.*, 1992). Important economical benefits that CMV can infect large chilies, cayenne peppers, tomatoes, cucumbers, melons, bananas, eggplants, chrysanthemum, lilies, and a number of other ornamental plants (Agrios, 1998).

CMV has a wide range of host, it spreads in all countries and strains of different biological properties have been reported from various places. CMV has many strains and differences between strains depending on the host species, resulting symptoms, transmission capability, and other properties and characteristics (Francki *et al.*, 1979; Agrios, 2005). Symptoms of CMV infection vary depending on the host species or CMV strains, so there appears to be a host to host characteristic of CMV infection. In cucumber plants (*Cucumis sativus*) and other *Cucurbitaceae* members, CMV infection causes mosaic and dwarf symptoms, and decreases the quantity and quality of the fruit. In tomato plants (*Lycopersicon esculentum*), CMV infections cause mosaic, dwarf, lamina leaf (*fern leaf*) (MacNab, Sherf, and Springer, 1983). In chili plants (*Capsicum annuum*), CMV infection causes mosaic symptoms, blistering, fruit malformation, and other viruses (TMV, TEV, PVY, CVMV) causing symptoms of complex viral infections (curly symptoms).

Information on the biological character of CMV isolates from Bali is still limited. Therefore it is necessary to conduct a study to find out whether CMV isolates originating from different host causes different symptoms of infection or specific symptoms in certain plants. The problems that must be answered, firstly, Can CMV isolates be isolated from some CMV infected plants? , Can pure CMV isolates be collected by viral purification using a plant indicator?, and can specific symptoms be seen in infected plants through biological testing of each type of CMV isolate ?

Based on these problems, this study was conducted with the objective of biologically characterizing the specific symptoms occurring in the plant due to the infection of CMV isolates from Bali so that they can add to the collection and knowledge of the biological character of the Bali CMV isolates, which can be used for further research and development of science and technology.

2. Materials and Methods

2.1 Exploration of symptomatic host samples infected with the virus

Survey and samples were taken from 9 districts/cities in Bali, namely: Jembrana, Buleleng, Tabanan, Badung, Gianyar, Bangli, Kelungkung, Karangasem and Denpasar City. Different host plants suspected CMV infection were collected and taken at least three samples. To get more variation isolates, samples were taken to obtain different symptoms or specific symptoms of infected with a virus in the form of mosaic, blistering, necrotic leaf/fruit and leaf malformation. The leaves syrup is stored in a glass tube containing CaCl₃ powder, and then sealed tightly until it is airtight. This hygroscopic material will cause the leaf samples to dry to be preserved but they do not affect the viability or intrinsic nature of the virus that may be contained therein.

2.2 Propagation of CMV isolates

The multiplication of isolates carried through mechanical inoculation of sap of symptomatic host plants. Inoculation of the virus in the mechanical way is done as follows: 1 gram each plant leaf of the CMV inoculums sources is crushed in a sterile mortar with 10 ml 1 phosphate buffer/phosphate buffer (1:10 b/v) buffer containing 1% β -*capthethanol* (0.01 M, pH 7.0) . The sap immediately inoculated on the *N. glutinosa* plant that is on the first leaf surface that has been fully opened, the first leaf has been sprinkled with 600 mesh carborundum. The inoculated faucet is preserved in a vector-borne insect visor until symptoms appear. The mosaic symptom as similar to mosaic symptoms in the next CMV source crop is selected to further purify the CMV isolates.

2.3 Purification of CMV isolates

Purification of the virus is carried out biologically to select CMV isolates. Plants are required to obtain pure CMV isolates are *Nicotiana glutinosa*, *Cucumissativus*, and *Chenopodium amaranticolor*. The method of purifying the CMV isolates was carried out as follows: samples from the field which have been propagated in *N. glutinosa* plant were infected to *C. sativus* cotyledone, and then infected to *C. amaranticolor* (repeated three times) plants. Furthermore, the virus obtained inoculated back to the plant *N. glutinosa* and *C. sativus* . Furthermore, the isolates obtained were maintained on *N. glutinose* and to confirm

the obtained CMV isolates serologic testing of isolates with *Indirect-Enzyme linked immune sorbent assay* (I-ELISA) using anti-CMV serum (AGDIA, USA).

2.4 CMV isolates collection through the Biological Testing on Indicator plants

The indicator plants were used to study the symptoms of infection by all isolates obtained were these plants, namely *C. annuum* (large chili), *Cucumis sativus* (cucumber), *L. esculentum* (tomato) , *Solanum melongena* (green egg plant) , *Vigna sinensis* (beans) , and *Cucurbita moschata* (pumpkin). All CMV-positive isolates were inoculated on several indicator plants. Differences isolates are expected to be identified based on the difference symptoms on indicator plants in order to obtain some collection of CMV isolates originating from the host plants.

3. Results and Discussion

3.1 Collection of isolates and symptoms of virus infected plants in the field

A collection of isolates from 9 districts/cities in Bali and from several different host plants as well as observation of symptoms occurring on plants suspected of being infected with CMV have been performed and the obtained data was shown in Table 1. Isolates were obtained from the field are 36 isolates derived from several host plants, namely cucumber (5 isolates), large chili (6 isolates), tomatoes (4 isolates), long beans (4 isolates), watermelon (4 isolates), pumpkin (4 isolates) and melon (4 isolates).

Virus infected plants in the field showed complex symptoms infected with several viruses simultaneously, but it is expected to be infected by CMV singly. Symptoms occurred in host plants that are suspected of being infected with CMV vary widely or differently, ranging from mild to severe mosaic, blistering, necrotic and leaf malformation (Table 1). Different symptom on the same host plant is expected to be obtained by different strains of CMV.

Table 1. Isolate from Bali, Host Plant and CMV infected Symptoms

Origin of isolate Regency / City	Host Plant	Host Symptoms in the field	Sample Code
1. Bangli			
	Cucumber	Mosaic	1 M
	Long beans	Necrotic	2 K
	Eggplant	Mosaic	3 Tr
	Pumpkin	Malformations	4 L
2. Karangasem			

	Watermelon	Malformations	5 S
	Cucumber	Malformations	6 M
	Pumpkin	Mosaic	7 L
	Melon	Necrotic	8 Me
3. Kelungkung			
	Tomato	Malformations	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	Malformations	15 Tr
	Tomato	Mosaic	16 T
5. Buleleng			
	Melon	Mosaic	17 Me
	Chili	Malformations	18 C
	Eggplant	Mosaic	19 Tr
	Long beans	Mosaic	20 K
6. Jembrana			
	Cucumber	Malformations	21 M
	Watermelon	Necrotic	22 S
	Melon	Mosaic	23 Me
	Chili	Necrotic	24 C
7. Tabanan			
	Tomato	Malformations	25 T
	Cucumber	Mosaic	26 M
	Pumpkin	Necrotic	27 L
	Chili	Mosaic	28 C
8. Badung			
	Melon	Mosaic	29 Me
	Chili	Blistering	30 C
	Long beans	Malformations	31 K
	Tomato	Malformations	32 T
9. Denpasar			
	Chili	Necrotic	33 C
	Watermelon	Mosaic	34 S
	Eggplant	Mosaic	35 Tr
	Long beans	Blistering	36 K

3.2 Result of isolate separation and biological testing of CMV on plant indicator

Plants from the field suspected of being infected subsequently purified/given virus purification with biological techniques to separate CMV from other viruses using specific indicator plants to gain more successful separation of the CMV virus with other viruses. All isolates obtained from the field were 36 isolates, only 18 isolates showed symptoms on *N. glutinosa* plant, while 18 other isolates showed no symptoms. Isolates that showed symptoms of mosaic symptoms were 10 isolates, 4 isolates showed malformation and 4 isolates showed necrotic symptoms (Table 2). The isolates showed symptoms on *N. glutinosa* plant were

isolates from cucumber (4 isolates), eggplant (3 isolates), pumpkin (1 isolate), chilli (3 isolates), tomatoes (2 isolates), long beans (2 isolates) and watermelon (3 isolates). The incubation period of *N. glutinosa* plant ranges from 11 days to 15 days after inoculation. Results of inoculation of isolate on *N. glutinosa* plant were shown in Table 2.

Table 2. The incubation period and type of symptoms on *N. glutinosa* plants infected by CMV Isolate from different host

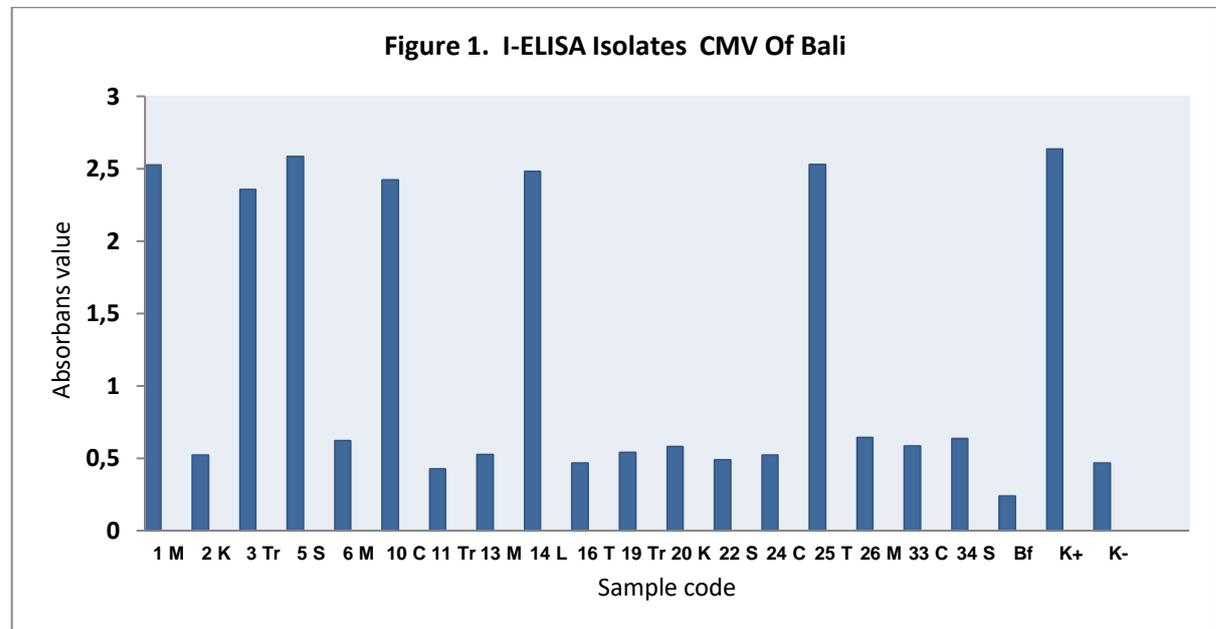
Sample Code	Host origin In the field	Period incubation (day after inoculation)	Symptoms on Plants indicator / <i>N. glutinosa</i>
1 M	Cucumber	12	mosaic
2 K	Long bean	14	necrotic
3 Tr	Eggplant	13	mosaic
5 S	Watermelon	11	malformation
6 M	Cucumber	11	malformation
10 C	Chili	14	mosaic
11 Tr	Eggplant	14	necrotic
13 M	Cucumber	12	necrotic
14 L	Pumpkin	13	mosaic
16 T	Tomato	14	mosaic
19 Tr	Eggplant	13	mosaic
20 K	Long bean	15	mosaic
22 S	Watermelon	13	mosaic
24 C	Chili	15	malformation
25 T	Tomato	12	malformation
26 M	Cucumber	11	mosaic
33 C	Chili	15	necrotic
34 S	Watermelon	12	mosaic

CMV performed systemic infections in many plants. The older organ or plant tissue that developed before the virus was infected was usually not affected by the presence of the virus, but the tissues or young cells that developed after viral infection were strongly affected and generally showed acute symptoms. Virus symptoms increased several days after infection, then decreased to a certain extent or until the plant died.

3.3 Serious Serological Test of CMV Isolates

The presence of CMV induced variation of symptoms that are very difficult to identify only on the basis of symptoms alone. In addition, it is also difficult to distinguish CMV isolate from other *Cucumovirus* (such as *Alfalfa mosaic virus*, *Tomato aspermy virus*, and *Peanut stunt virus*). To further ensure that the virus obtained is CMV, the isolates that have been successfully separated were then tested serologically by using the I-ELISA test. The isolate

sample was stated to be positive, if the absorbance result at the 405 nm wave length using the ELISA reader (Fig. 1)) has a value two times greater than the value of the negative control (Matthews, 2002).



Detection of the I-ELISA using antiserum CMV in this study found 6 isolates were CMV positive (Table3) which are derived from cucumber plant (CMV-MB isolate), eggplant (CMV-TRB isolate) watermelon plant (CMV-SB isolate), chili plant (CMV-CB isolate), pumpkin plant (CMV-LB isolate) and tomatoes (CMV-TB isolate). Other isolates also showed symptoms on plants *N. glutinosa*, after being tested using I-ELISA , the results were not CMV positive.

Table 3. Positive Isolates of CMV from different host

No.	Code Sample	Host origin	Type of symptoms on the plant indicator / <i>N. glutinosa</i>	I-ELISA	CMV isolate from 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	Tomato	Malformation	+	CMV-TB
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3.4 Symptoms in plants Indicators of the CMV Isolate-Isolate Biological Test

Symptoms resulted from the infection of six isolates of CMV from Bali on some important indicator plants are highly variable and until there are no symptoms. Symptoms appear variably from 6 days after inoculation to 13 days after inoculation with mosaic symptoms, severe mosaic, malformation and necrotic local (Table 4).

Table 4. Symptoms on indicator plants due to infection CMV isolates from Bali

Plant indicator	Isolates of CMV-Bali					
	CMV-MB	CMV-TRB	CMV-SB	CMV-CB	CMV-LB	CMV-TB
	Symptoms					
1. <i>Nicotiana glutinosa</i>	mosaic	malformation	mosaic	mosaic	malformation	mosaic
2. <i>Capsicum Annuum</i>	mosaic	mosaic	malformations	mosaic	mosaic	malformations
3. <i>Cucumis sativus</i>	mosaic weight	mosaic	mosaic weight	no symptom	mosaic	mosaic
4. <i>Lycopersum. esculentum</i>	malformation	malformation	mosaic	mosaic	malformation	malformation
5. <i>Vigna sinensis</i>	malformation	no symptom	no symptom	no symptom	no symptom	malformation
6. <i>Cucurbita Moschata</i>	mosaic	no symptom	malformation	mosaic	mosaic	malformation
7. <i>Chenopodium amaranticolor</i>	necrotic	necrotic	necrotic	necrotic	necrotic	necrotic

Severe mosaic symptoms occurred only in *Cucumis sativus* (cucumber) plants *due to* CMV-MB and CMV-SB isolate *infections*. Mosaic symptoms occurred in plants indicator *Nicotiana glutinosa* (due to isolates infection of CMV-MB, CMV-SB, CMV-CB, and CMV-TB), on *Capsicumannuum* (chili) due to isolate infection of CMV-MB, CMV-TRB , CMV-CB and CMV-LB , *Cucumis sativus* (cucumber) plant , (due to CMV-TRB isolate infection , CMV-LB and CMV-TB), *Lycopersum esculentum* (tomato) plant (due to CMV-SB isolate infection and CMV-CB), on *Cucurbitamoschata* (pumpkin) plant (due to isolate infection of CMV-MB, CMV-CB and CMV-LB). On plants *Vigna sinensis* (green beans) plant due to isolate infection of CMV-Bali did not

cause mosaic symptom, only cause symptoms of malformation (due to CMV-MB and CMV-TB isolate infection) and even isolate of CMV from Bali e.g CMV-TRB, CMV-SB, CMV-CB and CMV-LB isolates cause no symptom. On *Chenopodium amaranticolor* plant all isolate causes local necrotic symptoms.

4. Conclusions

The results showed that all isolates obtained from the field were 36 isolates, only 18 isolates showed symptoms on *N. glutinosa* plant, while 18 other isolates did not show symptom. The isolates showed symptoms of *N. glutinosa* plant were 4 isolates from cucumber plant, 3 isolates from eggplant, 1 isolate from pumpkin, 3 isolates from chilli, 2 isolates from tomato, 2 isolates from long beans and 3 isolates from watermelon.

The types of symptoms appeared variedly, e.g., 10 mosaic symptom isolates, 4 malformation symptom isolates of malformation and 4 necrotic symptom isolates. Detection of I-ELISA using antiserum CMV found 6 isolates were CMV positive, those are isolates derived from cucumber plants (CMV-MB isolate), eggplant (CMV-TRB isolate) watermelon (CMV-SB isolate), chili (CMV-CB isolate), pumpkin (CMV-LB isolate) and tomato (CMV-TB isolate).

The results of the CMV-Bali biological testing, mosaic symptoms occurred on indicator plant *Nicotiana glutinosa*, *Capsicum annuum* (large chili), *Cucumis sativus* (cucumber), *Lycopersum esculentum* (tomato) and *Cucurbita moschata* (pumpkin). Severe mosaic symptoms occurred only in the *Cucumis sativus* (cucumber) plant. In *Vigna sinensis* (long bean) isolates infection of CMV-Bali only caused symptoms of malformations and even no symptoms.

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