INCIDENCE AND DISTRIBUTION OF PEPPER-INFECTING VIRUSES IN THAILAND

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ABSTRACT

Incidence and distribution of plant viruses infecting peppers in Thailand were surveyed from 2014-2016 to obtain updated information to support development of effective diagnosis and disease management. Field surveys for virus infection were conducted in 15 provinces encompassing 40 pepper-growing locations. Total numbers of 1,319 samples from 33 varieties were tested for virus infection using enzyme-linked immunosorbent assay with locally produced and commercial antibodies specific to chilli veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco mosaic virus (TMV), tomato leaf curl New Delhi virus (ToLCNDV), tomato necrotic ring virus (TNRV), watermelon silver mottle virus (WSMoV) and pepper mild mottle virus (PMMoV). Of this sample number, 567 were positive to at least one pepper virus. Mean disease incidence (DI) indicated the highest value in the northern (93.41±8.62) followed by northeastern (92.60±9.99) and central (73.80±25.04) regions. The highest DI was in Uttaradit, Phitsanulok and Surin provinces (100%). Overall identified virus incidence was 42.99% whereas the highest value was in Chiang Rai province (72.31%). CMV was the major species among 3 surveyed regions (29.11%) followed by ChiVMV (11.83%), tospovirus serogroup IV (6.67%), PMMoV (5.53%), ToLCNDV (1.21%), PVY (1.14%), TMV (0.53%) and TNRV (0.45%). Mean disease severity showed the highest value in the northeastern (3.00±0.58) followed by northern (2.55±0.54) and central (2.05±0.86) regions. The Yok Siam pepper variety was found to be very susceptible to all viruses examined. Among mixed infections, incidence rate of ChiVMV + CMV was the highest at 41.8%. The information from this research provides useful information to support development of effective disease diagnosis and management for peppers in Thailand.

Key words: disease survey, plant virus, *Capsicum* spp.

INTRODUCTION

Pepper (*Capsicum* spp.) originated in regions of Southern Peru and Bolivia in South America. It is now one of the most widely cultivated vegetable crop worldwide and is grown extensively under various environmental and climatic conditions in more than 60 countries, covering a total annual production of approximately 34.5 million tons worldwide. The majority of chilies and peppers are produced in Asia and Europe with 64.9% and 11.5%, respectively (Lin et al. 2013; FAOSTAT 2016). The growing areas of peppers in Thailand covered more than 18,000 hectares and doubled by 2019, with over 60% being planted to small erect-fruited chili in 2016. The main

production area for small pepper is in the northeast followed by the north and the east of Thailand (DAE 2019). Peppers are used in some local dishes all over the country although the types and quantities used vary in different geographical areas. Two main pepper types are grown in Thailand; the Cayenne or big group (*C. annuum*) and the small hot group (*C. annuum* and *C. frutescens*) (Kraikruan et al. 2008). Generally, these are dried, ground and then processed into viscous liquid or powder, which is then used as spice. Viral diseases are considered a major limiting factor in pepper production contributing to yield losses and low quality. Many viruses known to infect peppers cause a wide range of symptoms such as mosaic, mottle, deformation, leaf spots, stunt, vein banding, necrosis, blistering and ring spots.

Pepper viruses in 13 provinces in Thailand were surveyed from 1989 to 1991 (Kittipakorn et al. 1993). Examination was conducted using enzyme-linked immunosorbent assay (ELISA) and the results indicated the prevalence of chilli veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco etch viruses (TEV), pepper mottle virus (PepMoV), tobacco mosaic virus (TMV), and pepper mild mottle virus (PMMoV). Updated information regarding infecting viruses is necessary for development of proper diagnostic tools and resistant cultivars for different pepper crops and locations. In addition, another part of our project sought to produce on-site test kits for single and mixed infections of major pepper viruses. Therefore, this study was conducted to investigate incidence and distribution of plant viruses infecting peppers in major production areas in the north, northeast and central Thailand.

MATERIALS AND METHODS

Survey of virus incidence. Field surveys of pepper virus infection were conducted from April 2014 to September 2016 in major pepper-growing areas among 15 provinces in the northern (Chiang Rai, Chiang Mai, Phrae, Uttaradit and Phitsanulok provinces), central (Nakhon Pathom, Kanchanaburi, Ratchaburi and Phetchaburi provinces) and northeastern regions of Thailand (Chaiyaphum, Nakhon Ratchasima, Buri Ram, Surin, Si Sa Ket and Ubon Ratchathani provinces) (Meteorological Department of Thailand 2020). Twenty samples were randomly collected per the area of one rai (2.53 rai = 1 acre) based on the guideline for surveillance for plant pests in Asia and the Pacific (McMaugh 2008). When plants were grown in rows within 1 rai, Format 1 was applied however, if plants were grown more than 1 rai, Format 2 was applied (Fig. 1). Pepper leaves were randomly collected at the fruiting stage (4-8 months) from the middle part of plant. Symptoms were recorded and percentage of infection was visually estimated based on the extent of leaf damage and disease scoring scales of 0-5.

Fig. 1. Field sampling design per the area of one rai based on the guideline for surveillance for plant pests in Asia and the Pacific (McMaugh 2008).

Virus identification using ELISA method. All collected pepper leaf tissue samples were diagnosed for virus infection using locally produced antibodies specific to ChiVMV, CMV, PVY, TMV, tomato leaf curl New Delhi virus (ToLCNDV), tomato necrotic ring virus (TNRV), watermelon silver mottle virus (WSMoV) and a purchased antibody to PMMoV (Agdia USA).

Indirect plate-trapped antigen ELISA (PTA-ELISA) was performed according to Crowther (2001) with minor modification. Wells in microtiter plates were coated with 50 µL/well of clarified, ground plant sap in sodium carbonate buffer, pH 9.6 (1:10 w/v) and incubated overnight at 4ºC. Leaf extract from healthy pepper leaf tissue was used as a negative control while positive controls were purchased from Agdia (USA). The coated wells were washed with phosphate buffered saline (PBS) and then 4% dried skimmed milk in PBS was added with 37ºC incubation for 1 h to block non-specific reaction. An optimum dilution of each virus-specific antibody was added at 50 µL/well and incubated for 2 h at 37ºC. Goat anti-rabbit IgG conjugated with alkaline phosphatase at a 1:30,000 dilution was added and incubated for 1 h at 37ºC. Each step was followed by three washes with PBST (PBS with 0.05%.Tween 20) at 5 min incubation. The enzyme reactivity was measured after 60 min incubation at 37°C with *p*-nitrophenyl phosphate (1 mg/mL) in diethanolamine buffer, pH 9.8. For anti-PMMoV antibody, double-antibody sandwich ELISA was performed according to the manufacturer (Agdia USA). Reactions from both PTA-ELISA and DAS-ELISA were measured spectrophotometrically at 405 nm using a MultiscanEX ELISA reader (Labsystems, Finland). A sample was considered positive if the absorbance was greater than twice of the negative control.

Disease incidence and severity. In each field, incidence and severity were estimated visually at several selected sampling sites. Virus symptom severity was scored on a scale of 0-5, based on the extent of leaf damage and percentage number of leaves showing symptoms such as mosaic, mottle, chlorotic blotches, leaf distortion, systemic chlorosis and yellow. The rating scales were as follows: L0 = no disease on leaf (0%), L1 = very mild (1-20%), L2 = mild (21-40%), L3 = severe (41-60%), L4 = very severe (61-80%) and L5 = almost dead (81-100%) (Fig. 2). Disease incidence, severity and infection index were calculated according to Allen et al. (1983). The data for DI and DS were analyzed by analysis of variance (ANOVA) at the significance level of 0.05 followed by Tukey's honest significant difference test.

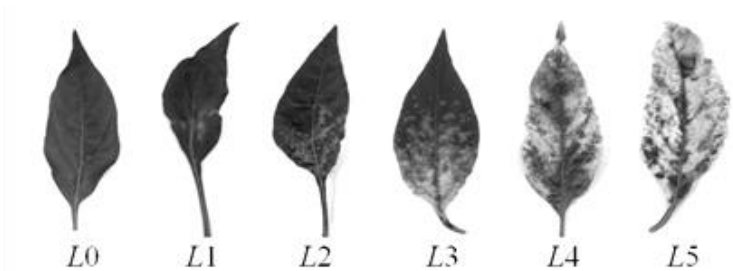

Fig. 2. Disease assessment score to determine the severity of symptoms: *L*0 = 0% (no disease on leaf), *L*1=1-20% leaf area (very mild), *L*2=21-40% leaf area (mild), *L*3=41-60% leaf area (severe), *L*4=61-80% leaf area (very severe) and *L*5=81-100% leaf area (almost dead).

RESULTS AND DISCUSSION

Detection and identification of virus diseases in the pepper samples. Pepper leaf samples collected from field surveys in fifteen provinces including forty locations were tested with specific antibodies to eight viruses as mentioned above. The results showed that 42.99% of the overall collected samples (567/1319) were positive to at least one virus. The incidence of viruses was highest for CMV (29.11%) followed by ChiVMV (11.83%), tospovirus serogroup IV (6.67%), PMMoV (5.54%), ToLCNDV (1.21%), PVY (1.14%), TMV (0.53%) and TNRV (0.46%) (Table 1). Based on the identified virus incidence and distribution, CMV was the major species among 3 surveyed regions. Since anti-WSMoV PAb, used for the detection of tospoviruses, recognizes more than one of the Tospovirus serogroup IV including capsicum chlorosis virus (CaCV) and WSMoV/GBNV (groundnut bud necrosis virus) positive samples from Agdia (USA), specific identification of the infecting species requires a more specific method.

Table 1. Detection of virus incidence in pepper samples collected from 15 provinces in Thailand, 2014-2016 survey

Region	Province	No. of samples collected	No. of virus incidence								Positive samples	Virus incidence (%)
			ChiVMV	CMV	PMMoV	PVY	TMV	TNRV	ToLCNDV	WSMoV*		
North	Chiang Rai	130	66	33	34	8	0	4	0	7	94	72.31
	Phrae	165	34	98	1	1	2	1	1	3	107	64.85
	Uttaradit	20	3	6	0	0	0	0	0	4	9	45.00
	Chiang Mai	75	3	0	10	0	4	1	5	10	25	33.33
	Phitsanulok	86	0	25	0	0	0	0	0	0	25	29.07
Total		476	106	162	45	9	6	6	6	24	260	54.6
Virus incidence (%)			22.27	34.03	9.45	1.89	1.26	1.26	1.26	5.04		
Central	Kanchanaburi	331	17	82	23	6	0	0	2	46	133	40.18
	Ratchaburi	18	3	5	0	0	0	0	0	0	7	38.89
	Nakhon Pathom	200	5	8	0	0	0	0	5	0	15	7.50
	Phetchaburi	14	0	0	0	0	0	0	0	0	0	0.00
Total		563	25	95	23	6	0	0	7	46	155	27.5
Virus incidence (%)			4.44	16.87	4.09	1.07	0	0	1.24	8.17		
North-east	Si Sa Ket	80	0	51	0	0	0	0	0	0	51	63.75
	Buri Ram	40	10	14	1	0	0	0	1	3	26	65.00
	Surin	40	15	15	2	0	0	0	2	4	23	57.50
	Nakhon Ratchasima	50	0	26	0	0	0	0	0	8	28	56.00
	Chaiyaphum	30	0	13	2	0	1	0	0	3	16	53.33
	Ubon Ratchathani	40	0	8	0	0	0	0	0	0	8	20.00
Total		280	25	127	5	0	1	0	3	18	152	54.3
Virus incidence (%)			8.93	45.36	1.79	0	0.36	0	1.07	6.43		
Total		**1,319**	**156**	**384**	**73**	**15**	**7**	**6**	**16**	**88**	**567**	
Virus incidence (%)			**11.83**	**29.11**	**5.53**	**1.14**	**0.53**	**0.45**	**1.21**	**6.67**		

* Anti-WSMoV antibody utilized in this experiment recognizes CaCV and WSMoV/GBNV positive samples from Agdia.

The highest percentage of virus incidence (72.31%) was observed in Chiang Rai province with six viruses detected including ChiVMV (66/130), PMMoV (34/130), CMV (33/130), PVY (8/130), Tospovirus serogroup IV (7/130) and TNRV (4/130); followed by Buri Ram (65%), Phrae (64.85%), Si Sa Ket (63.75%), Surin (57.50%), Nakhon Ratchasima (56%), Chaiyaphum (53.33%), Uttaradit (45%), Kanchanaburi (40.18%), Ratchaburi (38.89%), Chiang Mai (33.33%), Phitsanulok (29.07%), Ubon Ratchathani (20%) and Nakhon Pathom (8%). There was only one province, Phetchaburi, where no virus incidence was found in any sample (Table 1). Conversely, all viruses examined were detected in the samples from Phrae with the highest incidence of CMV (98/165) followed by ChiVMV (34/165) while the rest of virus incidences were observed in low numbers (1-3 positive samples).

Mixed infections. Twenty-seven combinations of mixed virus infections in the pepper samples are presented in Table 2. Interestingly, the highest number of mixed-infected samples was found in Chiang Rai at 41.8% (56/134) in which almost all of them contained ChiVMV. Among mixed infections, the combination of ChiVMV and CMV was found to be highest as 41.8%. The highest number of virus species in mixed infection was eight species whereas most of them contained 2-3 species of viruses (126/134).

Virus incidence and severity were highly variable among geographical locations. Symptoms observed in the surveyed areas were diverse which consisted of mosaic, mild mottle, mottle, chlorotic blotches, leaf distortion, systemic chlorosis and yellowing. However, the prominent symptoms of individual virus infection were: mosaic, mild mottling and systemic chlorosis associated with ChiVMV, CMV, PMMoV and PVY; mosaic and leaf distortion associated with TMV; leaf mottle and distortion associated with TNRV; mosaic, systemic chlorosis and yellowing associated with ToLCNDV; chlorotic blotches, systemic chlorosis and yellowing associated with Tospovirus serogroup IV. Some of the negative samples in ELISA displayed virus-like symptoms such as mosaic, blistering, deformation and size reduction. This might indicate the other causal agents for these symptoms, not only other viruses but also physiological disorders, therefore symptoms are not recommended to be used for virus identification.

The earlier survey indicated the presence in descending order of prevalence of ChiVMV (56.96%), CMV (26.67%), PVY (24.35%), TEV (11.88%), PepMoV (9.97%), TMV (4.86%), and PMMoV (1.92%) (Kittipakorn et al. 1993). From 2016 to 2019, pepper viruses including begomoviruses, ChiVMV, CMV, PVY, TMV and TNRV were detected in 13 provinces of Thailand (Laprom et al. 2019). These surveys revealed the incidence of the viruses infecting pepper in Thailand was high, being identified in nearly 70% (1,482/2,149) of the collected samples. The highest virus incidence was found in the central (96%), followed by northern (74.4%) and northeastern (52.8%) regions, respectively. Begomoviruses (32.7%), ChiVMV (21.5%), CMV (25.7%), TNRV (19.5%) were detected at varying rates whereas PVY, TMV and TSWV were not detected (31.0% negative samples). Compared to our observations, the results are consistent for ChiVMV and CMV which were present in all regions surveyed and these viruses were the most common in the mixed infection in both the present survey and Laprom et al. (2019). The percentage of PMMoV-infected samples increased from 1.92% from the previous survey (Kittipakorn et al. 1993) to 5.53%, especially in the north. Although the value was not very high, this virus can remain infective for many months in alternative weed hosts, infects some other solanaceous plants and be easily spread by contact (Agrios 2005). Therefore, it can lead to contamination in seed production and export. Since there was no antibody for this virus available in Thailand then, polyclonal and monoclonal antibodies were produced from PMMoV antigen and rapid test kits were developed for this virus and 4 pepper-infecting tobamoviruses (Phatsaman et al. 2020). Begomoviruses were not reported in the 1989-1991 survey which might be due to no evidence of symptoms associated with this pathogen observed in the production fields. However, increasing incidence and prevalence of begomoviruses were reported, not only in peppers but also in tomatoes and cucurbits, in 2014-2019 (Charoenvilaisiri et al. 2020; Laprom et al. 2019; Malichan et al. 2019).

Table 2. Mixed infection detected by ELISA in peppers collected from Chiang Rai (CRI), Chiang Mai (CMI), Phrae (PRE), Uttaradit (UTT), Phitsanulok (PLK), Nakhon Pathom (NPT), Kanchanaburi (KBI), Phetchaburi (PBI), Ratchaburi (RBR), Chaiyaphum (CPM), Nakhon Ratchasima (NMA), Buri Ram (BRM), Surin (SRN), Si Sa Ket (SSK) and Ubon Ratchathani (UBN) provinces.

Mixed infection of viruses	Northern region					Central region					Northeastern region					Virus-infected samples (%)*
	CMI	CRI	PLK	PRE	UTT	KBI	NPT	RBR	BRM	CPM	NMA	PBI	SRN	SSK	UBN	
ChiVMV, CMV	0	18	0	25	1	2	3	1	1	0	0	0	5	0	0	41.8
CMV, Tospovirus gr.IV	0	0	0	0	1	11	0	0	0	0	6	0	1	0	0	14.2
ChiVMV, Tospovirus gr.IV	0	3	0	0	0	4	0	0	0	0	0	0	2	0	0	6.7
ChiVMV, PMMoV	1	7	0	0	0	0	0	0	1	0	0	0	0	0	0	6.7
ChiVMV, CMV, PVY	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	4.5
CMV, PMMoV	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	2.9
ChiVMV, PVY	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2
ChiVMV, TNRV	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2
ToLCNDV, Tospovirus gr.IV	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2.2
ChiVMV, PVY, Tospovirus gr.IV	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, PVY, Tospovirus gr.IV,	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, Tospovirus gr.IV	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, PMMoV	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, ToLCNDV, Tospovirus gr.IV	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, TNRV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, CMV, PMMoV, TNRV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, PMMoV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7

Mixed infection of viruses	Northern region				Central region					Northeastern region					Virus-infected samples (%)*
	CMI	CRI	PLK	PRE	UTT	KBI	NPT	RBR	BRM	CPM	NMA	PBI	SRN	SSK	UBN	
ChiVMV, CMV, PMMoV, TNRV, Tospovirus gr.IV	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7
PMMoV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
CMV, PMMoV, Tospovirus gr.IV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.7
CMV, PVY, Tospovirus gr.IV	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, TMV, Tospovirus gr.IV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
PMMoV, TMV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, CMV, PMMoV, PVY, TMV, Tospovirus gr.IV, TNRV, ToLCNDV	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.7
TMV, ToLCNDV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
TMV, ToLCNDV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.7
TMV, ToLCNDV, Tospovirus gr.IV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
Total	**8**	**46**	**0**	**27**	**3**	**24**	**3**	**1**	**3**	**3**	**6**	**0**	**10**	**0**	**0**	

* Percentage of the particular mixed infection among the total number of mixed infected samples (134 samples).

Disease incidence and severity. The highest disease incidence (100±0.00) recorded was in Uttaradit, Phitsanulok and Surin provinces followed by Nakhon Ratchasima (98.22±2.52), Chiang Rai (96.97±4.29), Si Sa Ket (95.00±10.00), Buri Ram (92.50±10.61), Chaiyaphum (90.00±14.14), Phrae (89.01±8.31), Kanchanaburi (84.38±20.06), Chiang Mai (80.00±0.00), Ubon Ratchathani (77.50±3.54), Nakhon Pathom (50.50±0.00) and Ratchaburi (38.89±0.00). A significant effect of region location was observed with the highest incidence recorded in the northern region (93.41±8.62a) followed by northeastern (92.60±9.99a) and central regions (73.80±25.04b) which was significantly different from the former 2 regions.

Disease severity investigation, based on mean severity values, showed the highest severity in the northeastern (3.00±0.58a) followed by northern (2.55±0.54ab) and central regions (2.05±0.86b). Variation of severity was observed among the pepper samples with the highest DS in Surin (3.58±0.53) and the lowest in Ratchaburi province (0.94±0.06). Region-based analysis of mean infection index showed that virus diseases of peppers displayed moderate to very severe infection indices and corresponded to the disease severity reported above. The highest infection index was in the Northeast (60%) followed by the North (50.94%) while the Central had the lowest value (41.05%).

Incidence of viruses detected in different pepper varieties revealed the most susceptible variety was Yok Siam in which all viruses analyzed could be detected (Table 3). Most varieties were infected with at least one virus species except three varieties including the Saban-Nga, Rocket Chili and Chai Buri. However, some of these varieties might have been present in too small sample number to substantiate this conclusion. The severity was also affected by the growth stage of the pepper plant. In the northeastern region, most pepper plants had an average age of 3 months and were susceptible to the viruses, as shown by the highest DS value, followed by the northern and central regions where the average pepper plants during the collection periods were aged 5 and 6 months, respectively. The younger the age of the peppers, the higher the risk of virulence. Analysis of the relationship between disease incidence and severity from forty locations in fifteen provinces revealed that the severity increased linearly as incidence increased (data not shown).

Expansion and intensification of pepper cultivation also affected DI and DS. For example, peppers in the northeast were grown for industrial seasonings and most of them were in the Small hot group. The planting areas were large with frequency of planting spacing aiming to increase the number of pepper plants as observed in Chaiyaphum, Nakhon Ratchasima, Surin and Sisaket provinces. These planting conditions would likely enhance the field spread of viruses from the infested plant to neighboring plants. In the central region (Kanchanaburi and Nakhon Pathom provinces), peppers were planted wide apart with a furrow system for easy watering, whereas in the north, the Big pepper group was planted in separate rows to avoid fruit damage when handling such as in Phrae but in Chiang Mai, these were grown in greenhouses. This condition reduces potential transmission by insect vectors for several viruses and might be the reason for less severity of diseases in the Big pepper group.

In addition, the growing season also influenced disease occurrence and severity. Climate change is expanding the geographic range suitable for the viruses and vectors. Analysis of the relationship between DI and DS showed that severity increased linearly with increased incidence. Although the pepper-growing season in Thailand varies by geographical areas and it is not possible to definitively identify the proper pepper-planting period. Growing in late summer before the rainy season in order to break the cycle of disease is recommended to avoid virus infection (Pakuthai et al. 2015).

Table 3. Incidence of viruses detected in different pepper varieties using ELISA method.

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiV MV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
Capsicum annuum	Bell gr. / Big gr.	Spider	N	17	1	0	0	0	4	0	0	3	3
C. annuum	Bell gr./ Big gr.	Sunnyez	N	58	3	10	3	0	6	1	0	2	6
C. annuum	Cayenne/ Big gr.	Jomthong 2	N	1	0	1	0	0	0	0	0	0	1
C. annuum	Cayenne/ Big gr.	Manee-Karn	N	1	0	1	0	0	0	0	0	0	1
C. annuum	Cayenne/ Big gr.	Pichai	N/C	30	0	0	3	0	7	0	10	0	3
C. annuum	Cayenne/ Big gr.	Prik Chi Fa	C	39	0	0	3	0	0	0	10	0	2
C. annuum	Cayenne/ Big gr.	Prik Num	C	19	0	0	0	0	9	0	0	0	1
C. annuum	Cayenne/ Big gr.	Saban-Nga	N	2	0	0	0	0	0	0	0	0	0
C. annuum	Cayenne/ Big gr.	Salika	N	15	0	3	12	0	1	2	4	0	5
C. annuum	Cayenne/ Big gr.	Wat Bot	C	102	0	0	0	0	1	0	34	0	2
C. annuum	Cayenne/ Big gr.	Yellow Pepper	C	18	0	0	1	0	0	0	7	0	2
C. annuum	Cayenne/ Big gr.	Yok 31	N	41	0	2	3	0	0	0	8	0	3
C. annuum	Cayenne/ Big gr.	Yok Sawan	N	7	0	3	5	0	2	1	0	0	4
C. annuum	Cayenne/ Big gr.	Yok Siam	N	174	2	2	37	1	5	1	98	1	8
C. annuum	Small hot gr.	Bird Eye Pepper	N/C	56	0	6	25	8	1	0	29	1	6
C. annuum	Small hot gr.	Chinda	C	86	0	4	1	0	10	0	30	2	5

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiV MV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
C. annuum	Small hot gr.	Double Hot	N	5	0	2	4	0	0	1	2	0	4
C. annuum	Small hot gr.	Haurue	C	11	0	0	0	0	3	0	0	0	1
C. annuum	Small hot gr.	Hot Chili	N/NE	161	0	4	11	0	1	0	14	3	5
C. annuum	Small hot gr.	Maxi	N	2	0	1	0	0	0	0	0	0	1
C. annuum	Small hot gr.	Taiwan	C	30	0	0	9	5	20	0	14	0	4
C. annuum	Small hot gr.	Pha-Nom-Pai	N	3	0	3	0	0	1	0	0	0	2
C. annuum	Small hot gr.	Red Hot	N	8	0	2	4	0	0	0	0	0	2
C. annuum	Small hot gr.	Rocket Chili	N	1	0	0	0	0	0	0	0	0	0
C. annuum	Small hot gr.	Smile Hot	N	5	0	3	2	0	0	0	0	0	2
C. annuum	Small hot gr.	Star Hot	NE	40	0	2	15	0	4	0	13	2	5
C. annuum	Small hot gr.	Super Hot	N/NE	78	1	5	10	0	3	0	26	1	6
C. annuum	Small hot gr.	Three Cherry Pepper	C	37	0	0	0	0	3	0	11	0	2
C. frutescens	Small hot gr.	Amphawa	N/NE	86	0	1	5	0	1	0	58	0	4

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiV MV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
C. frutescens	Small hot gr.	Chai Buri	C	16	0	0	0	0	0	0	0	0	0
C. frutescens	Small hot gr.	Hmong	C	112	0	15	3	1	2	0	5	1	6
C. frutescens	Small hot gr.	Karen	C	31	0	2	0	0	0	0	1	0	2
C. frutescens	Small hot gr.	Pop	NE/C	27	0	1	0	0	4	0	10	0	3
Total				**1319**	**7**	**73**	**156**	**15**	**88**	**6**	**384**	**16**	
Virus incidence (%)					**0.53**	**5.53**	**11.83**	**1.14**	**6.67**	**0.45**	**29.11**	**1.21**	

* Geographical regions of Thailand; North (N), Central (C), and Northeast (NE)

** Anti-WSMoV antibody utilized in this experiment recognizes CaCV and WSMoV/GBNV positive samples from Agdia.

Note: Some samples were infected with more than one virus.

CONCLUSIONS

This study focused on the incidence of plant viruses infecting peppers in Thailand from 2014-2016 in 3 regions to obtain useful information for further effective disease management and virus-resistance breeding programs. Peppers were infected with several viruses with different levels of incidence and severity. A significant effect of province and region was observed on disease incidence. The following categories could be the main reasons for disease incidence and severity: growing season, growth stage, pepper variety and also geographical area of sampling. Severity increased linearly with increased incidence. Organized epidemiological knowledge can help determine virus occurrence and forecast possible diseases in order to prevent viruses from infecting peppers as well as provide useful information for breeding programs. Efforts made for phytosanitary system should be focused on early detection and diagnostics which will facilitate successful pepper production in Thailand.

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