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Occurrence and distribution of cucumber mosaic virus in cucurbits in Karanganyar, Central Java, Indonesia

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***Cucumber mosaic virus* (CMV) is an important pathogen in agricultural crops which is spread throughout the world with a wide host range. In Indonesia, CMV is also an important pathogen that infect various high economic value crops. Karanganyar regency is a horticultural production centers in Central Java. Its foremost products are cucurbits incuding cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), chayote (*Sechium edule*), angled luffa (*Luffa acutangula*) and bitter melon (*Momordica charantia*). Recently, cucurbits crops in the area suffered from disease with symptoms of virus infection, especially CMV, that is, mosaic, vein clearing, vein banding, malformation, etc. The disease is detrimental to farmers. This research aimed to determine whether the causal agent of disease in the crops is CMV. For that, the authors conducted a survey in the field, picked up sample crops showing symptoms, brought the sample crops to the laboratory, and checked for the presence of CMV by triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA). The results showed that of the 50 sample crops including 7 species of cucurbits showing symptoms of virus infection, 12 were detected to be infected by CMV. CMV was distributed evenly in cucurbits in Karanganyar regency.**

Key words: *Cucumber mosaic virus* (CMV), cucurbits, triple antibody sandwich enzyme-linked immunosorbent assay (TAS ELISA), Karanganyar, Indonesia.

INTRODUCTION

Cucumber mosaic virus (CMV), a member of *Bromoviridae* family is an important pathogen in agricultural crops which spreads throughout the world. The virus has a wide host range including 1200 species

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in over 100 plant families (Zitter and Murphy, 2009; Palukaitis et al., 1992). In agricultural crops, especially cucurbits (*Cucurbitaceae* family), CMV infection causes varied symptoms, commonly mosaic, vein clearing, vein banding and malformation (Zitter and Murphy, 2009). In Indonesia, CMV is also an important pathogen in agricultural crops. CMV was first detected in Indonesia in 1972 in Bogor, West Java which infected tobacco (Suseno and Lumanau, 1972). Hartana (1987) reported that CMV spread throughout Indonesia and infect cucurbits and other plants. Subsequent reports have shown that CMV was found widespread in various regions in Indonesia and infect a variety of agricultural crops. Outside Java island, CMV was reportedly detected in Sumatra (Bengkulu) to infect chili (Sutrawati, 2010; Sutrawati et al., 2012) and soybean (Damayanti and Wiyono, 2015), in Sulawesi to infect chili (Taufik et al., 2011) and in Bali to infect weeds (Pranatayana et al., 2014).

In Java island, CMV was detected infect soybean (Damayanti and Wiyono, 2015). In the Special Region of Yogyakarta Province, CMV was detected to infect cucurbits (Somowiyarjo et al., 1993; Daryono and Natsuaki, 2009) and cucumber (Septariani et al,, 2014). In West Java Province, CMV was detected to infect yardlong bean (Damayanti et al., 2009, 2010) and cucumber (Septariani et al., 2014). In Central Java province, CMV was detected to infect cucurbits in Klaten Regency (Daryono and Natsuaki, 2009), yardlong bean (Damayanti et al., 2009, 2010), and cucumber in Tegal and Sukoharjo Regency (Septariani et al., 2014).

Karanganyar Regency is a horticultural production center in Central Java. This area is a plateau with an average elevation of 511 m above sea level and located between 110° 40' - 110° 70' East Longitude and 7° 28'-7° 46' South Latitude. The horticultural commodities provide 40% of total regional income. The flagship product are cucurbits which include cucumber, melon, watermelon, pumpkin, chayote, angled luffa and bitter melon (Statistics Bureau of Indonsia, 2014). Recently, many cucurbits crops in the region suffered a kind of disease showing symptoms of viral infections such as mosaic, vein clearing, vein banding and malformation which are typical of CMV infection symptoms. The disease is very detrimental to farmers (Center for Monitoring Pests and Plant Diseases of Karanganyar, 2014), but so far, basic information and research on the existence and distribution of CMV infecting cucurbits in the region is not yet available.

This research aimed to find out the presence and distribution of CMV infecting cucurbits in Karanganyar Regency. Therefore, the authors conducted a survey of the field, then observed crops showing the symptoms as well as determined the disease incidence. They also picked up some samples of diseased crops representatively and brought them to the laboratory for detection of CMV using TAS ELISA. The results showed that CMV was detected in various species of cucurbits in Karanganyar Regency with evenly distribution.

MATERIALS AND METHODS

Study area

Karanganyar Regency consists of 17 sub-regions (districts), that is, Tasikmadu, Mojogedang, Colomadu, Gondangrejo, Kebakkramat, Jaten, Kerjo, Ngargoyoso, Jatipuro, Jenawi, Karanganyar, Karangpandan, Matesih, Tawangmangu, Jumantono, Jumapolo and Jatiyoso (Figure 1). During the survey (in the year 2014), cucurbits were found relatively evenly planted over the entire region. Cucurbits showing symptoms typical of CMV infection were also found distributed relatively evenly over the region. Firstly, 10 districts from where the data were collected were representatively determined. The districts were Tasikmadu, Mojogedang, Kerjo, Ngargoyoso, Karangpandan, Matesih, Tawangmangu, Jumantono, Jumapolo and Jatiyoso (Figure 1).

Selection of gardens, crops sampling, symptoms observation and visual assessment of disease prevalence and disease incidence

In each district, 5 field plots (gardens) of cucurbits crops having crops showing the symptoms of CMV infection were representatively determined as mentioned above. Disease prevalence was estimated according to Sydanmetsa and Mbanzibwa (2016) to obtain the percentage of the fields with at least one diseased crop as assessed visually (by symptoms observation). Then, disease incidence of each garden was estimated (computed) by dividing the number of crops with symptoms by the total number of crops on which observation were made per field. In each garden, one diseased crop was purposively determined. Then, each of the determined diseased crop was observed for symptoms and recorded.

Collection of leaf of sample crops

From each of the determined diseased crop in the above stage, young leaves showing the symptoms were cut off, inserted into plastic clip, labeled, and kept in ice box. After taking to the laboratory, the leaf samples were transferred to refrigerator prior next assay.

Detection of CMV by TAS ELISA

To ensure that the causal agent of the disease was CMV, sap of sample crops were immunoassay detected with CMV antiserum. Immunoassay was performed utilizing Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS ELISA) format using PathoScreen® Kit from Agdia Inc., following protocol from the manufacturer. Three leaf pieces of sample crops were grinded in extraction buffer at a 1:10 ratio (w/v). In brief, after sample wells were coated using polyclonal antibody, 100 µl of prepared samples were dispensed into sample wells. The plate was incubated for 2 h at room temperature, followed by 7 times washing with washing buffer. Here, monoclonal antibody mixture specific for general CMV was used as detection antibody. 100 µl of alkaline phosphatase conjugate was dispensed per well, then incubated for 2 h at room

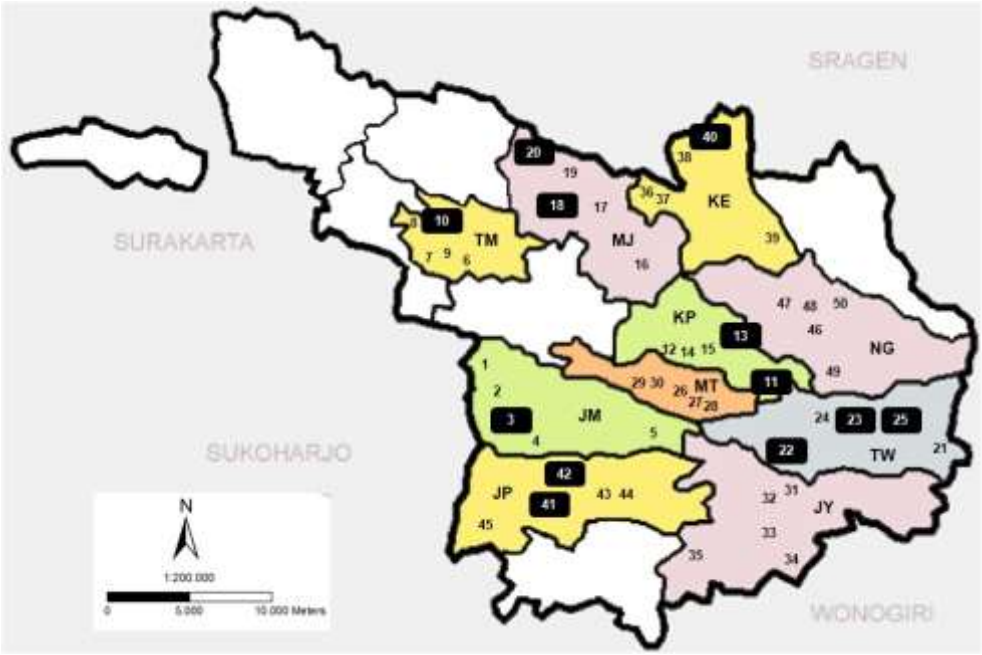

Figure 1. Map of Karanganyar regency and distribution of sample crops in the region. JM, Jumantono; TM, Tasikmadu; KP, Karangpandan; MJ, Mojogedang; TW, Tawangmangu; MT, Matesih; JY, Jatiyoso; KE, Kerjo; JM, Jumapolo; NG, Ngargoyoso. Numbers refer to serial number of sample crops or field plots. Numbers with dark backgrounds are sample crops in which CMV were detected (Table 1).

temperature, followed by 8 times washing with washing buffer. 100 µl of PNP substrate was dispensed into test well followed by incubation for 60 min (in dark). The developing color was observed by eyes, and the color intensity (absorbance value) was measured on an ELISA plate reader at 405 nm. The tested samples were stated as positively CMV infection if they have absorbance value of twice of the negative control.

RESULTS AND DISCUSSION

Variations of visual symptoms in the field

Fifty field plots (gardens) of cucurbits crops have been observed for their virus disease symptoms. The crops includes seven species of cucurbits, that is, cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), chayote (*Sechium edule*), angled luffa (*Luffa acutangula*) and bitter melon (*Momordica charantia*). Based on visual observations in the field, the cucurbits crops showed variation of symptoms which include: mosaic, vein clearing, vein banding and malformation (Table 1 and Figure 3). The symptoms are typical of CMV infection in cucurbits (Zitter and Murphy, 2009). However, these symptoms can also be caused by infection with other viruses in cucurbits, such as *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini lethal chlorosis virus* (ZLCV), *Zucchini yellow mosaic virus* (ZYMV) and other viruses (Ullman et al., 1991; Yuki et al., 2000; Lima et al., 2012; Romay et al., 2014; Sydanmetsa and Mbanzibwa, 2016).

Based on visual observations (Table 1), it can be seen that the cucurbits crops showing symptoms of viral infections (including CMV) are widely spread in Karanganyar Regency. The virus-like disease symptoms were seen in cucurbits in all of 10 fields that were surveyed. According to Sydanmetsa and Mbanzibwa (2016), this represented a disease prevalence of 100% as was assessed as the number of fields with at least a diseased plant in the total number of fields.

Visual assessment of virus disease incidence

Of the 50 field plots of cucurbits crops, their virus disease incidence were assessed based on visual observation of disese symptoms in the field (Table 1). From Table 1, it can be seen that the disease incidence varied among the field plots, ranged from 0.6 to 64%. The lowest disease incidence was found in the field number 40 of bitter melon crops in district Kerjo. In this field, bitter melon crops showed malformation and mosaic symptoms. The

Table 1. Crops sampling and symptoms observation of cucurbits crops in the field and the results of TAS-ELISA.

District	Field plot and location	Sample crop and serial number	Symptoms	Number of crops in a field plot	Number of diseased crops in a field plot	Disease Incidence	TAS-ELISA results		
							Color	Absorbance value	Judgment*
Jumantono	1. Sukosari	1. melon	malformation, mosic	27	3	11.1	clear	0.316	-
	2. Sukosari	2. cucumber	vein banding	135	5	3.7	clear	0.439	-
	3. Tugu	3. melon	mosaic	300	17	5.7	yellow	0.649	+
	4. Tugu	4. melon	mosaic, vein clearing	300	17	5.7	yellow	0.539	+/-
	5. Tunggulsari	5. melon	mosaic, vein banding	3850	27	0.7	yellow	0.526	+/-
Tasikmadu	1. Suruh	6. melon	malformation, vein banding	7200	67	0.9	clear	0.062	-
	2. Kaling	7. melon	mosaic, vein clearing	96	2	2.1	clear	0.391	-
	3. Karangmojo	8. melon	malformation, mosaic	560	5	0.9	yellow	0.573	+/-
	4. Ngijo	9. pumpkin	mosaic	12	4	33.3	clear	0.282	-
	5. Pandeyan	10. bitter melon	mosaic	1110	157	14.1	yellow	1.428	+
Karangpandan	1. Salam	11. cucumber	mosaic, vein banding	300	6	2.0	yellow	0.816	+
	2. Doplang	12. cucumber	malformation, vein clearing	180	3	1.7	clear	0.421	-
	3. Harjosari	13. Angled luffa	malformation, mosaic	80	4	5.0	yellow	0.694	+
	4. Doplang	14. melon	mosaic	8100	126	1.6	clear	0. 330	-
	5. Dayu	15. melon	malformation	56	1	1.8	clear	0.406	-
Mojogedang	1. Mojogedang	16. chayote	malformation	10	4	40.0	clear	0.377	-
	2. Pendem	17. cucumber	mosaic, vein clearing	233	9	3.9	clear	0.454	-
	3. Gentungan	18. cucumber	mosaic	340	15	4.4	yellow	0.839	+
	4. Munggur	19. angled luffa	mosaic	60	17	28.3	clear	0.384	-
	5. Munggur	20. angled luffa	mosaic, vein banding	180	3	1.7	yellow	1.876	+
Tawangmangu	1. Gondosuli	21. chayote	malformation	10	2	20.0	clear	0.444	-
	2. Tawangmangu	22. chayote	mosaic	28	4	14.2	yellow	0.84	+
	3. Blumbang	23. pumpkin	mosaic	167	7	4.2	yellow	2.106	+
	4. Blumbang	24. pumpkin	mosaic	295	11	3.7	clear	0.308	-
	5. Kalisoro	25. cucumber	mosaic	560	4	0.7	yellow	0.742	+
Matesih	1. Karangbangun	26. cucumber	mosaic	430	8	1.9	yellow	0.595	+/-
	2. Karangbangun	27. cucumber	malformation, mosaic	250	9	3.6	clear	0.381	-
	3. Koripan	28. cucumber	mosaic	560	13	2.3	clear	0.362	-
	4. Matesih	29. cucumber	mosaic	148	6	4.1	clear	0.39	-
	5. Matesih	30. cucumber	mosaic	80	3	3.8	clear	0.375	-
Jatiyoso	1. Beruk	31. chayote	malformation	20	14	70.0	clear	0.358	-
	2. Beruk	32. chayote	malformation, mosaic	15	7	46.7	clear	0.347	-
	3. Wonorejo	33. chayote	mosaic	24	9	37.5	clear	0.343	-
	4. Wonokeling	34. chayote	mosaic	12	2	16.7	clear	0.357	-
	5. Jatiyoso	35. chayote	mosaic	18	5	27.8	clear	0.362	-

Table 1. Contd.

Kerjo	1. Kutho	36.	watermelon	malformation	370	6	1.7	clear	0.395	-
	2. Kutho	37.	watermelon	malformation	200	16	8.0	clear	0.313	-
	3. Tawangsari	38.	watermelon	malformation	60	1	1.7	clear	0.353	-
	4. Plosorejo	39.	bitter melon	mosaic	48	4	8.3	clear	0.439	-
	5. Sumberejo	40.	bitter melon	malformation, mosaic	60	9	0.5	yellow	0.66	+
Jumapolo	1. Kwangsan	41.	cucumber	mosaic	280	7	2.5	yellow	0.646	+
	2. Bakalan	42.	cucumber	mosaic	440	13	3.0	yellow	0.85	+
	3. Jumapolo	43.	cucumber	malformation, vein banding	44	1	2.3	clear	0.321	-
	4. Jumapolo	44.	bitter melon	malformation	38	17	44.7	clear	0.326	-
	5. Lemahbang	45.	watermelon	malformation	525	7	1.3	clear	0.302	-
Ngargoyoso	1. Girimulyo	46.	chayote	mosaic	15	4	26.7	clear	0.378	-
	2. Kemuning	47.	chayote	malformation, mosaic	25	16	64.0	clear	0.472	-
	3. Segorogunung	48.	chayote	malformation, mosaic	18	6	33.3	clear	0.306	-
	4. Berjo	49.	chayote	malformation, mosaic	10	1	10.0	clear	0.122	-
	5. Segorogunung	50.	cucumber	malformation, mosaic	40	2	5.0	clear	0.219	-
Standard	CMV infected		nd*	nd	nd	nd	nd	yellow	0.539	+
	CMV free		nd	nd	nd	nd	nd	clear	0.326	-
	Extraction buffer		nd	nd	nd	nd	nd	clear	0.452	-

*****Annotation: +, Positif; -, Negatif; nd, no data. Blue columns are that the sample was CMV detected by TAS-ELISA.

highest disease incidence was found in the field number 47 of chayote crops in district Ngargoyoso. In this field, chayote crops also showed malformation and mosaic symptoms.

From Table 1, it can be seen that the disease showing symptoms tipical of virus infection had suffered cucurbits crops in Karanganyar Regency with disease incidence of moderate level, referring to a scale of Sydanmetsa and Mbanzibwa (2016). This corroborates the reports of Statistics Bureau of Indonsia (2014) and Center for monitoring pests and plant diseases of Karanganyar (2014) which reported that disease with such symptoms of virus infection had recently been seen in cucurbits in the regoin and caused economic loss to farmers.

Presence of CMV in sample crops based on TAS ELISA

Of the 50 field plots of cucurbits observed, from each was taken the best to represent sample crop. The sample crops were then visually observed and the symptoms were recorded. From parts of each sample crop (leaf pieces) were detected the presence of CMV by TAS ELISA using antibody for general CMV. The results of ELISA are presented in Table 1 (Figure 2). The table shows that of the 50 sample crops showing CMV infection symptoms, there were only 12 sample crops in which CMV was detected. The details are as follows: 1 melon crop showing mosaic; 2 bitter melon crops showing mosaic and malformation; 5 cucumber crops showing mosaic and vein banding; 2 angled luffa crops showing malformation, mosaic and vein banding; 1 chayote crop showing mosaic, and 1 pumpkin crop showing mosaic (Figure 3).

From Table 1 and Figure 3, it can be seen that the symptoms may vary between crops in the

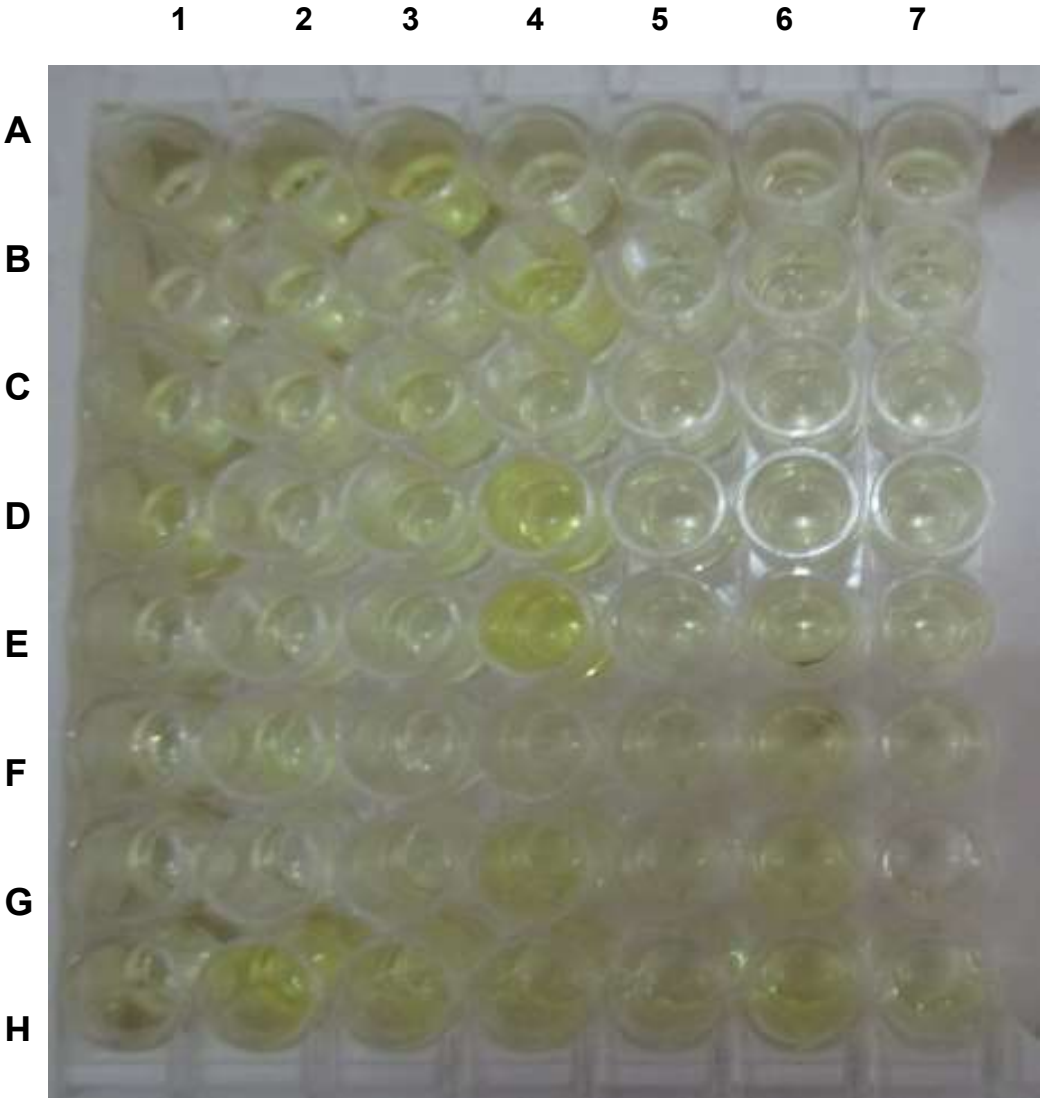

Figure 2. Visual appearance of TAS ELISA result. A1 (extraction buffer), B1 (negative control), D1 (positive control), G1 (1), H1 (2), A2 (3), B2 (4), C2 (5), D2 (6), E2 (7), F2 (8), G2 (9), H2 (10), A3 (11), B3 (12), C3 (13), D3 (14), E3 (15), F3 (16), G3 (17), H3 (18), A4 (19), B4 (20), C4 (21), D4 (22), E4 (23), F4 (24), G4 (25), H4 (26), A5 (27), B5 (28), C5 (29), D5 (30), E5 (31), F5 (32), G5 (33), H5 (34), A6 (35), B6 (36), C6 (37), D6 (38), E6 (39), F6 (40), G6 (41), H6 (42), A7 (43),B7 (44), C7 (45), D7 (46), E7 (47), F7 (48), G7 (49), H7 (50). Numbers in the brackets are the serial numbers of sample crops.

same species but similar symptoms may show in crops in different species. Several explanations could be given as follows. Theoritically, the appearance of viral infection symptoms of a crop in the field can vary depending on many factors. The main factors affecting the development of symptoms in the field include the virus strains, species/varieties of host crop, crop age and environmental factors (Green, 1984; Zitter and Murphy, 2009; Hull, 2014).

The other possible causes of variations in symptom is the presence of mixed infections. There were some reports of mixed infections in cucurbits causing variations of symptoms in the field. In Tanzania, Sydanmetsa and Mbanzibwa (2016) reported that mixed infections of three viruses, CMV, ZYMV and WMV on cucurbits caused varied symptoms that were mosaic, leaf curling, wrinkled leaves, stunted growth, green vein banding, yellow spots and yellow mottling. For examples, mixed infection in pumpkins caused mosaic, rugosity and green veinbanding; in cucumber caused green vein banding, yellowing and

Figure 3. Symptoms variation of various sample crops of cucurbits which was CMV detected. Numbers refer to the serial number of sample crops. The description of the symptoms are as follows: sample no 3, mosaic; sample no 10, mosaic; sample no 11, mosaic, vein banding; sample no 13, malformation, mosaic; sample no 18, mosaic; sample no 20, mosaic, vein banding; sample no 22, mosaic; sample no 23, mosaic; sample no 25, mosaic; sample no 40, malformation, mosaic; sample no 41, mosaic; sample no 42, mosaic (Table 1).

mosaic; whereas in watermelon, it caused leaf deformation. Similar phenomenon was also reported by Barbosa et al. (2016) in Brazil, in which mixed infection of CMV, WMV, ZYMV and PRSV on cucurbits showed a variety of symptoms in the field.

In Java and Central Java, there have also been reported mix infections of viruses in Cucurbits. Daryono and Natsuaki (2009) reported mix infection of CMV, *Cucumber green mottle mosaic virus* (CGMMV) and *Kyuri green mottle mosaic virus* (KGMMV) on melon and other cucurbits which induced a variety of mosaic symptoms. Septariani et al. (2014) also reported that a

variety of symptom including mosaic, chlorotic spotting, leaf curling, blistering, vein banding, reduction and distortion of leaf and fruit were observed on cucurbit which had mixed infected with *Squash mosaic comovirus* (SqMV), ZYMV and CMV. Variations of symptoms on cucurbits with mixed infected by viruses has also been reported in Yogyakarta by Somowiyarjo et al. (1993).Not all plants showing typical symptoms of CMV was detected as having CMV. The first possibility is that the crops were infected by distinct strains of CMV. Zitter and Murphy (2009) reported that distinct strains of viruses could differ serologically. The second possibility is that the crops were infected by other distinct cucurbits viruses. Many authors reported that cucurbits could be infected by many viruses, commonly CMV, PRSV, ZYMV, WMV and others both in single or mix infection that induce a variety of symptoms including symptoms of CMV infection such as mosaic, vein clearing, vein banding and malformation (Zitter and Murphy, 2009; Romay et al., 2014; Barbosa et al., 2016; Sydanmetsa and Mbanzibwa, 2016).

Distribution of CMV in the field

The result of ELISA indicated that CMV was detected in 7 districts out of 10 selected districts (Table 1 and Figure 1). From Figure 1, it can be said that CMV is distributed evenly in Karanganyar Regency. The first explanation that could be given is that CMV is seed borne virus. In general, Zitter and Murphy (2009) and Palukaitis et al. (1992) reported that CMV is a seed-borne virus in many crops with varied transmission rate. In India, Abdullahi et al. (2001) reported the efficiency of CMV transmission through seed in beans was 30%. In Indonesia, Nurhaelena (2013) reported that CMV was seed transmitted in cucurbits such as cucumber, squash, melons with efficiency ranged from 2 to 12%. In Karanganyar where this survay was conducted, many farmers used seeds for growing cucurbits which were derifed from the previous cucurbits crops (Center for Monitoring Pests and Plant Diseases of Karanganyar, 2014). Thus, the infected crops are sources of inoculums for the next growing crops. The second explanation that could be given is that CMV is insect borne. CMV has many insect vectors and the vectors have a broad host range (including within cucurbits). Palukaitis et al. (1992) and Zitter and Murpgy (2009) reported that in general, more than 80 aphid species could transmit CMV. Transmission efficiency varies with the aphid species, virus strains, host plant species, environmental conditions and time of the year. The authors also reported that CMV is transmitted mainly by the green peach aphid, *Myzus persicae*, and by *Aphis gossypii*. In Karanganyar, from the rough sampling done during the survey, some species of insect vectors such as Aphids and Myzus (Unpublished data) were found.

From the aspect of host plant species, CMV also spread relatively evenly among the species of cucurbits. Of the seven species of host crops which were selected for observation, that is, cucumber, melon, watermelon, pumpkin, chayote, angled luffa and bitter melon, CMV was detected in all species except in watermelon. It is suggested that, in addition to the relatively few number of sample crop for watermelon (4 out of 50 sample crops), their distribution were also relatively clustered, this made the crops to get small probability to get virus through insect vectors.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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