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OCCURRENCE OF *CHILI VEINAL MOTTLE VIRUS* (CHIVMV) IN INDONESIA AND RESPONSE OF CHILI GERMPLASMS TO CHIVMV INFECTION

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ABSTRACT

Viral diseases are considered to be the major limiting factors in chili production. *Chili veinal mottle virus* (ChiVMV) is one of the important viruses, which decrease yield significantly. Infection of ChiVMV is associated with mosaic and mottle disease of chili in Indonesia. The distribution and incidence of ChiVMV is reported from major chili growing areas in West Sumatra, West Java, Central Java, East Java, and South Kalimantan based on field surveys conducted in 2008 to 2009. Screening of 29 chili accessions against ChiVMV based on symptomatology and disease incidence (%) under screenhouse conditions showed that the genotypes were classified into 5 reaction groups, i.e. Highly Resistant (IPB C1, IPB C10, and PBC 521), Resistant (IPB C8, IPB C14, IPB C17, and Keriting Sumatra), Moderately Susceptible (IPB C48, IPB C60, Tegar, Toro, and Taring), Susceptible (IPB C6, IPB C15, and Tanjung), and Highly Susceptible (IPB C13, IPB C20, IPB C21, IPB C24, IPB C33, IPB C55, IPB C73, IPB C81, IPB C99, Tornado, Andalas, Tegak, Beauty Bell, and Polaris). Further effort should be made to identify the resistance gene that might be incorporated in the breeding program to improve chili yield.

Key words: *Capsicum annuum*, chili pepper, disease resistance, ELISA

INTRODUCTION

Chili is an important and essential component of the daily Indonesian diet. It is also an important commercial crop grown year-round mainly by small-scale farmers both in high and lowlands under rain-fed as well as irrigated conditions. In 2007, it was cultivated on a total area of more than 200 thousand ha producing about 1.13 million of fresh weight with an average yield of 10 ton per ha [Directorate General of Horticulture, Ministry of Agriculture Indonesia, 2008]. The production of chili is limited by a wide range of diseases. *Chilli veinal mottle virus* (ChiVMV) is one of the important pathogens commonly found in chili plants in Indonesia besides *Pepper yellow leaf curl begomovirus* (PepYLCV), *Cucumber mosaic cucumovirus* (CMV), *Ralstonia solanacearum*, *Phytophthora capsici*, and *Colletotrichum* spp. (Mariyono and Bhattarai, 2009; Taufik *et al*., 2005).

Mottle and mosaic disease caused by ChiVMV infection was first reported from Malaysia by Burnett in 1947 (Brunt *et al*., 1996), but it is now known widely spread in many countries in Asia including Korea, Taiwan, Thailand, Indonesia, Papua New Guinea, Phillippine, China, Bangladesh, India, Nepal, Sri Lanka and also in Australia, West and East Africa (Davis *et al.*, 2002; Shah *et al.*, 2001 Taufik *et al.*, 2005; Womdin *et al.*, 2001). The incidence of ChiVMV infection may reach 30% and may cause yield loss up to 95% and 30% in sweet chili and small chili, respectively based on field surveys conducted by Green (2004) in 16 Asian countries. ChiVMV is easily transmitted in the field by many aphid species in a non persistent manner (Ong *et al*., 1979). Infection of ChiVMV causes various symptoms in chili including irregular dark green spot on the leaf (mottle), vein banding, leaf malformation (Latifah *et al*., 2008 Siriwong *et al.*, 1995; Tsai *et al*., 2008), and reduction of fruit size (Shah and Khalid, 2001).

During chili disease monitoring in 2005 ChiVMV and PepYLCV was found to be the most prevalent viruses especially in Java (Taufik *et al*., 2005). In view of this scenario, an effort was made to screen available chili germplasm (Latifah *et al*., 2008 Millah, 2007; Riyanto, 2007). Further investigation is required especially to study the distribution of ChiVMV in several chili growing areas in Indonesia since previous field survey was only conducted in limited regions. Continuous breeding effort should also be made to screen and evaluate available chili germplasm so that breeders could get resistant material to incorporate resistance gene in highly susceptible cultivars as well for farmers to improve chili yield. This paper reports the current incidence of ChiVMV on field-grown chili in Indonesia and screening results of 29 chili accessions against ChiVMV.

MATERIALS AND METHOD

Field surveys and leaf sampling

Field surveys were conducted from 2008 to 2009 in a total of 23 fields in major chili growing areas in West Sumatra, West Java, Central Java, East Java and South Kalimantan (Table 1). Leaf samples exhibiting symptoms such as mosaic, mottle, or leaf malformation were collected. Each sample from different plants were kept in plastic bags in ice boxes during the survey, and later at 4^0C in a refrigerator before serological test was performed using ChiVMV, CMV, PVY, PMMV and ToMV antisera.

Table 1. Number of tested and virus-infected chilli pepper fields in five regions (provinces) in Indonesia.

Location of collected samples	Number of chillipepper fields infected by a given virus*					No. of tested fields	No. of infected fields
	ChiVMV	CMV	PVY	TMV	PMMV		
West Sumatera	4	1	0	0	0	4	4
West Java	1	1	1	0	0	2	2
East Java	5	3	7	2	1	8	8
Central Java	4	1	0	1	0	4	4
South Kalimantan	2	1	0	1	0	5	2

**Chili veinal mottle virus* (ChiVMV), *Cucumber mosaic virus* (CMV), *Potato Y virus* (PVY), *Tobacco mosaic virus* (TMV), *Pepper mild mottle virus* (PMMV).

Sap transmission for propagation and mechanical inoculation of virus isolates

Leaf samples giving positive reaction to ChiVMV based on ELISA result were selected for propagation of ChiVMV isolates. Selected samples with severely symptomatic leaves were

homogenised with a mortar and pestle with 1:10 ratio (w:v) of 0.01 M phosphate buffer (pH 7.0) separately, and the sap was immediately used for mechanical inoculation. Infected sap was applied on carborundum (600 mesh) dusted young leaves (3 weeks after planting) of propagation host (*C. annuum* var. Grosum cultivar Yolo Wonder) or tested chili varieties/lines (Table 4). A second inoculation was performed a week later to confirm ChiVMV infection. Control plants were inoculated with sap from healthy plant or even with buffer. Inoculated plants were kept in insect-free screenhouse for symptom development.

Host response.

Phenotypic data of host reaction was recorded in terms of symptom manifestation following mechanical inoculation of ChiVMV isolate Cikabayan (West Java) on plants of each cultivar/lines, placed under screen house conditions two weeks post inoculation.

Detection of ChiVMV.

Direct ELISA (DAS-ELISA) was performed following the method of Clark and Adam (1977) for screening of 29 chili accessions against ChiVMV. All seedlings of each chili accessions, i.e. 20 to 25 seedlings, were tested for ChiVMV. Detection was performed twice, i.e. a week post first inoculation and a week post second inoculation. A sample was considered positive when the mean absorbance value of the two wells used for each tested sample was greater than twice that of the healthy or buffer control. Disease incidence was measured as the proportion of number of plants giving positive ELISA reaction and total number of tested plants. The cultivar/lines were rated as HR (Highly Resistant, 0-10% infection), R (Resistant, 11-30% infection), MS (Moderately Susceptible, 31-50% infection), S (Susceptible, 51 – 70% infection), HS (Highly Susceptible, >70% infection) based on accumulative data of host response and ELISA values.

RESULTS AND DISCUSSION

Distribution of ChiVMV in chili growing area in Indonesia.

The infection of ChiVMV in Indonesia was reported by Taufik *et al*. (2005) in West Java, Central Java, and South Sulawesi. Further field survey conducted during 2008 to 2009 in West Java, Central Java, East Java, West Sumatera, and South Kalimantan revealed a wider spread and distribution of the disease in Indonesia. Most of the leaf samples collected showed very strong and obvious mosaic, mottle, and malformation symptoms. Virus diagnosis based on ELISA technique was able to detect ChiVMV, CMV, PVY, PMMV and TMV from leaf samples (Table 1). Infection of ChiVMV and CMV was found in almost all fields although with various disease intensity, whereas infection of PVY, PMMV and TMV was only detected in few fields. Symptoms of ChiVMV ranged from mild to severe mottle, with variation of leaf malformation, shoestring and leaf curling. ELISA further confirmed the incidence of the virus from 22 to 77% in areas surveyed during 2008 – 2009 (Table 2).

Although infection of ChiVMV is considered sporadic in almost all chili growing areas in Java, Sumatera, and Kalimantan, the virus has the potential to cause yield loss up to 65% (Subekti *et al*., 2006). Therefore, virus (ChiVMV) resistance is still a major goal of chili pepper breeding programmes.

Table 2. Incidence and symptoms of ChiVMV infection in major chili growing regions in Indonesia*

Location of collected samples	No samples	No samples infected by ChiVMV (% disease incidence)	Symptoms
West Sumatera	13	10 (77)	Mild to severe mottle, leaf malformation
West Java	9	2 (22)	Severe mottle, shoestring
East Java	79	20 (25)	Mild mottle
Central Java	26	14 (54)	Mild to severe mottle, shoestring
South Kalimantan	22	9 (41)	Mild mottle, leaf curling

*Disease incidence was calculated based on ELISA result

Response of chili germplasms to ChiVMV infection.

There were mainly 3 types of symptoms observed on the infected plants. Eight chili genotypes **(**IPB C48, IPB C15, IPB C21, IPB C73, IPB C6, Tegak, IPB C81, Andalas) were observed with mild mottle symptom, 7 genotypes (Tanjung, IPB C20, Tegar, Tornado, IPB C99, IPB C60, Toro) showed mild mottle with leaf malformation symptoms, and 6 genotypes (IPB C13, IPB C24, IPB C55, IPB C33, Polaris, Beauty Bell) showed severe mottle, leaf malformation and dwarfing of plants (Table 3). Similar symptoms, including mild to severe vein mottling, was reported by Shah *et al.* (2011) during screening of indigenous and exotic *Capsicum* genotypes against Pakistani isolate of ChiVMV.

Table 3. Symptom type of ChiVMV infection on 29 genotypes of chili (recorded 2 weeks after inoculation)

Symptom type	Chili genotype
No symptom	IPB C17, IPB C521, IPB C14, IPB C1, IPB C8, IPB C10, Taring, Keriting Sumatera
Mild mottle	IPB C48, IPB C15, IPB C21, IPB C73, IPB C6, Tegak, IPB C81, Andalas
Mild mottle with leaf malformation	Tanjung, IPB C20, Tegar, Tornado, IPB C99, IPB C60, Toro
Severe mottle with leaf malformation and plant dwarfing	IPB C13, IPB C24, IPB C55, IPB C33, Polaris, Beauty Bell

Eight out of 29 genotypes tested (IPB C17, IPB C521, IPB C14, IPB C1, IPB C8, IPB C10, Taring,Keriting Sumatera) showed no symptom until the last observation period (30 days after inoculation) (Table 4). However, when samples of these last 8 genotypes was tested by DAS-ELISA, infection of ChiVMV was detected although only from genotype Taring. Latent infection (infection with no visible symptoms) of viruses has been reported previously (Bashir and Hampton, 1996). *Virus cryptic* infection was discussed by Antoniw *et al.* (1990) to show the phenomenon of symptomless virus infection that may cause significant yield loss. Therefore it is very important to confirm virus infection in germplasms evaluation using reliable detection methods, especially when screening for source of virus resistance.

Chili genotypes varied greatly in their reaction to ChiVMV infection. The genotypes were classified into 5 reaction groups based upon symptom type and % disease incidence. Out of 29 genotypes tested, 3 were highly resistant (IPB C1, IPB C10, and PBC 521), 4 were resistant (IPB C8, IPB C14, IPB C17, and Keriting Sumatra), 5 were moderately susceptible (IPB C48, IPB C60, Tegar, Toro, and Taring), 3 were susceptible (IPB C6, IPB C15, and Tanjung), and 14 were highly susceptible (IPB C13, IPB C20, IPB C21, IPB C24, IPB C33, IPB C55, IPB C73, IPB C81, IPB C99, Tornado, Andalas, Tegak, Beauty Bell, and Polaris) (Table 4).

The reaction of chili accessions was also varied in terms of incubation period. The viral incubation period, indicated by days from inoculation time until first symptoms of the disease appear, in genotypes with HS reaction was shorter (7 to 12 DAI) than genotypes with MS reaction (14 DAI) whereas in genotypes with HR and R reaction symptoms were not visible.

Table 4 Response of 29 chili genotypes to ChiVMV infection.

No.	Species per genotype		Source of germplasm*	Incubation period (DAI)**	Disease incidence (%)	Response type
	Red Chili					
1.	*C. annuum*	IPB C1	PSPT C-17	No symptom	5.00	HR
2.	*C. annuum*	IPB C13	AVRDC	7	95.65	HS
3.	*C. annuum*	IPB C14	AVRDC	No symptom	13.04	R
4.	*C. annuum*	IPB C15	AVRDC	10	60.84	S
5.	*C. annuum*	IPB C17	AVRDC	No symptom	21.74	R
6.	*C. annuum*	IPB C24	AVRDC	7	91.30	HS
7.	*C. annuum*	IPB C48	AVRDC	10	47.37	MS
8.	*C. annuum*	PBC 521	AVRDC	No symptom	0.00	HR
9.	*C. annuum*	Tanjung	Local commercial	12	60.87	S
	Red curly chili					
10.	*C. annuum*	IPB C6	PSPT	12	65.22	S
11.	*C. annuum*	IPB C73	PSPT	12	78.26	HS
12.	*C. annuum*	Tegar	Local commercial	10	34.78	MS
13.	*C. annuum*	Keriting Sumatera	Local commercial	No symptom	26.09	R
14.	*C. annuum*	Tornado	Local commercial	10	88.24	HS
15.	*C. annuum*	Andalas	Local commercial	10	100.00	HS
	Small chili					
16.	*C. annuum*	IPB C8	AVRDC	No symptom	17.39	R
17.	*C. annuum*	IPB C10	AVRDC	No symptom	0.00	HR
18.	*C. annuum*	IPB C60	AVRDC	14	30.43	MS
19.	*C. frutescens*	Tegak	Local commercial	12	94.74	HS
20.	*C. frutescens*	Toro	Local commercial	14	35.29	MS
21.	*C. frutescens*	Taring	Local commercial	14	43.48	MS
	Ornamental chili					
22.	*C. pubescens*	IPB C20	AVRDC	7	95.65	HS
23.	*C. annuum*	IPB C21	AVRDC	7	100.00	HS
24.	*C. annuum*	IPB C33	AVRDC	7	100.00	HS
25.	*C. annuum*	IPB C55	AVRDC	10	82.61	HS

No.	Species per genotype		Source of germplasm*	Incubation period (DAI)**	Disease incidence (%)	Response type
26.	*C. annuum*	IPB C81	Local	7	95.65	HS
27.	*C. annuum* Sweet chili	IPB C99	AVRDC	7	92.30	HS
28.	*C. annuum*	Beauty Bell	Local commercial	7	85.00	HS
29.	*C. annuum*	Polaris	Local commercial	7	95.65	HS

[1] Source of germplasm : Centre for Breeding Program, IPB (PSPT); Asian Vegetable Research and Development Centre (AVRDC).
[2]Incubation period is days from inoculation time until first symptoms of the disease appear (DAI: days after inoculation).
[3]Disease incidence : No plants positive ELISA per total no plants tested x 100%.
[4]Response : Highly resistant (HR), resistant (R), moderately susceptible (MS), susceptible (S), highly susceptible (HS).

CONCLUSION

The management of viral diseases has always been focused on the control of insect-vector and the use of resistant varieties. Three chili genotypes, i.e. IPB C1, IPB C10, and PBC 521 were symptomless and negative for ChiVMV after ELISA and might be a useful source of resistance that can be used in the national breeding program.

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