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Incidence of cucumber viruses in Northern Thailand

Parichat Plapung and Prasartporn Smitamana*

[1]Biotechnology Program, Graduate School, Chiang Mai University, Chiang Mai, 50200, Thailand

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Virus diseases of cucumber are important limiting factors for cucumber production in Thailand and worldwide. A survey of cucumber viruses was conducted in six provinces in Northern Thailand: Chiang Mai, Chiang Rai, Phitsanulok, Lampang, Lamphun and Phayao during June 2007 to July 2008 in order to identify the major viruses infecting cucumber. Results indicated that the majority of cucumber plants in the major growing areas were infected with either single or mixed infections of viruses which resulted in various symptoms: mosaic, mild mosaic, mottling, vein-banding, interveinal chlorosis, vein clearing, malformation of leaves and fruit, and different combinations of symptoms. In this study, 335 cucumber leaf samples were collected and DAS-ELISA was used for virus identification. The results revealed that CMV I was the most prevalent virus detected from the cucumber growing areas in Northern Thailand. During June-October 2007, CMV I was the most commonly detected virus in all locations, while mixed infection of CMV I, SqMV, ZYMV and PRSV detected at an incidence of 22.47% was most commonly identified in the samples taken during January- August 2008.

Keywords: CMV, PRSV, ZYMV, TSWV, SqMV, CGMMV, symptoms, distribution, DAS-ELISA

Introduction

Cucumber (*Cucumis sativas* L.) is one of the most economically important vegetables and belongs to the Cucurbitaceae family which includes 118 genera and 825 species found in tropical and subtropical regions in the world (Jeffrey, 1999). In Thailand, cucumber is one of the most popular vegetables, is widely grown throughout the country and provides a lucrative income for growers. The important limiting factors for cucumber production are disease and pests, especially the viral diseases which cause economic losses worldwide. More than 35 virus diseases have been reported affecting cucurbit production (Providenti, 1996). ZYMV, PRSV-W and CMV were noted as the important viruses that infected cucurbits crop in some Hawaiian Islands

* Corresponding author: Prasartporn Smitamana; e-mail: psporn@gmail.com

(Ullman *et al.*, 1991). Single and mixed viral infections were detected in cucurbit crops which were greenhouse-grown in several Iranian regions. CMV and ZYMV were the most frequently detected, accounting for 21.2% and 18% of the infected plants, respectively. Double and triple infections involving different combinations of CMV, ZYMV, WMV-2, and TSWV were noted in 117 and 4 samples, respectively. Single, double and triple virus infections caused a range of different symptoms, e.g. mosaic, mild to severe yellow mottling, vein clearing, green vein banding**,** blistering, and leaf distortions (Massumi *et al.*, 2007). The enzyme-linked immunosorbent assay (ELISA) has been used for the plant virus detection for nearly four decades (Clark and Adams, 1977). Its high sensitivity and labor-savings capacity, especially in terms of testing large volumes of samples in a short period of time, makes this technique well accepted for the routine plant quarantine investigation worldwide. This report summarizes the distribution and incidence of the important cucumber viruses in the major cucumber growing areas in Northern Thailand in three successive growing seasons during 2007-2008.

Materials and methods

Field survey and samples collection

Cucumber leaf samples were collected on June 2007 to August 2008 from the same cucumber growing areas in Chiang Mai (5 locations), Chiang Rai (3 locations) Phayao (3 locations), Lamphun (2 locations), Lampang (1 locations) and Phitsanulok (2 locations) provinces. All leaf samples showing virus infection were collected and placed in plastic bags and kept in ice boxes for the further investigation.

Detection of viruses by DAS-ELISA

DAS-ELISA was performed using commercial reagents and antibodies (Agdia Inc., USA). The infected leaf tissues were ground using sterile mortars and pestles with the sample and extraction buffer at the ratio of 1:10, centrifuged at 8000 g for 5 min, then the supernatants were transferred to Eppendorf tubes and kept in an ice bath. Subsequently 100 µl of each clear sample were micro pipetted into each assigned microwell of a 96-microwell polystyrene plate coated separately with the following specific capture-antibodies: CMV serogroups I and II, PRSV, SqMV, TSWV, ZYMV and CGMMV. The plates were then kept inside a humid box and incubated for 4 h at room temperature (RT) or overnight at 4 ^{o}C. After 8x washing with washing buffer, 100 µl of prepared enzyme conjugate solution was added to each well

and incubated in a humid box for 2 h at RT. After 8x washing the plates with 1x phosphate buffered saline plus Tween 20 (PBST), 100 µl of purine nucleoside phosphorylase (PNP) solution was added into each test well and incubated in a humid box for 30 - 60 min at RT. The yellow reaction color was seen visually and read using an ELISA plate reader at 405 nm.

Results

Field survey and samples collection

Various symptoms and disease incidences as well as severity levels were found in virus-infected cucumber plants from the field survey during the rainy season (June – October 2007). Samples collected from the Chiang Mai areas showed mosaic, interveinal chlorosis and mosaic with translucent spots on the infected leaves whereas samples found in Lampang showed mixed yellowing and vein banding, mottling, vein clearing and malformed leaves. Symptoms of virus-infected plants from Chiang Rai areas were vein banding, chlorosis and distorted leaves. Virus-infected cucumber plants from Phitsanulok areas showed fewer symptoms than the other areas; only mosaic or mosaic with translucent spots were noted. Leaf samples collected during January-February 2008 from Lamphun, Chiang Mai and Phayao provinces showed various symptoms which depended on collection location. Mosaic was the major symptom which was found in all the fields of the Pa Sang and Wiang Nong Long districts, Lamphun province, along with the interveinal chlorosis symptom also observed in these fields. Mosaic and vein banding symptoms were most commonly found on the cucumbers planted in Chom Thong district, Chiang Mai province which were caused by mixed virus infections. In the Doi Saket areas, Chiang Mai province, mosaic, interveinal-chlorosis and yellowing were observed. Severe virus outbreaks were detected in the San Sai district due to the planting of cucurbits near cucumber fields. Less virus symptoms were found in the Mae Wang district, Chiang Mai province, as well as in Phayao province where interveinal chlorosis and mosaic symptoms were commonly observed. The majority of cucumber virus symptoms found in Phayao, Chiang Rai and Chiang Mai provinces during January–February and August 2008 included mosaic, yellowing and vein banding. Mosaic at a high incidence was regularly found in Phayao fields, mosaic and yellowing were commonly seen in Chiang Rai. However, in San Kamphaeng district, Chiang Mai province, where good spray programs of insecticides and fungicides were implemented, few virus infections were observed.

Detection of viruses by DAS-ELISA

DAS-ELISA technique was used for virus detection in the 117 cucumber leaf samples collected from major cucumber plantations in Chiang Mai, Chiang Rai, Phitsanulok and Lampang provinces during June – October, 2007. Chiang Mai's samples were singly infected with CMV I (10%) (Table 1 and Figure 1), while samples from Chiang Rai were infected with 10% each of the following single viruses CGMMV, PRSV, and TSWV, and the rest of the samples from these locations had 10% incidences of mixed infections of either TSWV and CGMMV or CMV I and SqMV. Single virus infections of either CMV I or PRSV, 16.6% each, was detected in Phitsanulok and the rest of were mixed infections of CMVI, SqMV, TSWV, PRSV and CGMMV found at an incidence of 16.6%. In the Lampang areas, single infections of CGMMV, CMV I, CMV II, PRSV and TSWV were identified at incidences of 3.3, 13.2, 5.5, 5.5, and 5.5% respectively, and the remaining positive samples were diagnosed as mixed infection with the two or three viruses combinations: a) Two viruses combination: 2.2% with CGMMV and TSWV, 4.4% with CMV I and PRSV, 3.3% with CMV I and TSWV; b) three virus combinations: 2.2 % with CGMMV, CMV I and TSWV, and mixed infections at 1.1% each with the following viruses; CGMMV, SqMV and TSWV; CMV I, PRSV, and SqMV; CMV I, SqMV and TSWV (Table 1).

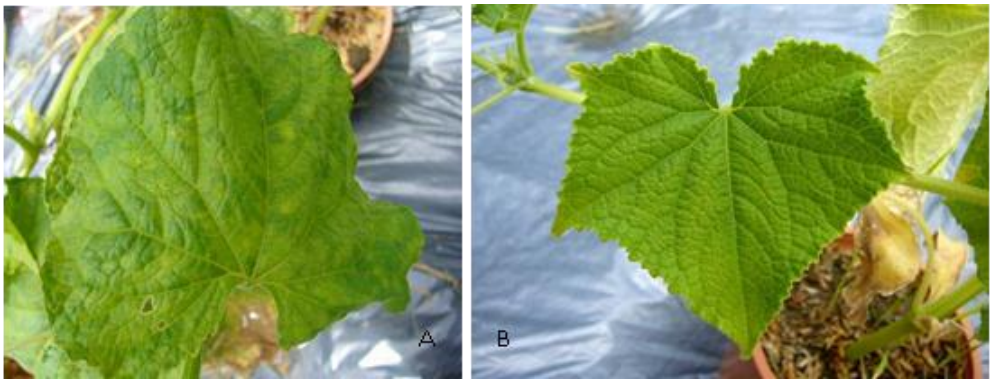

Fig. 1. Cucumber infected with CMV I showing mosaic (A) and mild mosaic (B) symptoms.

Details of the virus distribution as determined from the 218 samples collected from Chiang Mai, Phayao, Lamphun and Lampang provinces during January–February and August 2008 are shown in Table 2. Single virus infection was found higher than the samples collected in 2007 in all locations, especially in Phayao province where PRSV and SqMV were each found for the first time in this study at an incidence of 15.4% . Samples collected from Lampang province had single infections with the following viruses: CGMMV, CMV I,

PRSV and ZYMV as incidences of 4.6, 9.1, 9.1, and 4.6 % respectively. Different combinations of viruses were commonly detected in all surveyed areas and consisted of mixtures of two, three, four or five different viruses. The most common virus combination detected was CMV I, PRSV, SqMV and ZYMV which was found in all surveyed areas at incidences of 46.2% in Phayao, 40.9% in Lampang, 26.2% in Chiang Mai, and 7. 9% in Lamphun. Symptoms of various combinations of viruses in cucumber encountered in the 2008 survey are illustrated in Figure 2. It is noteworthy that ZYMV was found in most of the surveyed areas, especially in Chiang Mai. Single infection of CMV II was found only in the Chiang Mai and Lamphun areas which may be related to the close connection of the survey areas and the similar cultural practices of the farmers. CGMMV single infection was noted only in the Lampang area where continuous planting of the cucurbits throughout the year and reuse of the seeds are common grower practices.

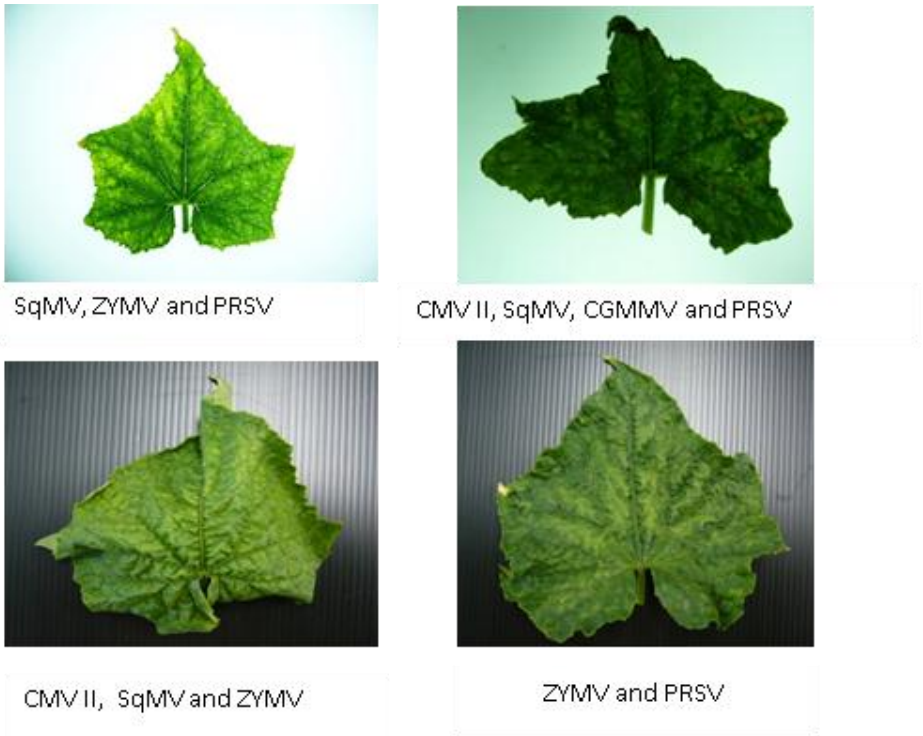

Fig. 2. Different symptoms in cucumbers caused by different mixed virus infections observed in the 2008 survey.

Combining of the data obtained from the 2007 and 2008 surveys, CMV I ranked as the most frequently found cucumber virus in Northern Thailand at

an incidence of 26.6%, follow by PRSV (25.1%), ZYMV (21.8%), SqMV (20.9%), CMV II (7.2%), CGMMV (6.0%) and TSWV (5.4%) (Table 3).

Table 3. Incidence of cucumber viruses detected in Northern Thailand during 2007-2008

Detected viruses	Jan - Oct 2007			Jan - Feb 2008			Total	Ranked
	VD[1]	TS[2]	%	VD[1]	TS[2]	%	(%)	
CMV I	28	117	23.9	61	218	28	26.6	1
PRSV	13	117	11.1	71.0	218	32.6	25.1	2
ZYMV	0	117	0	73.0	218	33.5	21.8	3
SqMV	5	117	4.3	65.0	218	29.8	20.9	4
CMV II	5	117	4.3	19.0	218	8.7	7.2	5
CGMMV	11	117	9.4	9.0	218	4.1	6	6
TSWV	17	117	14.5	1.0	218	0.5	5.4	7

[1]Type of virus detected, [2]Total collected samples

Discussion

Viral diseases cause important economic losses in commercial cucurbit production (Nameth *et al*., 1986). There have been no previous reports on the distribution and incidence of viruses in cucumber plantations in Northern Thailand. During 2007-2008, cucumber plants infected with from one to five different viruses were observed in most of the surveyed areas and caused various symptoms including mosaic, mild-mosaic, vein-clearing, vein-banding, interveinal-chlorosis, mottling, malformation and distortion. Zitikaite (2002) reported that CMV, CGMMV and ToRSV-infected cucumber in Lithuania showed a similar range of symptoms: vein-clearing, mosaic, mottling and malformation, deformation and stunting of plants. Different isolates of a virus may cause different symptoms (Davis and Mizuki, 1987), and the range of symptoms resulting from mixed viruses make it very difficult to identify viruses by symptoms alone. DAS-ELISA was used to evaluate the incidence of viruses from different areas in Northern Thailand. Interestingly, in 2007, only a low incidence (10%) of CMV I was detected in Chiang Mai, most likely because the plots were free from possible alternate hosts for viruses and other cucurbit crops were not grown in close proximity to the field surveyed. On the other hand, samples collected from Chiang Rai and Phitsanulok were positive for either single or mixed virus infections. In the case of Lampang, where cucurbits were planted all year round, a high incidence of virus infection was detected and most were caused by mixed viruses. In 2008, the survey was done during the cool period of the year and higher virus incidence was identified which was probably based on the presence of higher populations of

insect vectors (aphids, white flies, beetles, and thrips), and favorable climatic factors for virus symptom development. Be that as it may, the incidence of cucumber virus in Lamphun was lower than in the other provinces surveyed (Chiang Mai, Chiang Rai, Phayao, Lampang and Phitsanulok), most likely because the farmers used very rigorous insecticide spray program which resulted in a noticeable decrease of the vector population. High incidence of virus infection in Chiang Mai in 2008 was due to the overlap and repeated plantation of the crops, furthermore, the seedlings were prepared in the same area as the production plots so that virus infection at an early stage was possible. Major virus symptoms found in all Northern provinces were mosaic, mild mosaic, mottling, vein banding, vein clearing, interveinal chlorosis, yellowing and malformation of leaves. Mixed symptoms were normally detected in all cucumber growing areas and the high incidence of virus infection was mainly found in the areas where a continuous planting of the cucurbits like pumpkin, squash, luffa, melon and water melon occurred. Solanaceous crops including tomato, tobacco, chili and sweet pepper, grown in the surveyed areas could also have been a source of viruses which also infect cucumbers such as CMV, CGMMV, TSWV and TRSV. Most of the viruses detected in cucumber in Northern Thailand in this study were the same as those reported by Coutts (2006) in cucurbit plantations in Western Australia including CMV, PRSV, ZYMV, SqMV and WMV. CMV I was detected at the highest incidence in Northern Thailand in 2007-2008 probably due to its wide host range which includes 100 families and 1,200 species. In addition, more than 80 aphid species vector (Insecta:Hemiptera: *Aphidoidea*), including *Myzuspersicae* and *Aphis gossypii*, are capable of transmitting the virus in a non-persistent manner (Zitter, 2009). Moreover, CMV is one of the most common viruses infecting cucurbits in Oklahoma (Ali *et al*., 2012), Hawaiian Islands (Ullman *et al.*, 1991) and Costa Rica (Hord *et al*., 2001), especially in temperate or mediterranean climates. PRSV and ZYMV ranked second and third in incidence in most of the cucumber plantations and were limited to areas where cucurbits were continuously planted. On the other hand, CGMMV was found in only a limited number of the areas surveyed. This virus can be mechanically transmitted and seed borne so the use of virus-free seed must be implemented in order to minimize the spread of CGMMV in the future. Mixed virus infections of more than two viruses were generally found in all surveyed areas. This phenomenon is due to the possible mechanical transmission of one of these viruses, the tobamovirus, CGMMV, continuous growing of cucurbits in the same areas high population of alternate virus hosts e.g. tomato, tobacco, chilli pepper, and *Physalis spp.* and often high insect vector populations. Farmer virus management strategies including cleaning

tools, knives, cutters, scissors and pruning shears by dipping in a disinfectant, washing hands with an effective detergents an effective insect management program, crop rotation and integration of these practices should be used in order to minimize virus spread. Nevertheless, farmers often refuse to follow these recommendations because they fail to realize the importance of these preventative measures However, one virus management practice that is widely used by cucumber growers is the roughing virus-infected seedlings.

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Table 1. Incidence of single and mixed viruses found in infected cucumber in Northern Thailand during June-October, 2007

Locations[1]	Total sample	Number of virus infected samples														Total Infected samples/ percentage
		CGMMV	CMV I	CMV II	PRSV	TSWV	CGMMV + TSWV	CMV I+ PRSV	CMV I+ SqMV	CMV I+ TSWV	CGMMV + CMV I+ TSWV	CGMMV + SqMV+ TSWV	CMV I+ PRSV+SqMV	CMV I+ SqMV + TSWV	CGMMV+ CMV I+ PRSV+SqMV+ TSWV	
CNX	10	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
VD[2] (%)		0	10	0	0	0	0	0	0	0	0	0	0	0	0	10.0
CNR	10	1	0	0	1	1	1	0	1	0	0	0	0	0	0	5
VD (%)		10	0	0	10	10	10	0	10	0	0	0	0	0	0	50.0
PHIT	6	0	1	0	1	0	0	0	0	0	0	0	0	0	1	3
VD (%)		0	16.6	0	16.6	0	0	0	0	0	0	0	0	0	16.6	50.0
LAP	91	3	12	5	5	5	2	4	0	3	2	1	1	1	0	44.0
VD (%)		3.3	13.2	5.5	5.5	5.5	2.2	4.4	0	3.3	2.2	1.1	1.1	1.1	0	48.4
TS[3]	117	4	14	5	7	6	3	4	1	3	2	1	1	1	1	53.0
Total virus infected (%)		3.4	12.0	4.3	6.0	5.13	2.6	3.4	0.8	2.6	1.7	0.8	0.8	0.8	0.8	45.3

[1]CNX = Chiang Mai, CNR = Chiang Rai, PHIT = Phitsanulok and LAP = Lampang [2]VD = virus detected [3]TS= Total sample

Table 2. Incidence of single and mixed viruses found in infected cucumber in Northern Thailand during January–February and August 2008

Locations	Total sample	Number of virus infected samples																										Total Infected samples/ percentage
		CGMMV	CMV I	CMV II	PRSV	SqMV	ZYMV	CGMMV+CMV I	CGMMV+CMV II	CGMMV+ZYMV	CMV I+CMV II	CMV I+PRSV	CMV II+PRSV	CMV II+ZYMV	PRSV+SqMV	PRSV+ZYMV	CGMMV+CMV I+SqMV	CGMMV+PRSV+SqMV	CMV I+CMV II+PRSV	CMV II+PRSV+ZYMV	CMV II+PRSV+SqMV	CMV II+SqMV+ZYMV	PRSV+SqMV+ZYMV	CGMMV+CMV II+PRSV+SqMV	CMV I+CMV II+PRSV+ZYMV	CMV I+CMV II+SqMV+ZYMV	CMV I+PRSV+SqMV+ZYMV	CGMMV+CMV II+PRSV+SqMV+TSWV	
CNX[1]	107	0	1	3	3	3	10	0	1	0	1	0	0	2	1	2	0	1	1	1	1	2	1	0	1	0	28	0	63
VD[2 (%)]		0	1	3	2.8	2.8	9	0	0.9	0	1	0	0	2	1	2	0	0.9	0.9	0.9	0.9	1.9	0.9	0	0.9	0	26.2	0	58.8
PYA	13	0	1	0	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	12
VD (%)		0	8	0	15.4	15.4	0	0	7.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46.2	0	92.2
LPH	76	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	6	1	12
VD (%)		0	0	3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1.3		1.3	7.9	1.3	15.8
LAP	22	1	2	0	2	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	9	0	21
VD (%)		5	9	0	9.1	0	5	4.6	0	4.6	0	5	0	0	0	5	4.6	0	0	0	0	0	4.6	0	0	0	40.9	0	95.5
TS[3]	218	1	4	5	7	5	11	1	2	1	1	1	1	2	1	3	1	1	1	1	1	2	2	1	1	1	49	1	108
Total virus infected (%)		1	2	2	3.2	2.3	5	0.5	0.9	0.5	1	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.6	0.6	0.6	22.5	0.5	49.6

[1] Samples collection area, CNX = Chiang Mai, PYA = Phayao, LPH = Lamphun and LAP = Lampang [2]VD = virus detected TS[3] = Total sample