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Review article

Current viroid status in Thailand

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Abstract

<u>Importance of the work</u>: Since 2001, several viroids have been found and identified in Thailand in a wide range of economically important crops. However, there has not yet been any complete and up-to-date information published on the status of all viroids in Thailand.

<u>**Objectives**</u>: To provide current and comprehensive information on viroid status, epidemiology and economic impact in Thailand.

Results: Since 2001, four pospiviroids (chrysanthemum stunt viroid, citrus exocortis viroid, columnea latent viroid and pepper chat fruit viroid), one hostuviroid (hop stunt viroid), two apscaviroids (grapevine yellow speckle viroid 1 and 2) and one pelamoviroid (chrysanthemum chlorotic mottle viroid) have been found in Thailand.

<u>Main finding</u>: This review provided current, complete and up-to-date information on the status of all viroids in Thailand, never before compiled and published, particularly since most early reports are unavailable online.

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Introduction

Viroids are the smallest plant pathogens known, consisting of closed, circular, single-stranded, naked RNA (246–401 nucleotides in length) and lacking a protein capsid (Ding and Itaya, 2007). Unlike viruses, these infectious agents contain non-protein coding sequences (Navarro et al., 2012). Furthermore, viroids spontaneously form thermodynamic secondary structures that are highly stable and resistant to disinfectants, high temperatures and other extreme environmental factors (Brierley, 1952; Hollings and Stone, 1973; Dingley et al., 2003), making them almost impossible to eradicate (Kovalskaya and Hammond, 2014).

Viroids are taxonomically classified into two families—the Avsunviroidae and the Pospiviroidae—consisting of three and five genera, respectively. They are mainly transmitted by mechanical means such as contaminated farming implements, tools and equipment. Apart from vegetative propagation, several viroids can be transmitted through seed and pollen (Singh et al., 2003). Insect transmission is quite limited and inconsistent. There have been a few reports on potato spindle tuber viroid (PSTVd) and a few other pospiviroids such as tomato planta macho viroid (TPMVd) (Galindo et al., 1986). PSTVd has been reported to be transmitted by aphids at a very low rate; however, this insect transmission depends on simultaneous infection with potato leafroll virus (PLRV) or both PLRV and potato virus Y (PVY) (Salazar et al., 1995) by trans-encapsidation, viroid RNA enclosed with PLRV coat proteins (Salazar et al., 1995; Querci et al., 1997; Singh and Kurz, 1997) or velvet tobacco mottle virus (VToMV) (Francki et al., 1986). Tomato apical stunt viroid (TASVd) and TPMVd, another viroid, were transmitted by aphids and Myzus persicae, respectively. However, without helper viruses, the possibility of aphid transmission is dramatically reduced (Candresse et al., 2017). Disease symptoms vary from highly damaging to none, as a result of complex viroid-host-environment interactions (Owens et al., 2012). Unfortunately, viroid particles consist of only naked, single-stranded RNA without encoding any protein. Therefore, protein-based detection techniques, such as enzyme-linked immunosorbent assay, are unsuitable to detect this pathogen. Nucleic acid-based detection methods, such as reverse transcription polymerase chain reaction (RT-PCR and RT-qPCR), reverse transcription loop-mediated isothermal amplification and next generation sequencing, are still mainly used for viroid diagnosis or research.

Since the first discovery of this pathogen in 1971, several viroids have been reported to cause economic crop losses in potato, tomato, coconut, hop, several fruit trees and ornamental plants (Hammond, 2017). In Thailand, several viroids have been detected and reported in a variety of economic crops, including fruit trees, vegetable crops and ornamental plants. Disease symptoms and economic impacts vary depending on the viroid species and strain, plant host species or variety, plant age and climatic conditions (temperature and light intensity) (Randles, 2003; Flores et al., 2005). According to the Köppen climate classification, Thailand has a tropical savanna climate throughout most of the country and a tropical monsoon climate in the southern and eastern regions of Thailand (Climatological Group, 2015). Because of constant high average temperatures and relative humidity, viroid disease prevalence and phytosanitary impact are potentially high (Randles, 2003; Flores et al., 2005). For example, at temperatures below 25°C, most Columnea latent viroid (CLVd) isolates lose their infectivity and their titer accumulation is slowed. Likewise, the infected plants expressed fewer symptoms. In contrast, temperatures over 27°C induced a higher infection rate, viroid titer and symptom expression (Tangkanchanapas, 2020). Because the economic impact of viroids is more serious in warm or hot climates, such as in tropical countries, in Thailand, two pospiviroids—columnea latent viroid (CLVd) and pepper chat fruit viroid (PCFVd)—have caused serious yield losses with both tomato fruit and hybrid seed production (Tangkanchanapas, 2005; Marach, 2008; Reanwarakorn et al., 2011; Chambers et al., 2013; Tangkanchanapas et al., 2013a). Furthermore, compliance is required, especially for exported tomato (Solanum lycopersicum) and chili (Capsicum annuum) hybrid seeds, with a seed certification program (by field inspection, laboratory analysis and seed testing analyses such as RT-PCR or RT-qPCR) before a phytosanitary certificate can be issued. However, these two viroids have been intercepted with traded tomato and chili seeds during quarantine in several countries (Chambers et al., 2013). This has raised concerns regarding these diseases, especially CLVd of which isolates (or variants) in Thailand (tomato isolate) cause more severe symptoms than the ornamental isolates (most US and European isolates) and may have a different biology (host ranges, severity and yield losses) as well.

To date, eight viroids (four pospiviroids, one hostuviroid, two apscaviroids and one pelamoviroid) have been detected and reported in Thailand. A summary of the occurrence and geographical distribution of viroids in Thailand is presented in Table 1.

Table 1 Occurrence and geographical distribution of viroids in Thailand

Viroid	Host	Region	References
<u>Avsunviroidae</u>			
Pelamoviroid			
Chrysanthemum chlorotic	Chrysanthemum	Northern (Chiang Mai and	Supakitthanakorn et al., 2022
mottle viroid (CChMVd)		Chiang Rai)	
<u>Pospiviroidae</u>			
Pospiviroid			
Chrysanthemum stunt viroid	Chrysanthemum	Northeastern (Nakhon Ratchasima)	Netwong et al., 2020
(CSVd)		and Northern (Chiang Mai and	Supakitthanakorn et al., 2022
		Chiang Rai)	
Citrus exocortis viroid (CEVd)	Citrus spp. (very limited)	Central (Chai Nat)	Pomma, 2002; Reanwarakorn et al., 2003
	Tomato (only found in 2003	Northeastern (Khon Kaen)	Sangdee et al., 2003;
	and never again)		Tangkanchanapas et al., 2012, 2013;
			Bhuvitarkorn and Reanwarakorn, 2019
Columnea latent viroid (CLVd)	Tomato	Northeastern (Khon Kaen, Sakon	Tangkanchanapas, 2005;
		Nakhon, Udon Thani, Maha	Tangkanchanapas et al., 2012
		Sarakham, Kalasin, Mukdahan,	
		Nong Khai), Northern (Chiang Mai,	
		Chiang Rai, Lampang and Lamphun)	
Pepper chat fruit viroid (PCFVd)	Chili, Tomato	Northeastern (Khon Kaen and	Reanwarakorn et al., 2011;
		Nong Khai), Northern (Chiang Rai,	Tangkanchanapas et al., 2012, 2013
		Lampang and Lamphun) and central	
		(Nakhon Pathom)	
Hostuviroid			
Hop stunt viroid (HSVd)	Grapevine (very limited)	Western (Ratchaburi)	Rakdang, 2001
Apscaviroid			
Grapevine yellow speckle viroid 1	Grapevine	Northeastern	Hannok, 2004; Hannok
(GYSVd-1)	(very low disease incidence)	(Saraburi and Nakhon Ratchasima)	and Reanwarakorn, 2005;
			Tangkanchanapas et al., 2015, 2017
Grapevine yellow speckle viroid 2	Grapevine	Northeastern	Tangkanchanapas et al., 2015, 2017
(GYSVd-2)	(very low disease incidence)	(Saraburi and Nakhon Ratchasima)	

Viroids in citrus and grapevine

Several viroids have been found in various fruit trees such as lime (Citrus aurantifolia) and grapevine (Vitis spp.). Citrus exocortis viroid (CEVd), one of the most serious citrus pathogens, was the very first viroid to be discovered in Thailand in 2001 (Pomma, 2002). CEVd is a member of the Pospiviroid genus and the Pospiviroidae family. It was found in symptomless lime in three provinces: Chainat, Nakhon Pathom (central region), and Ratchaburi (western region), as shown in Fig. 1 (Pomma, 2002; Reanwarakorn et al., 2003). Indexing of plants, RT-PCR and Sanger sequencing were conducted to identify the CEVdinfected lime samples. The CEVd sample sequences shared 98.3% base pair similarity with a reported GenBank sequences— Accession J02053 (Pomma, 2002; Reanwarakorn et al., 2003). However, CEVd surveys conducted in citrus orchards (oranges and limes) from 2008 to 2010 revealed that the incidence was low in Thai citrus trees (Tangkanchanapas et al., 2010).

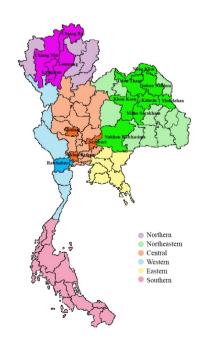


Fig. 1 Map of Thailand indicating regions and provinces where viroids have been detected

Hop stunt viroid (HSVd), a member of the *Hostuviroid* genus and the *Pospiviroidae* family, was among the first viroids to be reported in Thailand. It was found on the grapevine cultivars "White Malaga" and "Kyoho" in Ratchaburi province (Fig. 1) in 2001 and was associated with yellow speckle and/or vein-banding symptoms on leaves (Rakdang, 2001). The results of RT-PCR and Sanger sequencing techniques indicated that the HSVd-infected grapevine samples shared 99% sequence similarity with formerly reported GenBank sequences (Accession M35717) from a grapevine isolate from Japan (Rakdang, 2001). Furthermore, grapevine viroids surveys were conducted in vineyards in Saraburi and Nakhon Ratchasima provinces (Fig. 1) from February through March 2014; however, neither HSVd nor the above discussed CEVd, were found in any of the samples (Tangkanchanapas et al., 2015, 2017).

Grapevine yellow speckle viroid-1 (GYSVd-1), a member of the Apscaviroid genus and the *Pospiviroidae* family, is yet another grapevine viroid discovered in 2004 in Nakhon Ratchasima province (Fig. 1). The Thai isolate of GYSVd-1 shared 96% sequence similarity with GYSVd-1 Accession X06904, an isolate from the grapevine cultivar Cabernet franc in Australia (Hannok, 2004; Hannok and Reanwarakorn, 2005).

In grapevine viroid surveys in 2014, grapevine yellow speckle viroid-2 (GYSVd-2) and GYSVd-1 were detected on samples from all the major grapevine plantation sites (*Vitis vinifera* cvs. "Black Opal seedless", "Black Queen", and "Pok Dum") in Nakhon Ratchasima province (Fig. 1). Both GYSVd-1 and -2 positive leaf samples only showed yellow mottling spots and yellow speckled leaf symptoms (Fig. 2). These Thai variants of GYSVd-1 and GYSVd-2 shared 93–99% and 98–99% sequence similarities with reported GenBank sequences (GYSVd-1; AF059712, DQ371471, GQ995468, X87913, and X87920; and GYSVd-2; FJ490172, FJ597943, and JQ686716, respectively) (Tangkanchanapas et al., 2017). Notably, the new GYSVd-1 variants shared very low sequence similarity



Fig. 2 GYSVd-1 and -2 infections showing yellow speckle symptoms on grapevine leaves

(67.2–74.1%) to the former GYSVd-1 Thai variant (Accession AY639606). Possibly, the non-regulated GYSVd-1 might have been reintroduced into the country via imported propagative plant material. However, the disease incidence in all vineyards was very low at <1% (Tangkanchanapas et al., 2015, 2017).

In addition, in 2013 and 2014, because of the increasingly large amount of imported orange and lime fruits, citrus viroid surveys resulted in viroid interceptions on imported orange and lime fruits from China and Cambodia. Four viroids, CEVd, HSVd, citrus bent leaf viroid (CBLVd) and citrus dwarfing viroid (CDVd), were diagnosed using specific RT-PCR methods. CEVd and HSVd were not found, yet, CBLVd and CDVd were identified in 34.2% and 19.5% of the imported lime fruit, respectively, and in 62.3% and 75.3% of the imported orange fruit, respectively. In addition, both citrus viroids had never been reported before in Thailand. However, to date, the extent of the presence of these two viroids in Thailand is still unknown (Tangkanchanapas et al., 2014, 2018).

Viroids in vegetable crops

Hybrid seed production has been one of the most commercially successful businesses in Thailand, particularly with respect to tomato and pepper seeds (Office of Agricultural Regulation, 2021). This has resulted in Thailand currently becoming one of the major global seed production hubs (National Science and Technology Development Agency, 2020). Because of the high demand for new hybrid seed varieties, a large number of parent seeds have been imported into the country, resulting in a high risk of quarantine pests, such as viruses and viroids, being introduced. To date, for *Solanum* crops, such as tomatoes and chili pepper, two pospiviroids have been reported in Thailand.

CEVd was the first viroid to be reported in a vegetable crop in Khon Kaen province (Fig. 1) in 2003. This infectious agent caused typical viroid symptoms on tomato (*Solanum lycopersicum*) plants, such as a bunchy top and severe vein necrosis on the midrib, twig and stem (Sangdee et al., 2003). However, there has been no further information regarding the economic impact or disease incidence. Notably, from 2005 to 2012, CEVd was not detected during annual field inspections on entire tomato and pepper seed production sites for the issuing phytosanitary certificates. This possibly indicates that the incidence of CEVd on tomato and pepper production sites is very limited or non-existent in the country (Tangkanchanapas, 2005; Tangkanchanapas et al., 2012, 2013a; Tangkanchanapas, 2020).

Columnea latent viroid (CLVd), a second member of the Pospiviroid genus and the Pospiviroidae family, was first reported in 2005 from tomato hybrid seed production sites in Khon Kaen province (northeastern region) at a very low disease incidence (Fig. 1) (Tangkanchanapas, 2005). However, the first outbreak was subsequently eradicated by destroying all infected plants during annual field inspections (Tangkanchanapas et al., 2012). Originally, CLVd was found in symptomless lipstick vine (Columnea erythrophea), which has been reported to produce mild and intermediate symptoms on potato and tomato cv. Rutgers (Owens et al., 1978, 2003; Hammond et al., 1989). Notably, the first recorded Thai CLVd tomato variants (PC-2-Pa2, PC-2-Pa29 and PC-2-Pa54; Accessions DQ022677, DQ061193 and DQ061192, respectively) showed a very low sequence similarity to the two first described CLVd sequences that were submitted to GenBank (Accessions X15663 and AY222072), notably 90.5% and 90.5%, respectively (Tangkanchanapas, 2005). Furthermore, the Thai CLVd tomato isolate showed distinct symptoms on tomato cv. Rutgers, following biological indexing experiments. The isolate induced very severe stunting and strong leaf rugosity (Fig. 3) (Tangkanchanapas, 2005). From 2006 to 2008, CLVd was detected again on several occasions during annual field inspections on tomato hybrid seed production sites in northeastern Thailand (Accessions DO923058, DO923059, DQ923060 and DQ923061). These isolates had a very low sequence similarity with all former CLVd ornamental and tomato isolates at 92.0, 92.3 and 91.3%, respectively. Again, in this case, all infected plants were eradicated (Tangkanchanapas et al., 2012). In addition, the symptoms that this second tomato isolate produced were similar to those of the previous tomato isolate, albeit with higher severity, especially on tomatoes (Marach, 2008; Tangkanchanapas et al., 2012). Marach (2008) reported that this CLVd isolate caused very high yield losses in several commercial tomato cultivars by reducing the fruit size to around 50%. In addition, a high seed transmission rate (30%) was reported in tomato (Marach, 2008; Bhuvitarkorn and Reanwarakorn, 2019). From 2009 to 2012, numerous new CLVd variants have been reported, including one from tomato hybrid seed production sites in the northeastern provinces of Thailand, as well as an outbreak in tomato fruit production fields in northern Thailand (Tangkanchanapas et al., 2012, 2013a). Most of these isolates also induced very strong symptoms on bolo maka (Solanum stramoniifolium), a local vegetable and medical herb, whereas the ornamental and the first tomato isolate only showed mild symptoms or remained latent on this crop (Tangkanchanapas et al., 2012, 2013a, b) (Fig. 4).

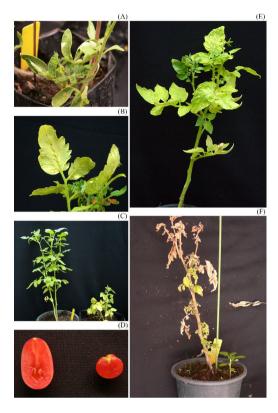


Fig. 3 Symptoms of CLVd isolate PC-2-Pa2 (Accession DQ022677) on tomato cv. Rutgers: (A) strong leaf rugosity; (B) vein necrosis; (C) severe stunting; (D) reduction of fruit size and seed abortion; (E) leaf epinasty and apical stunting; (F) Dieback of apical leaves (Tangkanchanapas, 2005)

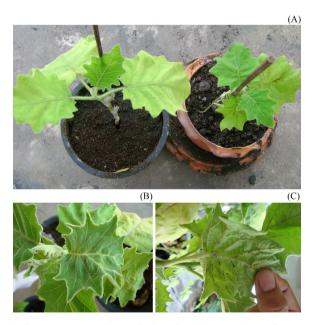


Fig. 4 Symptom comparison on bolo maka: (A) comparison of symptoms between two CLVd isolates, CLVd-bolo maka (right side) and CLVd first tomato isolate (left side); (B) CLVd-bolo maka showing severe apical stunting, leaf rugosity and epinasty; (C) CLVd-bolo maka showed severe vein necrosis (Tangkanchanapas et al., 2013a)

Furthermore, this CLVd strain caused severe reduction in fruit size on eggplants (Bhuvitarkorn and Reanwarakorn, 2019). Notably, during those surveys, the first tomato isolates from Thailand (Accessions DQ022677, DQ061193 and DQ061192) were never detected again, while the CLVd tomato isolates from the second outbreak were still detected, together with the isolates from the third outbreak on tomato production sites in northern and northeastern regions of Thailand (Tangkanchanapas et al., 2012, 2021). In addition, sequence similarity studies and phylogenetic tree analysis clustered all full sequences from the CLVd isolates in the

GenBank database into five groups: (1) Thai isolates from the second and third field inspection; (2) most European isolates obtained from ornamentals; (3) Thai isolates from the first, second and third field inspections; (4) European isolates obtained from tomatoes; and (5) minor European isolates found from ornamentals (Tangkanchanapas et al., 2012, 2021; Tangkanchanapas, 2021;) (Fig. 5). It is presumed that CLVd had been introduced to Thailand at least three times, most likely through contaminated imported tomato parent seeds, while viroid screening was barely conducted before all these parent seeds were released to the companies.

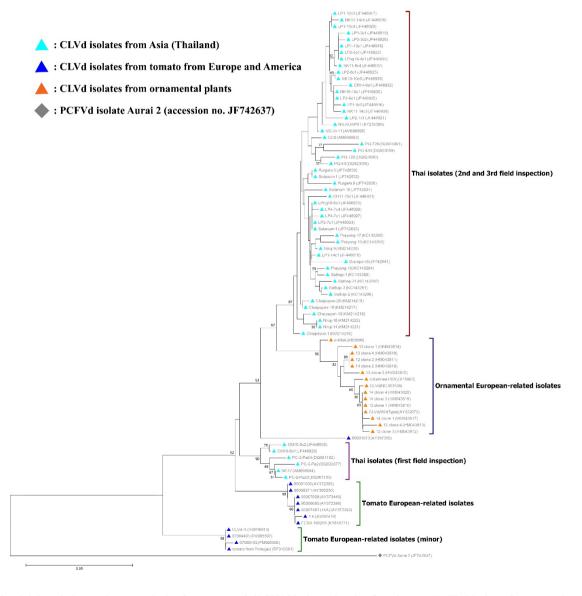


Fig. 5 Neighbor-joining phylogenetic tree analysis of sequences of all CLVd isolates showing five clusters: (1) Thai isolates from second and third field inspections; (2) European isolates found on ornamentals; (3) Thai isolates from first, second and third field inspections; (4) European isolates found on tomato; and (5) minor European isolates found on ornamentals, where tree constructed using MEGA version 7.0.21 with 1,000 bootstrap replicates and 50% cut-off value, with PCFVd isolate Aurai 2 (accession JF742637) used as outgroup (Tangkanchanapas, 2020)

Pepper chat fruit viroid (PCFVd), a member of the Pospiviroid genus and the Pospiviroidae family, is the second most damaging tomato viroid in Thailand. It was first recorded on both tomato fruit and at hybrid seed production sites in 2011 in Lampang (northern region) and Khon Kaen (northeastern region) provinces, as shown in Fig. 1 (Reanwarakorn et al., 2011; Tangkanchanapas et al., 2013b). The Thai variant shared 99% identity with the first PCFVd isolate (FJ409044), previously reported from sweet pepper in the Netherlands (Verhoeven et al., 2009). Since then, PCFVd has been detected quite frequently on both tomato and pepper production sites in the northern and northeastern regions of Thailand (Reanwarakorn et al., 2011; Tangkanchanapas et al., 2012). PCFVd causes serious problems and high yield losses in both tomato and chili pepper crops. Generally, it induces symptoms on tomatoes very similar to CLVd (Reanwarakorn et al., 2011; Tangkanchanapas et al., 2012, 2013b) (Fig. 6). Notably, PCFVd causes severe and more distinct symptoms on bolo maka than the other pospiviroids. It induces severe rugosity and malformation of bolo maka leaves, whereas the other pospiviroids mainly induce stunting on the whole plant with leaf epinasty and vein necrosis (Tangkanchanapas et al., 2012, 2013b), as shown in Fig. 7. In addition, sequence similarity studies and phylogenetic tree analysis clearly clustered all Thai PCFVd isolates into two groups by geographic location: (1) isolates from tomato and pepper hybrid seed production sites in northeastern Thailand; and (2) isolates from tomato and pepper production sites in northern Thailand (Fig. 8). It is possible that PCFVd was introduced to Thailand at least two twice through different pathways: northeastern PCFVd isolates from parent seeds for hybrid seed production export and northern PCFVd isolates from seeds for domestic consumption.

Furthermore, PCFVd and CLVd were repeatedly intercepted in tomato and chili pepper seeds exported from Thailand (Chambers et al., 2013; Constable et al., 2019; Plant Health Inspection Service (APHIS), 2019). As a result, several importing countries, such as Australia, USA and Japan, have imposed stricter requirements and regulations on all shipments of tomato, (bell) pepper and other Solanaceae vegetable seeds exported from Thailand. For this reason, export compliance with all quarantine requirements, including field inspection, laboratory analysis, and seed testing analysis, has become more stringent, resulting in more time-consuming and costly complications.



Fig. 6 Symptoms of PCFVd on tomato cv. Rutgers: (A) severe stunting and leaf yellowing; (B) apical stunting and vein necrosis on stem; (C) leaf rugosity and epinasty, as well as leaf distortion; and (D) necrosis on leaf veins and petioles

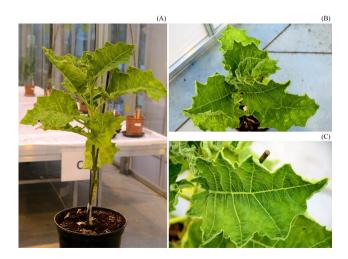


Fig. 7 Symptoms of PCFVd on bolo maka: (A) severe stunting; (B) apical stunting and malformation; (C) severe rugosity and malformation of leaves

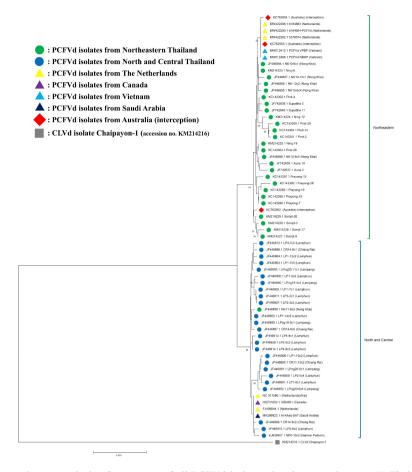


Fig. 8 Neighbor-joining phylogenetic tree analysis of sequences of all PCFVd isolates showing two clusters: (1) Thai isolates found in northeastern Thailand, along with three Netherlands isolates and three intercepted by Australia; (2) Thai isolates found in northern Thailand, along with other isolates consisting of two from Netherlands, one from Canada and one from Saudi Arabia, for tree constructed using MEGA version 11.0.13 with 1,000 bootstrap replicates and 50% cut-off value with CLVd isolate Chaipayon-1 (accession KM214216) used as outgroup

Viroids in ornamental plants

Most recently, two ornamental viroids have been reported in Thailand, namely chrysanthemum stunt viroid (CSVd) and chrysanthemum chlorotic mottle viroid (CChMVd).

CSVd, a member of the *Pospiviroid* genus and the *Pospiviroidae* family, is one of the most serious chrysanthemum and ornamental diseases. CSVd was first found in chrysanthemum in Nakhon Ratchasima province (Fig. 1) in 2020. It caused typical viroid symptoms such as stunting, leaf yellowing, malformation of flower, and reduction of flower size on chrysanthemum plants (Fig. 9). All CSVd Thai isolates had 98.3–99.7% similarity to previously reported GenBank sequences (Accessions AF394452, JQ685739, HQ891018, JX909290, JF414238, JF938538, Z68201, DQ094298, U82445, and FN646407) (Netwong et al., 2020). During 2019–2021, this viroid was found again in chrysanthemum cultivation areas in Chiang Mai and Chiang Rai provinces,



Fig. 9 CSVd natural infection symptoms on chrysanthemum: (A, B and C) stunting, leaf yellowing; (D, E and F) flower malformation and reduced flower size

Thailand (northern region; Fig. 1), with low disease incidence (3.3%). The new variants shared 98.7–99.9% similarity with previous CSVd variants deposited in the GenBank database (Supakitthanakorn et al., 2022). Lastly, CChMVd is another chrysanthemum pathogen that belongs to the *Pelamoviroid* genus and the *Avsunviroidae* family. Between 2019 and 2021, it was found in chrysanthemum production areas in Chiang Mai and Chiang Rai provinces, Thailand (northern region; Fig. 1), showing chlorotic spots and mottling on leaves mainly. However, the disease incidence was low at 4.3% for both CSVd and CChMVd in Chiang Mai province and 2.9% for CChMVd in Chiang Rai province. All Thai CChMVd variants shared 95.5–99.3% identity to previously reported GenBank sequences. The disease incidence was low at 3.3% for CSVd and 4.0% for CChMVd (Supakitthanakorn et al., 2022).

Conclusion

Thailand is a country with a range of diverse crops and agricultural products, including starch-based species, fruits, vegetables, oils and ornamental crops. International trade has increased with increasing globalization to include huge volumes of agricultural commodities that are both imported and exported, resulting in increased risks of introducing new plant pests, especially viruses and viroids, that may be associated with imported plant propagation materials. Furthermore, Thailand's climate, with its consistently high average temperature and long daylight hours, is favorable for viroid diseases, increasing the likelihood of their establishment. This has an impact on a variety of economic crops, particularly several Solanaceae species such as tomato and chili pepper.

The origin of viroids in Thailand is still mysterious, however, it is likely from imported plant propagation materials such as seeds, cuttings, or tissue cultures. It is possible that viroid transmission modes and agricultural commodity pathways are the primary factors in pest introduction. For example, seed-transmissible viroids (CLVd and PCFVd) are most likely introduced through imported parent seeds, while mechanical-transmissible viroids are probably introduced via imported vegetative propagation materials (cuttings, scions and buds). Although viroid symptom expressions on several major host plants are commonly observed and recognized, on the other hand, completely latent symptoms can be found in a high number of their minor host plants (particularly ornamentals, herbs and weeds), making monitoring and eradication of these diseases almost impossible. To conclude, the current status of most viroids in Thailand is still unclear, especially on fruit trees, ornamentals and solanaceous herbs and weeds. Further elucidation will require intensive surveillance and monitoring.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

- Bhuvitarkorn, S., Reanwarakorn, K. 2019. Pollen and seed transmission of *Columnea latent viroid* in eggplants. Eur. J. Plant Pathol. 154: 1067–1075. doi.org/10.1007/s10658-019-01728-9
- Brierley, P. 1952. Exceptional heat tolerance and some other properties of the chrysanthemum stunt virus. Plant Dis. Rep. 36: 243.
- Candresse, T., Verhoeven, J.T.J., Stancanelli, G., Hammond, R.W., Winter, S. 2017. Other pospiviroids infecting solanaceous plants. In: Hadidi, A., Flores, R., Randles, J.W., Palukaitis, P. (Eds.). Viroids and Satellites. Academic Press. Boston, MA, USA, pp. 159–168. doi. org/10.1016/B978-0-12-801498-1.00015-2
- Chambers, G.A., Seyb, A.M., Mackie, J., Constable, F.E., Rodoni, B.C., Letham, D., Davis, K., Gibbs, M.J. 2013. First report of *Pepper chat fruit viroid* in traded tomato seed, an interception by Australian biosecurity. Plant Dis. 97: 1386–1386. doi.org/10.1094/PDIS-03-13-0293-PDN
- Climatological Group. 2015. The Climate of Thailand. Climatological Group, Meteorological Development Bureau, Meteorological Department. Bangkok, Thailand.
- Constable, F., Chambers, G., Penrose, L., Daly, A., Mackie, J., Davis, K., Rodoni, B., Gibbs, M. 2019. Viroid-infected tomato and capsicum seed shipments to Australia. Viruses 11: 98. doi.org/10.3390/v11020098
- Ding, B., Itaya, A. 2007. Viroid: A useful model for studying the basic principles of infection and RNA biology. Mol. Plant Microbe Interact. 20: 7–20. doi.org/10.1094/MPMI-20-0007
- Dingley, A.J., Steger, G., Esters, B., Riesner, D., Grzesiek, S. 2003. Structural characterization of the 69 nucleotide potato spindle tuber viroid left-terminal domain by NMR and thermodynamic analysis. J. Mol. Biol. 334: 751–767. doi.org/10.1016/j.jmb.2003.10.015
- Flores, R., Hernández, C., Martínez de Alba, A.E., Daròs, J.-A., Di Serio, F. 2005. Viroids and viroid-host interactions. Annu. Rev. Phytopathol. 43: 117–139. doi.org/10.1146/annurev.phyto.43.040204.140243
- Francki, R.I.B., Zaitlin, M., Palukaitis, P. 1986. *In vivo* encapsidation of potato spindle tuber viroid by velvet tobacco mottle virus particles. Virology 155: 469–473. doi.org/10.1016/0042-6822(86)90208-4
- Galindo, J., Lopez, M., Aguilar, T. 1986. Significance of *Myzus persicae* in the spread of tomato planta macho viroid. Fitopatol. Bras. 11: 400–410.
- Hammond, R., Smith, D.R., Diener, T.O. 1989. Nucleotide sequence and proposed secondary structure of *Columnea* latent viroid: A natural mosaic of viroid sequences. Nucleic Acids Res. 17: 10083–10094. doi. org/10.1093/nar/17.23.10083
- Hammond, R.W. 2017. Economic significance of viroids in vegetable and field crops. In: Hadidi, A., Flores, R., Randles, J.W., Palukaitis, P. (Eds.). Viroids and Satellites. Academic Press. Boston, MA, USA, pp. 5–13. doi.org/10.1016/B978-0-12-801498-1.00001-2
- Hannok, P. 2004. Survey of Grapevine yellow speckle viroid in Thailand. M.Sc. thesis, Agriculture Biotechnology, Interdisciplinary Graduate Program, Kasetsart University Kamphaeng Saen Campus. Nakhon Pathom, Thailand.
- Hannok, P., Reanwarakorn, K. 2005. cDNA probe for grapevine yellow speckle viroid detection. Kasetsart J. (Nat. Sci.) 39: 46–52.

- Hollings, M., Stone, O.M. 1973. Some properties of chrysanthemum stunt, a virus with the characteristics of an uncoated ribonucleic acid. Ann. Appl. Biol. 74: 333–348. doi.org/10.1111/j.1744-7348.1973.tb07754.x
- Kovalskaya, N., Hammond, R.W. 2014. Molecular biology of viroid–host interactions and disease control strategies. Plant Sci. 228: 48–60. doi. org/10.1016/j.plantsci.2014.05.006
- Marach, S. 2008. Infectious clones of Columnea latent viroid and its effects on commercial tomato varieties. M.Sc. thesis, Agriculture Biotechnology, Interdisciplinary Graduate Program, Kasetsart University Kamphaeng Saen Campus. Nakhon Pathom, Thailand.
- National Science and Technology Development Agency. 2020. Strategic Master Plan Seed 2015–2024. National Science and Technology Development Agency. Pathum Thani, Thailand.
- Navarro, B., Gisel, A., Rodio, M.-E., Delgado, S., Flores, R., Di Serio, F. 2012. Viroids: How to infect a host and cause disease without encoding proteins. Biochimie 94: 1474–1480. doi.org/10.1016/j.biochi.2012.02.020
- Netwong, C., Tansuwan, K., Reanwarakorn, K. 2020. Detection of *Chrysanthemum stunt viroid* (CSVd) from chrysanthemum plants in the fields. Thai Agric. Res. J. 38: 23–32.
- Office of Agricultural Regulation 2021. Report on Quantity and Value of Imports and Controlled Seeds for the Year 2010. Office of Agricultural Regulation. Bangkok, Thailand.
- Owens, R.A., Flores, R., Di Serio, F., Li, S.F., Pallás, V., Randles, J.W., Sano, T., Vidalakis, G. 2012. Viroids. In: Andrew, M.Q.K., Michael, J.A., Eric, B.C., Elliot, J.L. (Eds.). Virus Taxonomy Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press. San Diego, CA, USA, pp. 1220–1234.
- Owens, R.A., Sano, T., Feldstein, P.A., Hu, Y., Steger, G. 2003. Identification of a novel structural interaction in Columnea latent viroid. Virology 313: 604–614. doi.org/10.1016/S0042-6822(03)00352-0
- Owens, R.A., Smith, D.R., Diener, T.O. 1978. Measurement of viroid sequence homology by hybridization with complementary DNA prepared *in vitro*. Virology 89: 388–394. doi.org/10.1016/0042-6822(78)90181-2
- Plant Health Inspection Service (APHIS). 2019. U.S. Federal Order for Pospiviroid. Phytosanitary Issues Management. Maryland, MD, USA.
- Pomma, S. 2002. Viroid detection in limes. M.Sc. thesis, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, Thailand.
- Querci, M., Owens, R.A., Bartolini, I., Lazarte, V., Salazar, L.F. 1997. Evidence for heterologous encapsidation of potato spindle tuber viroid in particles of potato leafroll virus. J. Gen. Virol. 78: 1207–1211. doi. org/10.1099/0022-1317-78-6-1207
- Rakdang, W. 2001. Detection of grapevine viroid in Thailand. M.Sc. thesis, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University Kamphaeng Saen Campus. Nakhon Pathom, Thailand.
- Randles, J.W. 2003. Economic impact of viroid diseases. In: Hadidi, A., Flores, R., Randles, J.W., Semancik, J.S. (Eds.). Viroids. CSIRO Publishing. Victoria, Australia, pp. 3–11.
- Reanwarakorn, K., Klinkong, S., Porsoongnurn, J. 2011. First report of natural infection of *Pepper chat fruit viroid* in tomato plants in Thailand. New Dis. Rep. 24: 6–6. doi.org/10.5197/j.2044-0588.2011.024.006
- Reanwarakorn, K., Pomma, S., Attathom, S. 2003. Evidence of citrus exocortis viroid in Thailand. Kasetsart J. (Nat Sci) 37: 453–459.
- Salazar, L.F., Querci, M., Bartolini, I., Lazarte, V. 1995. Aphid transmission of potato spindle tuber viroid assisted by potato leafroll virus. Fitopatologia 30: 56–58.

- Sangdee, A., Sirithorn, P., Thummabenjaporn, P. 2003. Viroid in tomato: New record in Thailand. Kaen Kaset 31: 200–210.
- Singh, R.P., Kurz, J. 1997. RT-PCR analysis of PSTVd aphid transmission in association with PLRV. Can. J. Plant Pathol. 19: 418–424. doi. org/10.1080/07060669709501070
- Singh, R.P., Ready, K.F.M., Nie, X. 2003. Biology. In: Hadidi, A., Flores, R., Randles, J.W., Semancik, J.S. (Eds.). Viroids. CSIRO Publishing. Collingwood, Australia, pp. 30–48.
- Supakitthanakorn, S., Vichittragoontavorn, K., Kunasakdakul, K., Ruangwong, O. 2022. Phylogenetic analysis and molecular characterization of chrysanthemum chlorotic mottle viroid and chrysanthemum stunt viroid from chrysanthemum in Thailand. J. Phytopathol. 170: 700–710. doi.org/10.1111/jph.13134
- Tangkanchanapas, P. 2020. Viroid-host interactions in Solanaceae. Ph.D. thesis, Faculty of Bioscience Engineering, Ghent University. Ghent, Belgium.
- Tangkanchanapas, P. 2005. Viroid detection in tomato (*Lycopersicon esculentum* Mill.) seed production plantation in Northeast of Thailand.
 M.Sc. thesis, Agriculture Biotechnology, Interdisciplinary Graduate Program, Kasetsart University Kamphaeng Saen Campus. Nakhon Pathom, Thailand.
- Tangkanchanapas, P., Haegeman, A., Höfte, M., De Jonghe, K. 2021. Reassessment of the *Columnea latent viroid* (CLVd) taxonomic classification. Microorganisms 9: 1117. doi.org/10.3390/ microorganisms9061117
- Tangkanchanapas, P., Juenak, H., De Jonghe, K. 2018. First reported occurrence of citrus bent leaf viroid and citrus dwarfing viroid on imported oranges from China and lime fruits from Cambodia. Virus Dis. 29: 416–417. doi.org/10.1007/s13337-018-0466-0
- Tangkanchanapas, P., Kongchuensin, M., Saelor, N., Noochoo, S., Juenak, H. 2014. Interception of citrus viroid on imported orange from republic of China and lime from Cambodia. Thai Agric. Res. J. 32: 252–267.
- Tangkanchanapas, P., Reanwarakorn, K., Juenak, H., De Jonghe, K. 2017. First report of *Grapevine yellow speckle viroid-2* infecting grapevine (*Vitis vinifera*) in Thailand. New Dis. Rep. 36: 6–6. doi.org/10.5197/j.2044-0588.2017.036.006
- Tangkanchanapas, P., Reanwarakorn, K., Kirdpipat, W. 2013a. The new strain of *Columnea latent viroid* (CLVd) causes severe symptoms on Bolo Maka (*Solanum stramonifolium*). Thai Agric. Res. J. 31: 53–68.
- Tangkanchanapas, P., Reanwarakorn, K., Kirdpipat, W. 2013b. Detection of *Columnea latent viroid* (CLVd) and Pepper chat fruit viroid (PCFVd). Thai Agric. Res. J. 31: 107–122.
- Tangkanchanapas, P., Saelor, N., Noochoo, S., Juenak, H., Reanwarakorn, K. 2015. Development of detection technique for grapevine yellow speckle viroid 1 and 2 (GYSVd-1 and 2) causing grapevine disease by RT-PCR method. Thai Agric. Res. J. 33: 68–84.
- Tangkanchanapas, P., Srithongchai, W., Warawichanee, K., Srichart, W. 2012. Interception of *Columnea latent viroid* (CLVd) in Imported Tomato Seed. Department of Agricultural. Bangkok, Thailand.
- Tangkanchanapas, P., Warawichanee, K., Srichart, W., Khampanich, W., Pongsapich, P. 2010. Development of Viroid Detection on Citrus Propagation, Annual Research Report 2010. Department of Agricultural, Bangkok, Thailand.
- Verhoeven, J.T.J., Jansen, C.C.C., Roenhorst, J.W., Flores, R., de la Peña, M. 2009. *Pepper chat fruit viroid*: Biological and molecular properties of a proposed new species of the genus *Pospiviroid*. Virus Res. 144: 209–214. doi.org/10.1016/j.virusres.2009.05.002