International Journal of Agricultural Technology 2023 Vol. 19(5):2349-2366
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Host range and graft-transmission of *Columnea latent viroid* in eggplant rootstocks

Kungwon, P. [1,2], Sinhabandhu, S.[3] and Reanwarakorn, K.[1,2,3*]

[1]Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand; [2]Center of Excellence on Agricultural Biotechnology: (AG-BIO/MHESI), Bangkok 10900, Thailand; [3]Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

Kungwon, P., Sinhabandhu, S. and Reanwarakorn, K. (2023). Host range and graft-transmission of *Columnea latent viroid* in eggplant rootstocks. International Journal of Agricultural Technology 19(5):2349-2366.

Abstract The host range study showed that CLVd could infect plants from the *Asteraceae*, *Compositae*, *Cucurbitaceae*, and *Solanaceae* families. However, only the *Cucurbitaceae* and *Solanaceae* expressed abnormal symptoms. These results might be attributed to differences in plant species, inoculation conditions, and viroid isolates. After grafting, we found that three out of four eggplant cultivars survived with varying numbers of plants. Tomato scion leaves expressed crinkled leaves and necrosis on the vein, petiole, and stem 2 weeks after grafting. The grafts became severely stunted, with some scions dying 1 month after grafting. Our findings regarding the host range could be useful to make farmers more aware of CLVd reservoirs and the importance of adhering to adequate hygiene standards to avoid unintended viroid dissemination.

Keywords: *Columnea latent viroid*, Eggplant, Graft transmission

Introduction

Viroids are the tiniest plant pathogens, with circular single-stranded RNAs and unable to code for any proteins (Adkar-Purushothama and Perreault, 2020; Flores *et al.*, 2005). One of the most important members of the *Pospiviroid* family members that should be detected during the seed importation process is *Columnea latent viroid* (CLVd). It causes substantial yield loss in tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*) and pepper (*Capsicum annuum*). In addition, CLVd can be easily transmitted through seeds, mechanical inoculation and even contaminated tools. Common symptoms caused by CLVd involve apical shoot stunt, leaf rugosity, necrosis of the leaf vein, petiole, and stem, as well as plant stunting. On the other hand, ornamental plants show no sign of symptoms when infected with CLVd

* **Corresponding Author:** Reanwarakorn, K.; **Email:** reanwarakorn.k@gmail.com

(Owens *et al.,* 1978; Spieker, 1996). The distribution of CLVd is heavily influenced by germplasm propagated vegetatively. However, controlling viroid propagation in the field can be challenging at times. Since no treatment can directly control viroids diseases (Barba *et al.,* 2003), one of the key strategies is to avoid pathogen introduction via propagation and planting materials that are free of viroid.

Tomatoes are one of the world's most important vegetable crops. They are, however, susceptible to a variety of soil microorganisms, including bacterial wilt, waterlogging, fusarium wilt, and nematode. On the other hand, eggplant roots have more vitality and are more resistant to bacterial and fungal attacks. The traditional breeding or transgenic plants developed to solve such challenges are time-consuming and limited in certain countries. As a result, the grafting technique became an alternate way that was employed to tackle these problems in many crops. (Albacete *et al.*, 2015; Cohen and Naor, 2002; Palada and Wu, 2005; Rouphael *et al.,* 2017), with additional crop performance benefits, notably increased yields (Chung and Lee, 2007; Musa *et al.,* 2020; Rivero *et al.,* 2003). Commercial tomato grafting was initially proven in the early 1960s (Lee and Oda, 2003). It has since become a common tomato crop farming practice in many regions of the world (Lee *et al.,* 2010; Mudge *et al.,* 2009).

The transplantation of plant organs, plant grafting, is the technique of cutting and fusing plant tissues from at least two different plants together to allow vascular continuity between rootstocks and scions (Melnyk and Meyerowitz, 2015; Pina and Errea, 2005). The whole sliced surface of the scion and rootstocks should be in close contact to prevent air from becoming trapped in the areas. Intraspecific grafting is more common. However, many cases of interspecific grafting were also reported, such as grafting of eggplants onto tomato hybrids (Khah *et al.,* 2006) and melon grafted onto *Cucurbita* rootstocks (Cohen *et al.,* 2002). Apart from the benefits of plant grafting, because CLVd can be transmitted mechanically, the propagation of this viroid may occur when rootstocks become contaminated unknowingly, thus accidentally spreading the disease.

The aim of study was to investigate the host ranges in economically important cultivars and transmission of CLVd from eggplant rootstocks via grafting.

Materials and methods

Viroid source and plant materials

The viroid source for inoculum was the *Columnea latent viroid* (CLVd) isolate NK-KUKPS1 accession no. KY235369 was inoculated onto young Rutgers tomato (*Solanum lycopersicum*) and used as a fresh inoculum. The mechanical inoculation was carried out by grinding CLVd-infected tomato leaves in 0.1 M phosphate buffer (pH 9.0) 1 g: 10mL. The prepared inoculum was applied to young tomato leaves that had previously been dusted with carborundum powder. The inoculated plants were then kept in an insect-proof greenhouse

For graft-transmission assays, three eggplant cultivars were used as rootstocks: long eggplant (*Solanum melongena*), small eggplant (*Solanum melongena*), wild pea eggplant (*Solanum torvum* Sw.) and thornless pea eggplant (*Solanum torvum* Sw.). CLVd was inoculated onto eggplants as described (three plants per experiment). The plants were then maintained in an insect-proof greenhouse. All experiments were done at the greenhouse located at the Department of Plant Pathology, Kasetsart University, Kamphaeng Saen Campus.

Host range of CLVd

Economically plants from various families, as shown in Table 1, were used in this study to investigate the host range of CLVd. Inoculation was carried out by rubbing 0.5 mL of CLVd inoculum onto the leaves of the selected plant cultivars, followed by rinsing with tap water. The second inoculation was carried out five days after the first inoculation for cucurbits and seven days after the first inoculation for other plant species to ensure plant infection. The inoculated plants were maintained in the net house at 28° to 35°C with a photoperiod of 12-14 h per day. An insecticide spraying program was implemented weekly. The biological activity was determined by the appearance of symptoms every week up to 12 weeks post-inoculation. The leaves of all inoculated plants were collected, and viroid infection was determined using the RT-PCR technique.

Transmission by grafting with infected rootstocks

CLVd was mechanically inoculated onto eggplant rootstocks as previously described. The presence of a viroid was confirmed using RT-PCR. One month after the inoculation, grafting was performed. The apical tips of Rutgers tomato scions (10-20 cm in length) were cut at 70-80 degrees with a sterilized blade. The rootstocks were inserted with the rubber tubing to allow the cut portion of the scion or splice to come into contact with each other. The scions were top-grafted onto eggplant rootstocks and covered with plastic for

14 days to maintain humidity. The grafted plants were then transferred into growing pots and kept in an insect-proof greenhouse.

CLVd detection by reverse transcription-polymerase chain reaction (RT-PCR)

The following RT-PCR steps were performed. Thermo Scientific RevertAid Reverse Transcriptase [RT] (Thermo Scientific™) synthesized cDNA using specific primer pairs designed for CLVd with additional primer pairs for Nad as an internal control. The PCR reaction was performed using the *Taq* DNA polymerase (Thermo Scientific™) under the following conditions: 96 ℃ for 40 sec, 54 ℃ for 40 sec, 72 ℃ for 40 sec, and a final extension at 72 ℃ for 7 min. All steps were performed according to the manufacturer's protocols in a Biometra T1 model (Biometra GmbH). CLVd-specific primer pairs (cCL-P2: CTGCAGCCATGCAAAGA and hCL-P2: GGTCAGGTGTGAACCAC) were expected to amplify PCR products of 370 bp (Marach, 2008), while Nad primers (Nad2-1a: GGACTCCTGACGTATACGAAGGATC and Nad2-1b: AGCAATGAGATTCCCCAATATCAT) were expected to amplify 188 bp (Thompson *et al.,* 2003). The RT-PCR amplification products were analyzed using gel electrophoresis on a 2% agarose gel with the staining dye RedSafeTM Nucleic Acid Staining Solution (iNtRON Biotechnology). A Gel Documentation UV-transilluminator was used to visualize the results under UV light (SYNGENEU Genesis3).

Results

Host range of Columnea latent viroid

The host ranges of CLVd were studied in 45 cultivars from 14 families using mechanical inoculation, with 27 cultivars tested to be the host for CLVd infection using RT-PCR (Table 1). Among these cultivars, CLVd mainly infected *Cucurbitaceae* host plants with no symptoms. The majority of these cultivars have yet to be identified as natural hosts. At 12 weeks after inoculation, the cucumbers *C. cucumis* L. cv. 'Bingo' and 'Nato' showed stunting, short internodes, and signs of leaf deformation. At a later stage, more symptoms were noticed, such as abnormal flower growth, less pollen, and overproduction of female flowers (Figure 1). Watermelons, as well as *G. aurantiaca* and *T. erecta L*., showed no signs of infection. Most *Solanaceae* species expressed severe leaf necrosis and stunting in *S. melongena*. We also confirmed that *S. stramonifolium* Jacq. (bolo maka), *Datura metel* (datura) and *N. glutinosa* (tobacco) could be infected by CLVd with severe leaf necrosis, leaf crinkle, and stunting symptoms (Figures 2, 3 and 4).

Table 1. The infectivity of CLVd mechanical inoculation on different plant species

Family	Scientific name	Common name	Symptoms	RT-PCR
Amaranthaceae	*Amaranthus tricolor* L.	Chinese spinach	ns	-
	Celosia argentea L. cv. Soi Gai	Cockscomb	ns	-
	Celosia argentea L. cv. Ngon Gai	Cockscomb	ns	-
Apocynaceae	*Catharanthus roseus* (L.)	Rose periwinkle	ns	-
	Adenium obesum Balf.	Desert rose	ns	-
Asteraceae	*Emilia sonchaifilia* (L.)	Emilia	ns	+
	Gynura aurantiaca	Purple passion	ns	-
	Tagetes erecta L.	Marigold	ns	-
Balsaminaceae	*Impatiens balsamina* L.	Garden balsam	ns	-
Capparidacea	*Cleome gynandra* L.	Bastard mustard	ns	-
Caricaceae	*Carica papaya* L. cv. Holland	Papaya	ns	-
Chenopodiaceae	*Chenopodium amaranticolor*	Chenopodium	ns	-
Compositae	*Zinnia elegans* Jacq.	Zinnia	ns	-
	Helianthus annuus L.	Sunflower	ns	-
	Cosmos spp.	Cosmos	ns	+
Cruciferae	*Brassica oleracea* L.	Chinese Kale	ns	-
Cucurbitaceae	*Cucumis sativus* L. cv. Bingo	Cucumber	st, L-dis, ab-fl, ab-pol, sh-int	+
	Cucumis sativus L. cv. Nato	Cucumber	st, L-dis, ab-fl, ab-pol, sh-int	+
	Cucumis sativus L. cv. Mae Wang	Cucumber	ns	-
	Cucumis melonis L. cv. Charentais	Melon	ns	+
	Cucumis melonis L. cv. Green Net T778	Melon	ns	+
	Cucumis melonis L. cv. Honey Dew 1348	Melon	ns	+
	Cucumis melonis L. cv. Pot Orange T1957	Melon	ns	+
	Cucumis melonis L. cv. Golden lady 1383	Melon	ns	+
	Lannatus citrullus L. cv. Showing175	Watermelon	ns	-

Family	Scientific name	Common name	Symptoms	RT-PCR
	Lannatus citrullus L. cv. Torpido	Watermelon	ns	-
	Laganaria siceraria (Molina) Standley	Bottle gourd	ns	+
	Luffa anguigulata	Angle luffa	ns	+
	Luffa cylindrica (L.) M. Roem	Smooth luffa	ns	+
	Cucurbita pepo	Zucchini	ns	+
	Momodica charantia L.	Bitter gourd	ns	+
Labiatae	*Ocimum sanctum* L.	Holy basil	ns	-
	Ocimum O. basilicum L.	Sweet basil	ns	-
Passifloraceae	*Passiflora edulis*	Passion fruit	ns	-
Solanaceae	*Solanum stramonifolium* Jacq.	Bolo maka	sev-st, L-nec, L-crk	+
	Solanum indicum L.	Brihati	ns	-
	Solanum torvum Sw.	Makhuea phuang	ns	-
	Datura metel	Datura	sev-st, L-nec, L-roll	+
	Nicotiana tabacum	Tobacco	ns	-
	Nicotiana glutinosa	Tobacco	st, L-nec	+
	Solanum melongena L. cv. Farmers Long	Eggplant	st, L-yel, L-nec	+
	Solanum melongena L. cv. RPG	Eggplant	st, L-yel, L-nec	+
	Solanum melongena L. cv. Jamaica	Eggplant	st, L-yel, L-nec	+
	Solanum lycopersicum cv. Rutgers	Tomato	sev-st, L-nec, L-dis, sh-int, ab-fl	+
Umbelliferae	*Eryngium foetidum* L.	Long coriander	ns	-

ab-fl (abnormal flower), ab-pol (abnormal pollen), L-crk (leaf crinkled), L-dis (leaf distortion), L-nec (leaf necrosis), L-roll (leaf rolling), L-yel (leaf yellowing), sev-st (severe stunt), sh-innode (Shorten internode), st (stunting), ns (no symptoms

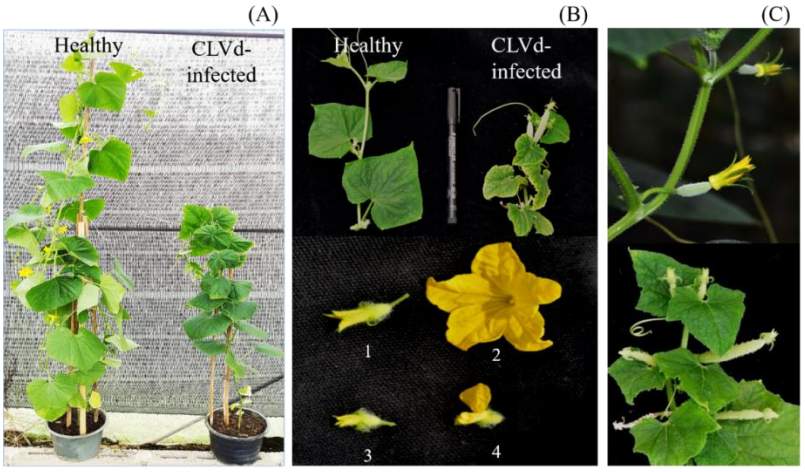

Figure 1. The symptoms of CLVd on CLVd-infected cucumber at 12 weeks after inoculation. A. stunting, short internode and leaf rolling symptoms; B. [upper] leaf distortion such as reducing of leaf size and leaf crinkling, [lower] CLVd-infected cucumber showed flower size reduction (B3 and B4), abnormal formation of male flower petals and do not expand (B4) compared to healthy (B1 and B2); C. [upper] healthy female flowers and [lower] overproduction of female flowers with undeveloped petals

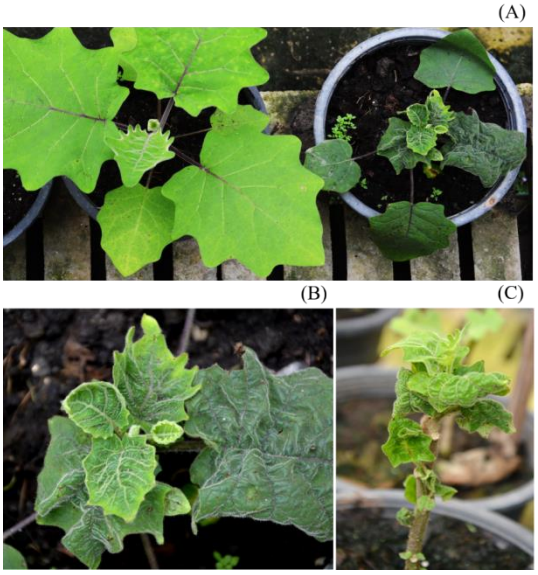

Figure 2. CLVd symptoms at 12 weeks after inoculation on *Solanum stramonifolium* Jacq. (bolo maka). A. severe stunt symptoms (right) compared to healthy plant (left); B. dark green color of mature leaves, crinkling and leaf rolling symptoms; C. severe stunt symptom

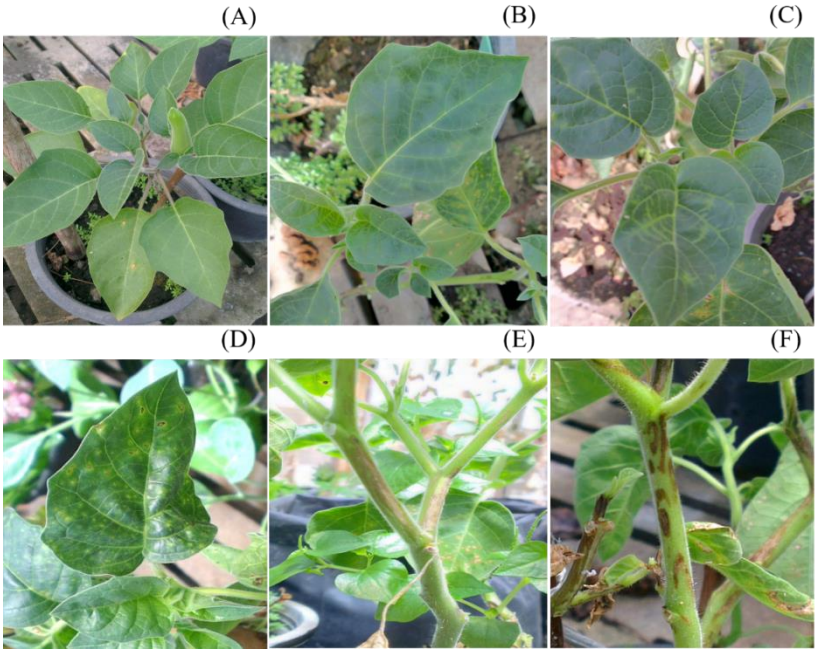

Figure 3. CLVd symptoms on *Datura metel* (datura): A. healthy plants; B. and C. vein yellowing at 2 weeks after inoculation; D. yellow spot with necrotic spot on mature and young leaves, leaf distortion; E. brownish lesion on stem and petioles at 8 weeks after inoculation; F. severe necrotic lesion on stem and petioles at 12 weeks after inoculation

Graft-transmission of Columnea latent viroid in eggplant rootstocks

Four different eggplant cultivars (long eggplant, small eggplant, wild pea eggplant, and thornless pea eggplant) were used as rootstock, where Rutgers tomato were used as scions in grafting experiments. However, we found that three out of four eggplant cultivars (small eggplant, wild pea eggplant, and thornless pea eggplant) survived the following grafting with varying numbers of plants (Table 2). When healthy tomato scions were grafted onto healthy small eggplant rootstocks or wild pea eggplant rootstocks, four grafted plants survived. In grafted plants where thornless pea eggplants were used as root stock, three survived. In grafted plants where CLVd-infected eggplants were used as rootstocks, the survived plants were 4, 2, and 4, when small eggplant, wild pea eggplant, and thornless pea eggplants were used as rootstocks, respectively. In grafted plants where CLVd-infected tomato used as scions and grafted onto healthy eggplant rootstocks, lower survival rate was observed. When small eggplants were used as rootstock, three grafted plants survived. In wild pea eggplant and thornless pea eggplant used as rootstocks,

only 2 plants from each grafting experiment survived. Tomato scion leaves expressed crinkled leaves and necrosis on the vein, petiole, and stem at 2 weeks after grafting (Figure 5). The grafts became severely stunted compared to healthy grafted tomato plants. None of the symptoms were observed in control grafted tomato plants where healthy scions grafted onto uninoculated rootstocks (Figure 6).

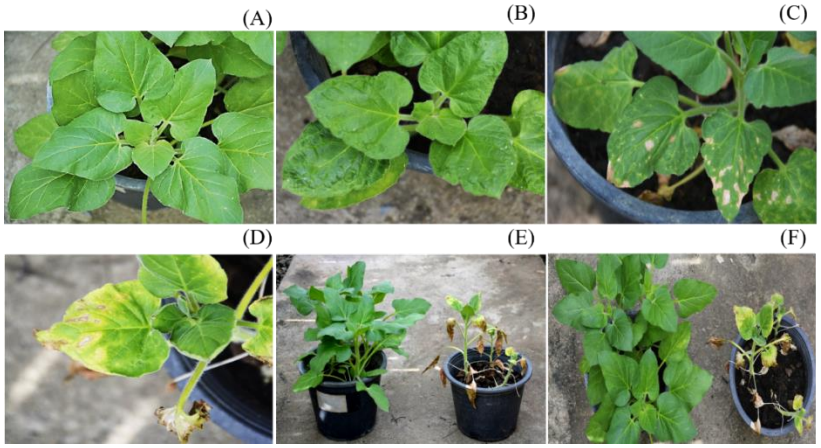

Figure 4. CLVd symptoms at 7 to 30 days after inoculation on *Nicotiana glutinosa* (tobacco). A. healthy plants; B. leaf mosaic and crinkling at 7 days after inoculation; C. necrotic lesions on leaves at 14 days after inoculation; D., E., F. leaf yellowing, necrosis and plant die at 30 days after inoculation

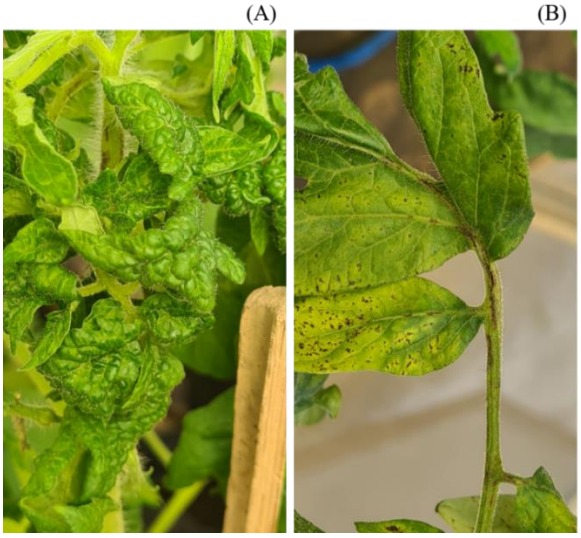

Figure 5. Symptoms of CLVd on tomato leaves at 2 months post grafting. A. crinkled leaves; B. necrosis on leaf, leaf vein and stem

Table 2. Eggplant rootstocks used for CLVd graft transmission

Treatmentsa/	Grafting on eggplant rootstockb/			
	Small eggplant	Long eggplant	Wild pea eggplant	Thornless pea eggplant
H/H	4/5	0/5	4/5	3/5
CL/H	4/5	0/5	2/5	4/5
H/CL	3/5	0/5	2/5	2/5

a/: Rootstock/scion, H (healthy), CL (CLVd infection), b/: Survived grafted plants/total number of grafted plants

When small eggplants were used as rootstocks, fruits obtained from healthy control grafted plants had the highest fruit weight of 3.53±1.10 g, whereas fruits obtained from healthy tomato scions grafted onto CLVd-infected rootstocks had the lowest fruit weight of 1.73±0.59 g. Fruits obtained from control grafted plants had a fruit width and length of 1.50±0.26 cm and 1.33±0.21 cm, respectively. Fruits obtained from healthy tomato scions grafted onto CLVd-infected rootstocks had a fruit width and length of 1.33±0.62 cm and 1.20±0.35 cm, respectively. For plant height, healthy control grafted plants had the greatest plant height of 111.58±1.26 cm, whereas healthy tomatoes grafted onto CLVd-infected rootstocks and CLVd-infected tomato grafted onto healthy rootstocks had plant heights of 27.46±1.80 and 24.97±1.87 cm, respectively (Figure 6A and Table 3).

Figure 6. Viroid-graft transmissions when eggplants were used as rootstocks at 2 months post grafting. Left (Healthy tomato scion top grafted onto healthy eggplant rootstocks), Middle (Healthy tomato scion top grafted onto CLVd-infected rootstocks), Right (CLVd-infected tomato scion top grafted onto healthy eggplant rootstocks), A. small eggplant rootstock; B. wild pea eggplant rootstock; C. thornless pea eggplant rootstock

Table 3. Comparison of fruit weight, width and length as well as plant height obtained from grafted tomato plants on eggplant rootstocks

Eggplant rootstock	Treatmentsa/	Average value ± SDb/			
		Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Plant height (cm)
Small eggplant	H/H	3.53a ± 1.10	1.65a ± 0.26	1.45a ± 0.21	111.58b ± 1.26
	CL/H	1.73a ± 0.59	1.16a ± 0.62	1.11a ± 0.35	27.46a ± 1.80
	H/CL	-	-	-	24.97a ± 1.87
Wild pea eggplant	H/H	7.45b ± 3.02	2.36b ± 0.47	1.80a ± 0.21	119.18b ± 1.52
	CL/H	1.79a ± 0.67	1.26a ± 0.37	1.17a ± 0.41	25.2a ± 0.28
	H/CL	-	-	-	27.4a ± 1.20
Thornless pea eggplant	H/H	7.28b ± 3.39	2.16b ± 0.39	1.90b ± 0.25	113.53b ± 0.57
	CL/H	2.05a ± 0.89	1.32a ± 0.24	1.24a ± 0.26	24.8a ± 1.47
	H/CL	-	-	-	25.6a ± 1.06

a/: grafted plants with rootstock/scion, b/: confidence interval at $p < 0.05$, H (healthy), CL (CLVd infection), - (no fruits obtained), value followed by the same letter are not significantly different

When wild pea eggplants were used as rootstocks, fruits obtained from healthy control grafted plants had the highest fruit weight of 7.45±3.02 g, whereas fruits obtained from healthy tomatoes grafted onto CLVd-infected rootstocks had fruit weight of 1.79±0.67 g. Fruits obtained from control grafted plants had a fruit width and length of 2.04±0.47 cm and 1.66±0.21 cm, respectively. Fruits obtained from healthy tomatoes grafted onto CLVd-infected rootstocks had a fruit width and length of 1.32±0.37 cm and 1.25±0.41 cm, respectively. For plant height, grafted control plants had the highest height of 119.18±1.52 cm. In contrast, healthy tomatoes grafted onto CLVd-infected rootstocks and CLVd-infected tomatoes grafted onto healthy rootstocks had plant heights of 25.20±0.28 and 27.40±1.20 cm, respectively (Figure 6B and Table 3).

When thornless pea eggplants were used as rootstocks, fruits obtained from healthy control grafted plants had the highest fruit weight of 7.28±3.39 g, whereas fruits obtained from healthy tomatoes grafted onto CLVd-infected rootstocks had fruit weight of 2.05±0.89 g. Fruits obtained from control grafted plants had a fruit width and length of 2.04±0.39 cm and 1.75±0.25 cm, respectively. Fruits obtained from healthy tomatoes grafted onto CLVd-infected rootstocks had a fruit width and length of 1.30±0.24cm and 1.22±0.26 cm,

respectively. For plant height, grafted control plants had the greatest plant height of 113.53±0.57 cm, whereas in healthy tomatoes grafted onto CLVd-infected rootstocks and CLVd-infected tomatoes grafted onto healthy rootstocks had plant heights of 24.80±1.47 and 25.60±1.06 cm, respectively (Figure 6C and Table 3).

Among three different eggplant cultivars used as rootstocks, grafted plants where wild pea eggplants were used had the highest average plant height of 119.18 cm, followed by thornless pea eggplant with an average plant height of 113.53 cm and small eggplant with an average plant height of 111.58 cm, respectively. However, when CLVd-infected rootstocks were used, all plants suffered severe stunting and had a low average plant height. Similar results were also observed when CLVd-infected scions were grafted onto healthy rootstocks. Fruits obtained from grafted plants where wild pea eggplants were used as rootstocks had the highest fruit weight, followed by those obtained from thornless pea eggplant. When small eggplants were used as rootstocks, fruits produced the lowest fruit weight (Figure 6 and Table 3).

ANOVA revealed statistically significant differences between treatments. A Tukey HSD post hoc study found significant ($p<0.001$) differences in plant height, fruit weight, and fruit width among treatments. There were no significant differences in fruit weight, fruit width, or fruit length between control grafted plants when small eggplants were used as rootstock and healthy tomato grafted onto CLVd-infected rootstocks. Fruit weight obtained from control grafted wild pea eggplant differed statistically from those obtained from healthy tomato grafted onto CLVd-infected rootstocks. A similar result was also found in the thornless pea rootstock treatments. Only the thornless pea eggplant treatments showed a significant fruit width and length difference. When the findings were compared across the different cultivars, there were no statistically significant differences in those healthy tomatoes grafted onto CLVd-infected rootstocks plants.

Discussion

CLVd infection symptoms may vary depending on the host plant, viroid strain, and environment. In this study, we found that *Cosmos* spp. (cosmos) could be infected with CLVd without showing symptoms which contrary to the findings of Matsushita (Matsushita and Tsuda, 2015). In *Solanaceae* species, however, plants expressed severe leaf necrosis and stunting in *S. melongena*, similar to the study by Matsushita and Tsuda in 2015 (Matsushita and Tsuda, 2015). These finding indicated limited host ranges and that the crops primarily affected were tomato (*S. lycopersicum*), potato (*S. tuberosum*), eggplant (*S.*

melongena), chili (*C. annuum*), petunia (*Petunia x hybrida*), bolo maka (*S. stramonifolium*), gynura (*G. aurantiaca*), and cucumber (*C. sativus*), which provided slightly updated information to support other studies (Matsushita and Tsuda, 2016; Tansuwan and Reanwarakorn, 2018; Verhoeven *et al.*, 2004).

Viroid infection causes complicated host responses, including crosstalk between the hormone and defensive signaling pathways (Owens and Hammond, 2009). According to the previous report, their host range includes vegetable crops and ornamentals (Hammond *et al*., 1989; Tangkanchanapas *et al*., 2013). Although these diseases do not often cause noticeable symptoms, they are significant for developing good propagating material (Papayiannis, 2014; Tangkanchanapas *et al.*, 2013). Most CLVd-infected test plants from the *Asteraceae*, *Cucurbitaceae*, *Compositae*, and *Solanaceae* families were asymptomatic. Consequently, contaminated seeds or vegetatively propagated plants may act as carriers for the undetected spread of viroids to important crops like tomato and eggplant (Van Bogaert *et al*., 2017). According to our findings, the differences in these results could be attributed to plant species, inoculation conditions, and the viroid isolate.

Plant grafting is an old propagation process of joining different parts from different plant varieties together (Goldschmidt, 2014). Its application has been widely used in various agricultural aspects such as asexual reproduction for plants, improving fruit quality and productivity, and increasing resistance to plant diseases. Tomato is one of the most important vegetables globally. However, it is susceptible to many plant diseases, including bacterial and fungal diseases, specially grown during the rainy season. Plant grafting technique is then used to overcome such a problem. Eggplant rootstock is an excellent candidate for interspecific tomato grafting (Max *et al*., 2009). In this study, three out of four eggplant cultivars survived the following grafting with varying numbers of plants. Incompatibility between eggplant rootstocks and scions, as well as rootstocks being too weak, could have been the cause of this incidence. The results agree with Kawaguchi *et al*. (2008), who reported the moderately compatibility of several tomato-eggplant rootstock-scion combinations (Kawaguchi *et al*., 2008). Although, some other graft combinations were successful despite the unsmooth graft union (Wutscher, 1979). Interspecific incompatibility involves cellular recognition, injury response, plant growth regulators, and incompatibility toxins (Andrews and Marquez, 1993), which might inhibit the wound healing and vascular regeneration process. In addition, slow growth of tomato grafting in the early stage was observed that could have been due to the adjustment time required by the plants, which was consistent with Kariada and Aribawa in 2017 (Kariada

and Aribawa, 2017). Plant grafting, however, could play a vital role in the spread of many plant diseases.

As shown in previous studies, eggplants are susceptible to CLVd infection (Bhuvitarkorn and Reanwarakorn, 2019; Matsushita and Tsuda, 2015). According to the results in this study, CLVd can be easily propagated through mechanical grafting from rootstocks to scions, which was consistent with Chung *et al*. (2001) who studied *Chrysanthemum stunt viroid* in chrysanthemum (Chung *et al*., 2001). Tomato scion leaves expressed crinkled leaves and necrosis on the vein, petiole, and stem 2 weeks after grafting. The grafts became severely stunted, with some scions dying 1 month after grafting. The severity of the CLVd isolate used in this study was known as it was grouped into group 1, CLVd-tomato Asian lineage recently (Tangkanchanapas *et al*., 2021). None of the symptoms were observed on healthy scions grafted onto uninoculated rootstocks. Because viroids do not encode any proteins, their long-distance movement could be resulted from interactions with cellular factors. Gómez and Pallás (2004) demonstrated that the phloem protein 2 (CsPP2) interacted with the *Hop stunt viroid* and translocated to the scion following grafting (Gómez and Pallás, 2004). To minimize tissue clumping in the rootstocks, this study did not confirm if viroids were transported from infected scions to rootstocks because the grafted plants with infected scions on healthy rootstocks were severely stunted. However, Bani *et al*. (2010) revealed the long-distance movement of *Citrus exocortis viroid* by grafting inoculated scions onto susceptible rootstock. The viroid was observed in rootstock 4 months after infection (Bani *et al*., 2010). Another report also confirmed the movement of *Apple scar skin viroid* (Kim *et al.*, 2006) to rootstock. In this study, tomato fruit was obtained from grafted plants on infected rootstock, except for the grafted plants using infected scions on healthy rootstocks. There were few differences in the fruit weight, width, or length obtained from CLVd-infected tomato plants grafted onto small eggplant rootstocks compared to the healthy control. The reason could be due to the age of the scion plants at the time of grafting, which was closing to the flowering stage. As a result, the viroid travels vertically (Matsushita *et al.*, 2018), it might not reach the developing flower yet. Furthermore, Zhu *et al.* (2002) reported that PSTVd was not presented in developing flowers. On the other hand, mature flowers retained the viroid in the parenchyma cells of the sepals but not in the petals, stamens, styles, or ovaries (Zhu *et al*., 2002).

Our findings suggested that CLVd was easily transmitted by grafting. However, mechanical transmission by contaminated instruments should not be ruled out. CLVd has been observed to be transferred by contaminated razor blades (Laomanotham and Reanwarakorn, 2019). *Apple scar skin viroid* (Kim

et al., 2006), *Citrus exocortis viroid* (Kyriakou, 1992), *Hop stunt viroid* (Terai *et al.*, 1990) and *Peach latent mosaic viroid* (Hadidi *et al*., 1997) have also been documented to be spread by infected equipment. Finally, the host range data provided by our study could be useful for farmers to become aware of CLVd reservoirs and the importance of adhering to adequate hygiene standards to avoid unintended viroid dissemination.

Acknowledgements

This research is supported by the Center of Excellence on Agricultural Biotechnology, Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (AG-BIO/MHESI) and the Department of Plant Pathology, Kasetsart University, Kamphaeng Saen campus, Thailand.

References

Adkar-Purushothama, C. R. and Perreault, J. P. (2020). Current overview on viroid–host interactions. Wiley Interdisciplinary Reviews. RNA, 11(2): e1570.

Albacete, A., Martínez-Andújar, C., Martínez-Pérez, A., Thompson, A. J., Dodd, I. C., Pérez-Alfocea, F. (2015). Unravelling rootstock×scion interactions to improve food security. Journal of Experimental Botany 66:2211-2226.

Andrews, Marquez. (1993). Graft incompatibility. In: Horticultural Reviews, Wiley-Interscience, pp.183-218.

Bani Hashemian, S. M., Barbosa, C. J., Serra, P. and Duran-Vila, N. (2010). Effects of resistance of *Eremocitrus glauca* and *Microcitrus australis* to viroid infection: replication, accumulation and long-distance movement of six citrus viroids. Plant Pathology 59:413-421.

Barba, M., Ragozzino, E. and Navarro, L. (2003). Viroid elimination by thermotherapy and tissue culture. In: Viroids, Collingwood, CSIRO Publishing, pp. 318-323.

Bhuvitarkorn, S. and Reanwarakorn, K. (2019). Pollen and seed transmission of *Columnea latent viroid* in eggplants. European Journal of Plant Pathology 154:1067-1075.

Chung, H. D. and Lee, J. M. (2007). Rootstocks for grafting. In: Horticulture in Korea, Korean Society for Horticultural Science, pp. 162-167.

Chung, B. N., Park, G. C., Kim, H. R. and Kim, J. S. (2001). *Chrysanthemum stunt viroid* in *Dendranthema grandiflorum*. The Plant Pathology Journal 17:194-200.

Cohen, R., Horev, C., Burger, Y., Shriber, S., Hershenhorn, J., Katan, J. and Edelstein, M. (2002). Horticultural and Pathological Aspects of Fusarium Wilt Management Using Grafted Melons. HortScience 37:1069-1073.

Cohen, S. and Naor, A. (2002). The effect of three rootstocks on water use, canopy conductance and hydraulic parameters of apple trees and predicting canopy from hydraulic conductance. Plant, Cell & Environment 25:17-28.

Flores, R., Hernández, C., Martínez de Alba, A. E., Darós, J-A. and Di Serio, F. (2005). Viroids and viroid-host interactions. Annual Review of Phytopathology 43:117-39.

Goldschmidt, E. e. (2014). Plant grafting: new mechanisms, evolutionary implications. Frontiers in Plant Science 5:727.

Gómez, G. and Pallás, V. (2004). A long-distance translocatable phloem protein from cucumber forms a ribonucleoprotein complex in vivo with Hop stunt viroid RNA. Journal of virology 78:10104-10110.

Hadidi, A., Giunchedi, L., Shamloul, A. M., Poggi-Pollini, C. and Amer, M. A. (1997). Occurrence of Peach Latent Mosaic Viroid in Stone Fruits and Its Transmission with Contaminated Blades. Plant Disease 81:154-158.

Hammond, R., Smith, D. R. and Diener, T. O. (1989). Nucleotide sequence and proposed secondary structure of Columnea latent viroid: a natural mosaic of viroid sequences. Nucleic Acids Research 17:10083-10094.

Kariada, I. K. and Aribawa, I. B. (2017). Grafting of Tomato with Eggplant Rootstock at Penyabangan Village Payangan Subdistrict of Gianyar Bali. IInd International Conference on Sustainable Agriculture and Food Security: A Comprehensive Approach (ICSAFS), West Java, Indonesia 625-630 p.

Kawaguchi, M., Taji, A., Backhouse, D. and Oda, M. (2008). Anatomy and physiology of graft incompatibility in solanaceous plants. The Journal of Horticultural Science and Biotechnology 83:581-588.

Kim, H. R., Lee, S. H., Lee, D. H., Kim, J. S. and Park, J. W. (2006). Transmission of *Apple scar skin viroid* by grafting, using contaminated pruning equipment, and planting infected seeds. Plant Pathology Journal, 22:63-67.

Khah, E. M., Kakava, E., Mavromatis, A., Chachalis, D. and Goulas, C. (2006). Effect of grafting on growth and yield of tomato (Lycopersicon esculentum Mill.) in greenhouse and open-field. Journal of Applied Horticulture 8:3-7.

Kyriakou, A. P. (1992). Incidence in Cyprus of citrus exocortis viroid and its mechanical transmission. Plant Pathology 41:20-24.

Laomanotham, S. and Reanwarakorn, K. (2019). Movement and horizontal transmission of *Columnea latent* viroid. King Mongkut's Agricultural Journal, 37:32-42.

Lee, J. M., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L. and Oda, M. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. Scientia Horticulturae 127:93-105.

Lee, J. M. and Oda, M. (2003). Grafting of herbaceous vegetable and ornamental crops. Horticultural Reviews 28:61-124.

Marach, S. (2008). Infectious clones of *Columnea latent viroid* and its effects on commercial Tomato Varieties. (Master Thesis). Kasetsart University, Bangkok, Thailand.

Matsushita, Y. and Tsuda, S. (2015). Host ranges of *Potato spindle tuber viroid*, *Tomato chlorotic dwarf viroid*, *Tomato apical stunt viroid*, and *Columnea latent viroid* in horticultural plants. European Journal of Plant Pathology 141:193-197.

Matsushita, Y. and Tsuda, S. (2016). Seed transmission of *potato spindle tuber viroid, tomato chlorotic dwarf viroid, tomato apical stunt viroid,* and *Columnea latent viroid* in horticultural plants. European Journal of Plant Pathology 145:1007-1011.

Matsushita, Y., Yanagisawa, H. and Sano, T. (2018). Vertical and Horizontal Transmission of Pospiviroids. Viruses 10:706.

Max, J. F. J., Horst, W. J., Mutwiwa, U. N. and Tantau, H-J. (2009). Effects of greenhouse cooling method on growth, fruit yield and quality of tomato (*Solanum lycopersicum* L.) in a tropical climate. Scientia Horticulturae 122:179-186.

Melnyk, C. W. and Meyerowitz, E. M. (2015). Plant grafting. Current Biology 25:183-188.

Mudge, K., Janick, J., Scofield, S. and Goldschmidt, E. (2009). A history of grafting. In: Horticultural Reviews, New York, Van Nostrand Reinhold Company, pp. 437-493.

Musa, I., Rafii, M. Y., Ahmad, K. and *et al.* (2020). Effects of Grafting on Morphophysiological and Yield Characteristic of Eggplant (*Solanum melongena* L.) Grafted onto Wild Relative Rootstocks. Plants 9:1583.

Owens, R. A. and Hammond, R. W. (2009). Viroid Pathogenicity: One Process, Many Faces. Viruses 1:298-316.

Owens, R. A., Smith, D. R. and Diener, T. O. (1978). Measurement of viroid sequence homology by hybridization with complementary DNA prepared in vitro. Virology 89:388-394.

Palada, M. C. and Wu, D. L. (2005). Increasing off-season tomato production using grafting technology for peri-urban agriculture in Southeast Asia. Ist International Conference and Exhibition on Soilless Culture, Singapore, 742 p.

Papayiannis, L. C. (2014). Diagnostic Real-Time RT-PCR for the simultaneous detection of *Citrus exocortis viroid* and *Hop stunt viroid*. Journal of Virology Methods 196:93-99.

Pina, A. and Errea, P. (2005). A review of new advances in mechanism of graft compatibility–incompatibility. Scientia Horticulturae 106:1-11.

Rivero, R. M., Ruiz, J. M. and Romero, L. (2003). Role of grafting in horticultural plants under stress conditions. Journal of food agriculture and environment 1:70-74.

Rouphael, Y., Kyriacou, M. C. and Colla, G. (2017). Vegetable Grafting: A Toolbox for Securing Yield Stability under Multiple Stress Conditions. Frontiers in Plant Science 8:2255.

Spieker, R. L. (1996). A viroid from Brunfelsia undulata closely related to the Columnea latent viroid. Archives of Virology 141:1823-1832.

Tangkanchanapas, P., Haegeman, A., Höfte, M. and De Jonghe, K. (2021). Reassessment of the *Columnea latent viroid* (CLVd) Taxonomic Classification. Microorganisms 9:1117.

Tangkanchanapas, P., Reanwarakorn, K. and Kirdpipat, W. (2013). The new strain of *Columnea latent viroid* (CLVd) causes severe symptoms on bolo maka (*Solanum stramonifolium*). The Thai Journal of Agricultural Science 31:53-68.

Tansuwan, K. and Reanwarakorn, K. (2018). Seed transmission of Columnea latent viroid in cucumber. The Thai Journal of Agricultural Science 36:130-140.

Terai, Sano, Shikata. (1990). Back-inoculation of plum dapple fruit disease and graft-transmission of peach dapple fruit disease. Annual of the Phytopathological Society of Japan 56:428.

Thompson, J. R., Wetzel, S., Klerks, M. M., Vašková, D., Schoen, C. D., Špak, J. and Jelkmann, W. (2003). Multiplex RT-PCR detection of four aphid-borne strawberry viruses in Fragaria spp. in combination with a plant mRNA specific internal control. Journal of Virological Methods 111:85-93.

Van Bogaert, N., Smagghe, G., Maes, M., De Backer, M. and De Jonghe, K. (2017). Phylogeny of five predominant pospiviroid species in Belgium. European Journal of Plant Pathology 149:25-33.

Verhoeven, J. th. J., Jansen, C. C. C., Willemen, T. M., Kox, L. F. F., Owens, R. A. and Roenhorst, J. W. (2004). Natural infections of tomato by *Citrus exocortis viroid*, *Columnea latent viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid.* European Journal of Plant Pathology 110:823-831.

Wutscher, H. K. (1979). Citrus Rootstocks. In: Horticultural Reviews, New York, Van Nostrand Reinhold Company, pp. 237-269.

Zhu, Y., Qi, Y., Xun, Y., Owens, R. and Ding, B. (2002). Movement of Potato Spindle Tuber Viroid Reveals Regulatory Points of Phloem-Mediated RNA Traffic. Plant Physiology 130:138-146.

(Received: 18 February 2023, Revised: 10 September 2023, Accepted: 13 September 2023)