DETECTION AND IDENTIFICATION OF CUCURBIT VIRUSES IN YOGYAKARTA *)

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Intisari

Intensifikasi budidaya labu-labuan ternyata diikuti oleh peningkatan jumlah tanaman yang menderita sakit yang diduga disebabkan oleh virus. Untuk mengetahui jenis virus penyebab mosaik tersebut, 83 sampel tanaman yang menunjukkan gejala mosaik atau gejala yang sejenis dikumpulkan dari Daerah Istimewa Yogyakarta dan sekitarnya dari September 1991 sampai Mei 1992. Sampel yang terkumpul direaksikan dengan antibodi poliklonal terhadap *cucumber mosaic virus* (CMV), *watermelon mosaic virus* 2 (WMV-2), *watermelon strain of papaya ringspot virus* (PRSV-W) dan *zucchini yellow mosaic virus* (ZYMV) dalam dot-immunobinding assay (DIA).

Antibodi terhadap CMV bereaksi dengan 31 sampel timun, waluh, semangka, gambas dan pare. Antibodi terhadap WMV-2 bereaksi dengan 29 sampel waluh dan semangka. Tiga sampel tanaman waluh dan semangka bereaksi dengan antibodi terhadap PRSV-W, sedangkan 10 sampel wuluh dan pare bereaksi dengan antibodi terhadap ZYMV. Empat dari 20 sampel waluh yang bereaksi positif dengan antibodi terhadap WMV-2 juga bereaksi dengan antibodi terhadap CMV.

Data yang diperoleh memberikan petunjuk bahwa di daerah penelitian terdapat paling sedikit empat jenis virus yang dapat membahayakan budidaya labu-labuan. Untuk mengurangi kerugian akibat serangan virus tersebut, disarankan agar sanitasi lingkungan ditingkatkan, terutama dengan cara tidak membiarkan tumbuh tanaman *cucurbitaceae* yang terinfeksi virus.

Kata kunci: *Cucurbitaceae*, potyvirus, dot-immunobinding assay.

Abstract

While the demand for high quality of cucurbit products increases, the commercial production of the crop has been increasingly faced with unknown viruses causing mosaic. To identify the viruses, 83 samples of cucurbit plants showing foliar mosaic or related symptom were collected from fields in Yogyakarta Special Territory from September 1991 to May 1992. The samples were then reacted with rabbit polyclonal antibodies against cucumber mosaic virus (CMV), watermelon mosaic virus 2 (WMV-2), papaya ringspot virus strain watermelon (PRSV-W) and zucchini yellow mosaic virus (ZYMV) in indirect dot immunobinding assay (I-DIA).

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CMV was detected in 31 samples of *Cucumis sativus, Cucurbita maxima, Citrullus lunatus, Luffa cylindrica,* and *Momordica charantia*. Of these 31 samples, 4 samples were infected doubly by WMV-2. WMV-2 was detected in 29 samples of *C. maxima* and *C. lunatus,* while PRSV-W was detected in 3 samples of *C. maxima* and *C. lunatus*. Ten samples of *C. maxima* and *L. cylindrica* were infected by ZYMV only.

The results indicated that in addition to CMV, at least three potyviruses, namely WMV-2, PRSV-W and ZYMV, are potentially importance in this area. This is the first report on the occurence of cucurbitaceous potyviruses in Yogyakarta.

While the characteristics of the viruses must be further studied, sanitary programme should be improved to minimize the yield losses caused by the viruses.

Key words: potyvirus, cucurbits, dot immunobinding assay.

Introduction

More than 50 viruses belonging to 11 virus groups, namely comovirus, cucumovirus, nepovirus, necrovirus, tymovirus, geminivirus, tobamovirus, carlavirus, rhabdovirus, tomato spotted wilt virus and potyvirus, have been reported to be able to infect naturally or experimentally to one or more species of cucurbits (Lovisolo, 1980; Honda *et al,* 1988), of which only cucumber mosaic virus (CMV) is known previously to infect the commercialiy grown cucurbits in Yogyakarta. In fact, CMV is one of the common viruses attacking cucurbits with an extensive host range. Several strains of CMV are usually present in the same area and some strains are known to be specialized to certain cucurbits.

In Yogyakarta, commercial production of cucurbits specifically, cucumber (*Cucumis sativus* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. *et.* Nakai] and melon has significantly increased in the last 15 years. Since that time, the cucurbit growers cite viruses as one of their most important problems.

This study was undertaken to asses the curent composition of cucurbitaceous viruses in Yogyakarta, and more specifically to determine the possible presence of potyviruses.

Materials and Methods

Sample collection.-- Foliar samples, including a young leaf and a fully expanded mature leaf, were collected in Yogyakarta from cucurbit plants in commercial fields from September 1991 to May 1992. The number of samples collected from Kotamadya Yogyakarta, Bantul, Sleman and Kulon Progo were 11, 8, 16, and 30, respectively.

Additionally, a number of 18 samples were also collected from five fields in Central Java.

Polyclonal antibodies.--- Rabbit polyclonal antibodies against ZYMV, WMV-2, PRSV-W and CMV were the same as those employed in the previous papers (Somowiyarjo *et al,* 1985; 1986, 1989). All the antibodies were kindly provided by Prof. Nobumichi Sako of the Saga University, Japan.

The assays were done using crude antisera diluted with extract of healthy plants at their respective titer. Alkaline phosphatase-labelled affinity purified goat anti rabbit IgG (Sigma) was kindly provided by Dr. Keiko T. Natsuaki of the Tokyo University of Agriculture, Japan.

Dot-immunobinding assay (DIA).-- Nomenclature of the reagents and solutions of DIA was adopted from those described by Hibi and Saito (1985). The assays were carried out on nitrocellulose membranes (NCM) (BioRad) which were cut to appropriate size and marked with a grid of 1 × 1 cm using a soft pencil. They were then dipped in 0.02 M Tris-HCl buffer containing 0.5 M NaCl, pH 7.5 (TBS) for 15 min and dried on filter paper for 5 min before deposition of the test antigen (sample).

The protocol of DIA was the same as that adopted, by Bantari and Goodwin (1985) with assay condition as that modified by Somowiyarjo *et al.* (1989). Throughout the experiment, only the antigen (2 μl) was applied individually to each grid of NCM by using a micropipet. Other reagents were applied to NCM by transferring the NCM onto a glass plate and covered with about 20 μl of reagent/grid. All reagents were incubated at room temperature (about 25 C). Following the first incubation on NCM with antigen (field sample) in 0.05 M carbonate buffer, pH 9.6, for 5 min, the unoccupied binding site on NCM was saturated by incubation with 3% bovine serum albumin (BSA) (Sigma) in TBS (blocking buffer) for 30 min. The NCM was then washed by rinsing briefly in distilled water and shaking gently for 20 min in each of two changes of TBS containing 0.05% Tween 20 and 2% polyvinylpyrrolidone (TBS-TPO). The washed NCM was further incubated sequentially with virus-specific antibody and Alkaline phosphatase-labeled affinity-purified immunoglobulin from goat antiserum against rabbit IgG. The incubation time for each reagent was 60-90 min, and washing procedure between the step was the same as that after incubation with blocking buffer. Finally, the NCM was incubated with color development solution which was prepared by mixing fast red TR salt (Sigma) and naphthol AS-MX phosphatase (Sigma) as recommended by Bantari and Goodwin (1985). The reaction was

stopped within 30-60 min by washing the NCM in distilled water, and the NCM was then air-dried for visual observation and storage.

Enzyme-linked immunosorbent assay (ELISA).-- Indirect-ELISA was done principally with a method of Koenig (1981) with the assay condition as that used in the previous report (Somowiyarjo *et al.*, 1985). The assays were carried out in polystyrene microtitre plates (Immunolon II). Three times of 3-minute washing were done between each step of the assays. The washing solution was 0.02 M PBS, pH 7.4, containing 0.05% Tween 20 and 0.5% polyvinyl pyrrolidone 40,000 (PBS-TPO). The plates were successively incubated with: (1) antigen (field sample) in 0.05 M carbonate buffer, pH 9.6, for 4 h at 25 C; (2) virus-specific antiserum diluted in PBS-TPO for 18 h at 6 C; (3) enzyme-labeled conjugate in 0.02 M PBS, pH 7.4 for 4 h at 25 C; and (4) 1 mg/ml of p-nitrophenyl phosphatase in 10% of diethanolamine, pH 9.8 for 1.5 h at 25 C. The ELISA absorbance values were measured with ELISA reader (Multiskan) at a wavelength of 405 nm.

Results and Discussion

1. Effect of extraction buffer.-- Despite the wide applicability of DIA, its effective use requires precise knowledge of experimental conditions. Extraction buffer is one of the critical condition of DIA because it influences the availability of antigen and compounds that may have any deleterious effects on interaction of antigen-antibody. Therefore, three kinds of extraction buffers, namely 0.05 M sodium carbonate buffer, pH 9.6, PBS and TBS, were examined to provide an optimum condition of virus detection.

It was found that the three extraction buffers tested were suitable for DIA (data not shown). The result was in agreement with the finding from studies of other serological techniques that show the usefulness of the three buffers to extract antigen from infected tissues (McLauglin and Barnet, 1979; Somowiyarjo *et al., 1985).*

2. Comparison of DIA and ELISA.-- As a tool for virus detection, ELISA has been found to be very sensitive and specific. The following experiment was conducted to compare the applicability of DIA and ELISA for detecting cucurbit viruses. The experiment was done by comparing the reactivity of 8 field samples with the same antisera in the two serological tested. The data given in table 1 showed that DIA was applicable to detect the viruses tested.

The principles of DIA are almost the same as those of ELISA, differing only in that antigen or antibody is absorbed to nitro-

cellulose membrane (NCM) and that the product of the enzyme reaction is insoluble (Hawkes *et al.*, 1982; Hibi and Saito, 1985; Banttari and Goodwin, 1985), DIA, although still undergoing modification and development. It has several advantages over ELISA which make the technique seems especially appropriate for large-scale application. The assay is rapid and simple in operation, and does not require any special equipment (Lange, 1986). Unlike ELISA which needs relatively large volume of samples (100-200 μl), DIA requires only minute amount of samples (1-2 μl) per assay. In addition, DIA can be employed for diagnosis by post (Somowiyarjo, 1992) which is very suitable for the use in the developing countries. These advantages have contributed to the increasing use of DIA for detecting many plant viruses (Powell, 1987; Smith and Banttari, 1987; Somowiyarjo, 1989, 1992).

3. Distribution of cucurbit virus.-- Data in table 2 show that the composition of cucurbit virus varied among the crops tested. One the basis of these results, the viral disease of cucurbit in Yogyakarta are caused by CMV, WMV-2, PRSV-W and ZYMV and other unknown virus. Among was them, the only virus intensively studies was CMV (Semangun, 1989). This woork was the first report of cucurbitaceous potyviruses, namely WMV-2, PRSV-W and ZYMV in Yogyakarta.

Although in the agroecosystem the status of pathogen always varies from time to time, recently potyviruses have been reported as ''key pest'' in several cucurbit cultivating areas (Namely *et al.*, 1986; Sako *et al.*, 1989). Therefore, the task of managing these viruses is critical to cucurbit production. It is clear that management of the diseases cannot rely on a single method and that complete control will not possible. An integrated program employing any or all combinations of available methods should be explored. While such program has not been available, reducing the virus inoculum by destroying infected crops and wild cucurbits in the vicinity of production area is strongly suggested.

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Table 1. **Comparison of enzyme-linked immunosorbent assay (ELISA) and dot-immunobinding assay (DIA) for detecting cucumber mosaic virus (CMV) watermelon mosaic virus 2 (WMV-2) watermelon strain of papaya ringspot virus (PRSV-W) and zucchini yellow mosaic virus (ZYMV) in cucurbit samples collected from Yogyakarta.**

No. Sample	Reactivity to antisera against *)			
	CMV	WMV-2	PRSV-W	ZYMV
36	0,208/ –	0.631/ +	0.291/ –	0.988/ +
38	0.212/ –	0.281/ –	0.185/ –	0.457/ –
39	0.145/ –	0.346/ –	0.178/ –	0.591/ +
48	0.227/ –	0.517/ +	0.334/ –	1.112/ +
61	0.414/ –	0.518/ +	0.412/ –	0.850/ +
69	0.208/ –	0.207/ –	0.742/ +	0.708/ +
82	0.204/ –	0.181/ –	0.179/ –	0.634/ +
Healthy (control)	0.208/ –	0.151/ –	0.159/ –	0.210/ –

*)Notes: Numerator : Values of ELISA absorbance
Denumerator = The results of visual observation on nitrocellulose membrane after processed in DIA (– = no reaction; + = positive reaction).

Table 2. **Distribution of cucurbit viruses in the different crops in Yogyakarta as determined by dot-immunobinding assay.**

Crops	No. of samples tested	No. of samples infected with				
		CMV	WMV-2	PRSV-W	ZYMV	Unknown
Cucumis sativus	5	4	0	0	0	1
Cucurbita maxima	34	9	20 (4)*	2	2	5
Citrullus lunatus	22	6	9	1	0	6
Luffa cylindrica	13	4	0	0	8	1
Momordica charantia	9	8	0	0	0	1
Total	83	31	29 (4)	3	10	14

*Doubly infected with CMV.