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DISEASE INCIDENCE OF MELON LEAF CURL IN EAST JAVA AND SPECIAL PROVINCE OF YOGYAKARTA

KEJADIAN PENYAKIT DAUN KERITING MELON DI JAWA TIMUR DAN DAERAH ISTIMEWA YOGYAKARTA

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ABSTRACT

Geminivirus infection has caused severe losses on various economically important crops. The disease incidence on melon has been observed since 2004, and widespread at melon production centers in East Java and Special Province of Yogyakarta. In East Java and Special Province of Yogyakarta, the Disease Incidence of leaf curl on melon reached 100% and 14.3 % respectively in 2008. Detection of the causal agent using primers of CPA5 and CPA2 resulted viral specific DNA fragment of 770 bp.

Key words: Disease Incidence, Geminivirus, Melon leaf curl

INTISARI

Infeksi penyebab penyakit yang disebabkan oleh geminivirus telah menyebabkan kerugian secara ekonomi berbagai jenis tanaman penting yang dibudidayakan. Kejadian penyakit pada tanaman melon telah diamati sejak tahun 2004, dan tersebar secara luas di pusat penanaman melon di Jawa Timur maupun Daerah Istimewa Yogyakarta (DIY). Di Jawa Timur dan Daerah Istimewa Yogyakarta (DIY), pada tahun 2008 kejadian penyakit daun keriting melon mencapai 100% dan 14,3%. Penyebab penyakit telah dideteksi menggunakan teknik polymerase chain reaction. Amplifikasi Fragmen DNA virus dari tanaman yang terinfeksi dihasilkan dengan ukuran 770 bp menggunakan sepasang primer CPA5 dan CPA2.

Kata kunci: daun keriting melon, geminivirus, kejadian penyakit

INTRODUCTION

The disease incidence of melon yellow leaf curl has been known since 2004 in East Java and the Special Province of Yogyakarta (Julijantono, 2005). The symptoms of the disease are yellowing, curling, and stunting. The causal agent of disease was transmitted by tobacco whitefly *Bemisia tabaci* (Genn) (Hemiptera: Aleyrodidae) with its abundant population in melon production centers occurred in the dry season. Preliminary symptomatological studies suggested that the disease was associated with geminivirus (Julijantono, 2006).

The particle morphology of geminivirus group is commonly different from other plant virus diseases. It is geminate and isometric (Bock, 1982) with single stranded (ss) DNA (Harrison, 1985; Lazarowits, 1987). Based on its genome structure, vector transmitted, and host plants, geminivirus can be divided into four genera, i.e. Mastrevirus, Curtovirus, Begomovirus, and Topocuvirus (van Regenmortel *et al*., 2000; Hull, 2002). Begomovirus is a geminivirus with dicotyl host plant, transmitted by whitefly (*B. tabaci)* and has bipartite or monopartite genome (Harrison, 1985).

The disease caused by Begomovirus can be spread faster, because Begomovirus was transmitted by *B. tabaci* as a vector. The development of effective, sensitive, and fast detection method would be useful to prevent the endemy of this disease (Rojas *et al.*, 1993). Detection method based on nucleic acid analysis has been used to identify and to detect Begomovirus widely. Nucleic acid hibridization technique (Polston *et al.*, 1989; Gilbertson *et al*., 1991; Hidayat *et al*., 1993; Bendahmane *et al*., 1995) and polymerase chain reaction (PCR) technique using universal primer can be used to identify Begomovirus from different plants and different places (Chiemsombat *et al.*, 1990; Rojas *et al*., 1993; Wyatt & Brown, 1996; Roye *et al*., 1997; Hidayat *et al*., 1999; Sudiono *et al*., 2004). The main crops infected by geminivirus transmitted whitefly are tomato, potato, eggplant, hotpepper, okra, cassava, common bean, lima bean, mung bean, cowpea, blackgram, watermelon,

squash, and melon (Muniyapa, 1980; Brown, 1994).

At present, Begomovirus has infected the family of Cucurbitaceae world wide. For example, disease incidence caused by Squash Leaf Curl Virus (SLCV) has been reported by Duffus and Stenger (1998), Cucurbit Leaf Curl Virus (CuLCV) with another name Cucurbit Leaf Crumple Virus (CuLCrV) by Brown *et al*. (2000), Melon Chlorotic Leaf Curl Virus by Brown *et al*. (2001), and Squash Yellow Mottle Virus in America by Karkashian *et al*. (2002). In Zacapa valley Guatemala, the disease occurred in 2000 in the melon production centers with incidence reached 70–80% (Brown *et al*., 2001). In Asia, the characteristic of Begomovirus has not been well documented (Mansoor *et al*., 2000; Samretwanich *et al*., 2000; Khan *et al*., 2001; Muniyappa *et al*., 2003). The aims of the research were to document the disease incidence of melon leaf curl in the melon production centers in East Java and the Special Province of Yogyakarta and to develop a method for the detection of melon leaf curl.

MATERIALS AND METHODS

Disease Incidence and Infected Plants Collection

Disease incidence observations were carried out by a direct survey in East Java and the Special Province of Yogyakarta. In East Java, the survey was done in Ngawi and Ponorogo Regencies, while in the Special Province of Yogyakarta, the survey was done in Bantul, Sleman, and Kulonprogo Regencies, in March and June 2008. In melon production centers, usually the farmers grow melon two times up to three times every year. The farmers commonly start the first planting in February, the second planting in May and the third planting in September. The disease incidence in this study was observed at the age of 35 days up to 55 days after planting. For five locations, observation was done at least in one location, and from one location, five sectors were taken. One sector was an area of 20 m^2 consisting of two rows of melon population. The disease incidence was calculated as:

$$I = \frac{\text{Total infected plants}}{\text{Total plants observed}} \times 100\%$$

Observation was done based on the symptom variation of disease, i.e. curling, yellowing, and stunting. Infected melon leaves from each location were collected for PCR detection.

Disease Detection with PCR Method

DNA extraction was done by modified Dellaporta *et al.* (1983). Leaf samples with ± 50 mg in weight were placed on 2 ml microtube, and 500 µl buffer extract containing Tris 0.1 M (pH 8), EDTA 0.05 M, NaCl 0.5 M and 5 µl β-mercapto ethanol was added. Then, the leaf samples were extracted using micropestle and 33 µl SDS 20%, was added and mixed with vortex, and incubated for 10 minutes at 65°C. 160 µl potassium acetate (KoAC) 5 M was added and mixed with vortex and centrifuged 12.000 rpm at 4°C for 5 minutes. Supernatant was taken and 0.5 volume cold iso-propanol was added and gently mixed. Then, centrifuged 12.000 rpm at 4°C for 15 minutes. The pellet was washed with an addition of 500 µl ethanol 70% and centrifuged 12.000 rpm at 4°C for 5 minutes. After centrifugation, the pellet was dried in a vaccum dessicator for 15 minutes, then 50 µl sterile ddH_2O was added.

PCR method was used to amplify gemini-virus genome with CPA5 (5'-ATGTCGAAGCGTCCA GCAGA-3') and CPA2 (5'-TTAATTCGTCACT-GAGTCAT- 3') primers. DNA amplification was done following Rojas *et al*. (1993). Every PCR reaction with a total volume of 20 µl contained: 1 µl DNA, 1 µl CPA5 and CPA2 primers, 0.1 µl *Taq polymerase*, 2.0 µl 10 X PCR buffer, 0.4 µl dNTP Mix 10 mM, 0.8 µl $MgCl_2$ 50 mM and dd H_2O 13.7 µl. DNA amplification with PCR system ABI 9700 contained 30 cycles and three steps as follows. DNA separation at 94°C for 3 minutes, primers annealing at 50°C for 30 seconds and DNA synthesis at 72°C for 1 minute (Rojas *et al*., 1993). In the last cycle, 10 minutes was added and it was saved at 4°C.

PCR products were run on 1% gel agarose in buffer Tris-borate EDTA (TBE) 0.5 X at 75 volt (Maniatis *et al*., 1989) and visualized on UV transiluminator after soaking in 0.5 µl ethidium bromide.

RESULTS AND DISCUSSION

Disease Incidence of Melon Leaf Curl

Disease incidence of melon leaf curl, in the melon production centers in East Java (Ngawi, Ponorogo) and Special Province of Yogyakarta (Sleman, Bantul, Kulonprogo) were different. In 2008, disease incidence in East Java ranged from 0 to 100% while in the Special Province of Yogyakarta it ranged from 0 to 14.3% (Table 1 and Table 2). One of the factors that might have been contributing to the different in level of disease

incidence was the common practice of the farmers. Farmers in East Java continuously plant melon all year around while in DIY commonly farmers plant melon after paddy planting. Disease Incidence at the second planting on June is higher. In melon production centers, the factors that affected the distribution of disease caused by Begomovirus are the high population of vector, availability of inoculum source from infected plants at the first planting, susceptible varieties, continuous melon planting, vector migration from other plants, and seedling infection.

Disease Detection with PCR Method

Disease detection of melon leaf curl with PCR method using CPA5 and CPA2 primers produce DNA fragment of 770 bp (Figure 1). Blast analysis

Table 1. Disease incidence of melon leaf curl in several melon production centers in March 2008

No.	Location				Variety	Population*	Age (DAT)	Disease Incidence (%)
	Province	District	County	Village				
1.	East Java	Ngawi	Pitu	Pitu	Action	3500	21	0
					Leader	3500	21	0
2.	East Java	Ngawi	Paron	Jambe	Action	6000	35	1.25
3.	East Java	Ngawi	Paron	Jambe	Action	13000	33	0
4.	East Java	Ngawi	Paron	Blego	Action	4000	14	0
5.	East Java	Ngawi	Paron	Gelung	Action	3000	50	0.7
					Japonica	3000	50	1.4
6.	East Java	Ngawi	Geneng	Sb. Rejo	Action	16000	22	6.1
7.	East Java	Ngawi	Kasreman	Geneng	Action	11000	25	73.3
8.	East Java	Ngawi	Kasreman	Geneng	Action	4000	25	31
9.	East Java	Ngawi	Kasreman	Geneng	Action	4000	25	33.2
10.	East Java	Ngawi	Sukorejo	Sukorejo	Action	1300	60	100
11.	East Java	Ngawi	Sukorejo	Sukorejo	Action	5000	55	100
12.	Special Province of	Sleman	Moyudan	Moyudan	M-1000	50000	50	0
13.	Special Province of Yogyakarta	Bantul	Srandaan	Babakan	M-1000	3000	32	17
14.	Special Province of Yogyakarta	Bantul	Srandaan	Babakan	M-1000	3000	32	5
15.	Special Province of Yogyakarta	Bantul	Srandaan	Babakan	M-1000	6000	32	0
16.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	5250	40	0.8
17.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	2250	40	0
18.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	2250	40	0

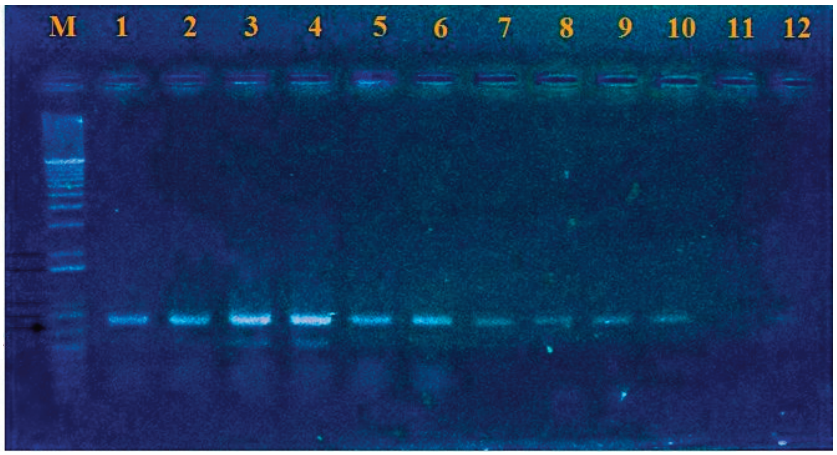

Figure 1. DNA amplification fragment resulted from several melon production areas using PCR method;. 1 kb DNA Marker (M), samples from Pitu Ngawi (1), Paron Ngawi (2), Kasreman Ngawi (3), Geneng Ngawi (4), Babadan Ponorogo (5), Lengkong Sukorejo Ponorogo (6), Nampan Sukorejo Ponorogo (7), Sleman (8), Bantul (9), Kulonprogo (10), healthy plant (11), positive control (12)

Table 2. Disease incidence of melon leaf curl in several melon production centers in June 2008

No.	Location				Variety	Population*	Age (DAT)	Disease Incidence (%)
	Province	District	Subdistrict	Village				
1.	East Java	Ngawi	Paron	Gelung	Action	8500	22	17.3
2.	East Java	Ngawi	Paron	Gelung	Star	3000	28	5.4
3.	East Java	Ngawi	Paron	Gelung	Action	4000	21	1.4
4.	East Java	Ngawi	Paron	Gelung	Star	9000	36	5.9
5.	East Java	Ngawi	Geneng	Kersikan	Action	4000	30	73.4
6.	East Java	Ngawi	Geneng	Kersikan	Action	14000	42	100
					Star	16000	42	100
7.	East Java	Ngawi	Geneng	Gunting	Action	4000	50	100
8.	East Java	Ngawi	Geneng	Kersikan	Star	6000	52	77
9.	East Java	Ngawi	Geneng	Kersikan	Action	6000	55	66
10.	East Java	Ngawi	Geneng	Kersikan	Action	6000	55	98
11.	East Java	Ngawi	Geneng	Kersikan	Action	14000	32	26
12.	East Java	Ngawi	Geneng	Kersikan	Star	4000	36	100
13.	East Java	Ngawi	Geneng	Kersikan	Star	7000	40	30
14.	East Java	Ngawi	Geneng	Kersikan	Action	4000	40	100
15.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	3750	55	2.5
16.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	3750	58	14.3
17.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	3000	50	0
18.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	1500	50	2
19.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	1500	55	3.1
20.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	6500	55	0
21.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	Action	4500	45	0
22.	Special Province of Yogyakarta	Kulonprogo	Lendah	Gulurejo	M-1000	3500	50	0

* Population based on number of packages (number of seeds are 550–1000 for every package)

of PCR product sample from infected melon revealed that CPA5 and CPA2 primers amplified *squash leaf curl Philiphines virus*. This fragment was a specific geminivirus (Kuakoon, personal communication)

In this study we concluded that the causal agent of melon leaf curl in melon production centers was geminivirus. This was the first report on the high occurrence of disease in melon production centers. In East Java disease incidence ranged from 0 to 100% and in the Special Province of Yogyakarta it ranged from 0 to 14.3%.

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