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REVIEW

Citrus Viroids and Minor Citrus Viruses in Japan

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Abstract

Citrus species grown in Japan are affected by several viruses and viroids, including satsuma dwarf virus, citrus vein enation virus, citrus exocortis viroid, and other citrus viroids. Recently, a comprehensive review was made on the occurrence, history, and research of the following three major citrus viruses in Japan: satsuma dwarf virus, citrus tatter leaf virus (apple stem grooving virus), and citrus tristeza virus (Iwanami 2022). As a sequel, this study reviews the occurrence, history, and research development of citrus viroids as well as minor citrus viruses in and around Japan.

Discipline: Agricultural Environment
Additional key words: quarantine program, transboundary pest, virus elimination

Introduction

Several citrus viruses and citrus viroids (CVds) are widely distributed and seriously affect citrus production in Japan. Some of these pathogens were apparently disseminated with unchecked budwoods from Japan to neighboring areas, where Japanese citrus cultivars are popular among growers. Recently, a comprehensive review was made on the occurrence, history, and research activities of the following three major citrus viruses in and around Japan: satsuma dwarf virus, citrus tatter leaf virus (apple stem grooving virus), and citrus tristeza virus (Iwanami 2022). As discussed in that paper, many citrus viruses and viroids are apparently disseminated around Japan, and it is reasonable to pay attention to the occurrence and research trends of these pathogens not only in Japan but also around Japan (Iwanami 2022). As a sequel, I herein present a comprehensive review of the occurrence and history of citrus viroids and minor citrus viruses in Japan as well as recent advances in related research in Japan and in China, Korea, and Taiwan. Further, some relevant insights on these citrus pathogens in the Philippines are also mentioned.

Citrus exocortis viroid

1. Occurrence

(1) Occurrence in Japan

Exocortis was first described in 1948 as the bark-scaling of trifoliate orange rootstock (Duran-Vila 2017). This disease was initially detected in some trees in a citrus variety collection at the National Citrus Research Institute in Okitsu, Shizuoka, Japan, by a visiting researcher (Dr. W. P. Bitters) in 1963 (Tanaka 1981a). Subsequent surveys conducted throughout Japan indicated that many citrus cultivars grown in Japan were affected by exocortis (Tanaka 1981a). However, the results should be interpreted carefully because the identification of exocortis was based only on the biological indexing involving Etrog citron. It is possible that some of the exocortis-like symptoms observed on Etrog citron trees in these surveys were induced by other viroids. A more precise RT-PCR-based survey indicated that CVd-II (HSVd variant) and CVd-III occur frequently, but CEVd is relatively rare in Japan (Ito et al. 2003).

(2) Occurrence outside of Japan

CEVd has been detected worldwide, including in all citrus-producing regions near Japan (i.e., in China,

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Korea, and Taiwan) (Dattaraji et al. 2017). However, in China, the incidence of CEVd infections decreased dramatically following the implementation of a virus-free scheme in 2001 (Zhou 2018). CEVd and CVd-II (HSVd) are commonly found simultaneously infecting different citrus cultivars in Taiwan (Lin et al. 2015).

2. Biological and molecular biological properties

CEVd induces bark-scaling symptoms in susceptible citrus cultivars (Duran-Vila 2017), including trifoliate orange, which is commonly used as a rootstock in and around Japan. The symptoms associated with a CEVd infection of Etrog citron Arizona 861-S1 reportedly include severe stunting and epinasty as well as vein and petiole necrosis (Duran-Vila et al. 1993). CEVd has a wide host range that includes tomato (*Solanum lycopersicum* L.); identification of CEVd by indexing on tomato was commonly practiced in 1970s in Japan (Tanaka 1981a). Among woody plants, CEVd can infect citrus and grapevine; however, CEVd infections of grapevine have been observed in China but not in Japan (Jiang et al. 2017).

3. Taxonomy and strains

(1) Taxonomy

The first studies on the CEVd nucleotide sequence indicated this viroid has an RNA genome comprising 371 nucleotides, with a predicted rod-like secondary structure (Duran-Vila 2017). According to the arbitrary criteria adopted by the International Committee on Taxonomy of Viruses (i.e., <90% nucleotide sequence identity, host range, and symptoms on host plants), CEVd was classified as a distinct viroid species in the genus *Pospiviroid* of the family *Pospiviroidae*.

(2) Strains

Isolates of CEVd that induce severe and mild symptoms during infections of tomato plants have been divided into class A and class B, respectively (Duran-Vila 2017). The nucleotide sequences of the class A isolates differ by at least 26 nucleotides from the nucleotide sequences of the class B isolates (Visvader & Symons 1985). Such differentiation of strains has not been examined in the Japanese CEVd isolates. Therefore, further research might reveal the diversity among the Japanese isolates.

4. Detection

(1) Biological assays

Biological indexing using Etrog citron Arizona 861-S1 (citron test) was common in Japan until the 1980s (Tanaka 1981a). Since then, the transmission to gynura (*Gynura aurantiaca* DC.) and tomato was used to accelerate the indexing of CEVd (Kano & Yamaguchi 1985).

(2) PCR-related detection

Biological indexing was rapidly replaced by various molecular biology-related methods in the 1990s, including RT-PCR assays, which have been commonly used to detect CEVd (Ito et al. 2002a). These molecular methods have been modified and refined. For example, a simpler one-step multiplex RT-PCR assay was developed in China (Wang et al. 2009).

5. Control

Preventing infections is the only viable option for controlling exocortis. The establishment of CEVd-free mother trees is the first step. CEVd-free plants were previously obtained via shoot-tip grafting *in vitro* (Navarro et al. 1975) or by semi-micrografting (Takahara et al. 1986). Transmission through sap during the pruning of plant materials in fields can be prevented by disinfecting tools in a solution consisting of 2% sodium hydroxide and 2% formaldehyde (Garnsey 1967). Subsequent research revealed the efficacy of sodium hypochlorite (household bleach) for disinfecting knives to eliminate potato spindle tuber viroid (Singh et al. 1989). The use of household bleach is apparently considerably more practical than the use of toxic chemicals (e.g., sodium hydroxide and formaldehyde). Furthermore, sodium hypochlorite is useful for eliminating HSVd-plum (Shimomura & Kusano 1997). Thus, household bleach containing sodium hypochlorite is routinely used as a disinfectant in many citrus laboratories in Japan (the author's observation). However, the effect of sodium hypochlorite on CEVd has not been demonstrated in Japan. Consequently, its efficacy for controlling CEVd should be thoroughly investigated.

Citrus viroids

1. Taxonomy and strains

(1) Recognition of citrus viroids

In the late 1980s, viroids that were distinct from CEVd were detected in citrus tissues by sequential polyacrylamide gel electrophoresis, and they were classified into the following four groups: citrus viroids I, II, III, and IV (CV-I, CV-II, CV-III, and CV-IV) (Duran-Vila et al. 1988); however, these groups have been more commonly abbreviated as CVd-I, CVd-II, CVd-III, and CVd-IV. The more common abbreviations are used in the following paragraphs. Subsequent molecular characterizations revealed the existence of new viroids that were designated as citrus viroid V (CVd-V) (Serra et al. 2008), citrus viroid OS (CVd-OS) (Ito et al. 2001),

and citrus viroid VII (Chambers et al. 2018).

(2) Taxonomy and variants

CVd-I includes three distinct variants, namely, CVd-Ia, CVd-Ib (Duran-Vila et al. 1988), and CVd-I-LSS (Ito et al. 2000). CVd-Ib was later designated as citrus bent leaf viroid (CBLVd) (Ashulin et al. 1991). Previous studies in Japan confirmed that CVd-Ia and CVd-I-LSS are related to CBLVd (Hataya et al. 1998, Ito et al. 2000). Currently, it is considered that CVd-Ia and CVd-I-LSS are also CBLVd variants (Zhou et al. 2020). CBLVd has been classified in the genus *Apscaviroid* of the family *Pospiviroidae* (Di Serio et al. 2017, Table 1).

CVd-II comprises hop stunt viroid (HSVd)-related variants, including CVd-IIa, CVd-IIb, and CVd-IIc (Reanwarakorn & Semancik 1999). CVd-IIb induces cachexia disease, whereas CVd-IIa does not. Additionally, CVd-IIc has commonly been found in xyloporosis-infected materials from Israel (Reanwarakorn & Semancik 1999). HSVd belongs to the genus *Hostuviroid* of the family *Pospiviroidae* (Di Serio et al. 2017, Table 1).

CVd-III includes CVd-IIIa and CVd-IIIb. Infections by CVd-IIIb can decrease the size of citrus trees grafted on trifoliate orange without affecting fruit quality (Semancik et al. 1997). For this reason, CVd-III was redesignated as citrus dwarfing viroid (Vernière et al. 2004), which belongs to the genus *Apscaviroid* of the family *Pospiviroidae* (Di Serio et al. 2017, Table 1).

CVd-IV was renamed as citrus bark-cracking viroid in 2007 (Kunta et al. 2007) because it consistently induces bark-cracking symptoms in trifoliate orange rootstocks (Vernière et al. 2006). This viroid species has been classified in the genus *Cocadviroid* of the family *Pospiviroidae* (Di Serio et al. 2017, Table 1).

CVd-V was first detected in *Atalantia citroides* plants that were inoculated with several CVds (Serra et al. 2008). This new viroid has a GC-rich genome consisting of 293 or 294 nucleotides (Serra et al. 2008). CVd-V was likely overlooked until it was discovered because its genome is similar to the CVd-II and CVd-III genomes in terms of size. CVd-V was found also in Japan (Ito & Ohta 2010). CVd-V belongs to the genus *Apscaviroid* of the family *Pospiviroidae* (Di Serio et al. 2017, Table 1).

A viroid that was originally reported in Japan as CVd-OS (Ito et al. 2001) was later redesignated as CVd-VI (Ito et al. 2013). Moreover, CVd-VI variants with 92.1%-94.3% sequence identity were found in Japanese persimmon (*Diospyros kaki* Thunb.) in Japan, indicating the possible wide host range of CVd-VI (Nakaune & Nakano 2008). This viroid has been classified in the genus *Apscaviroid* of the family *Pospiviroidae* (Di Serio

Table 1. Taxonomy of citrus exocortis viroids and citrus viroids[a]

Family	Genus	Species	Common name (Abbreviation)	Variant[b]	References
Pospiviroidae	*Pospiviroid*	*Citrus exocortis viroid*	Citrus exocortis viroid (CEVd)	CEVd Class A	Visvader & Symons (1985)
				CEVd Class B	Visvader & Symons (1985)
	Apscaviroid	*Citrus bent leaf viroid*	Citrus bent leaf viroid (CBLVd)	CVd-Ia	Duran-Vila et al. (1988)
				CVd-Ib	Duran-Vila et al. (1988)
				CVd-I-LSS	Ito et al. (2000)
		Citrus dwarfing viroid	Citrus dwarfing viroid (CDVd)	CVd-IIIa	Semancik et al. (1997)
				CVd-IIIb	Semancik et al. (1997)
		Citrus viroid V	Citrus viroid V (CVd-V)	CVd-V	Serra et al. (2008)
		Citrus viroid VI	Citrus viroid VI (CVd-VI) (synonym: CVd-OS)	CVd-VI	Ito et al. (2013)
		Citrus viroid VII[c]	Citrus viroid VII (CVd-VII)	CVd-VII	Chambers et al. (2018)
	Hostuviroid	*Hop stunt viroid*	Hop stunt viroid (HSVd)	CVd-IIa	Reanwarakorn & Semancik (1999)
				CVd-IIb	Reanwarakorn & Semancik (1999)
				CVd-IIc	Reanwarakorn & Semancik (1999)
	Cocadviroid	*Citrus bark cracking viroid*	Citrus bark cracking viroid (CBCVd) (synonym: CVd-IV)	CBCVd	Kunta et al. (2007)

[a]Only viroids referred in the text are listed.
[b]Shown in abbreviation
[c]Assigned tentatively (Zhou et al. 2020)

et al. 2017, Table 1).

A novel citrus viroid that was recently discovered in an asymptomatic Lisbon lemon (*Citrus* × *limon* L. Burm. f.) tree in New South Wales, Australia, was designated as CVd-VII (Chambers et al. 2018). There are no other reports of the detection of CVd-VII in any other countries. So far, CVd-VII represents a tentative species *Citrus viroid VII* in the genus *Apscaviroid* of the family *Pospiviroidae* (Zhou et al. 2020, Table 1).

2. Occurrence

A survey of 217 citrus trees in Japan showed that CVd-II (HSVd variant) was the most frequently detected viroid, followed by CVd-III. In addition, CVd-I-LSS (CBLVd variant), which is a variant of CVd-I, and CVd-VI (CVd-OS) were also frequently detected in some species or varieties, whereas CVd-Ib and CVd-IV were detected only occasionally (Ito et al. 2003). The results of an RT-PCR assay indicated CVd-V was present in 44 of the 275 tested citrus trees, suggestive of the spread of the viroid to some extent within commercial orchards in Japan (Ito & Ohta 2010). CVd-VII has not been detected in Japan.

One-step multiplex RT-PCR assays of 40 field samples of sweet oranges, mandarins, mandarin hybrids, and lemons in China revealed that CVd-II (HSVd variant) and CVd-III were the most frequently detected viroids (95% and 82.5%, respectively), which is consistent with the related findings in Japan. Additionally, CVd-Ib (CBLVd) was detected only occasionally (30%) and CVd-IV was undetected (Wang et al. 2009). In a subsequent study, CVd-IV and CVd-V were also detected at the same frequency in 3 of 42 samples in another field survey in China according to the same one-step multiplex RT-PCR assay (Cao et al. 2010). Remarkably, all but one sample that tested positive for CVd-IV or CVd-V originated from scions imported from Japan (Cao et al. 2010). Furthermore, seven CVd-I-LSS (CBLVd variant) isolates were detected in different citrus hosts in Pakistan and China (Wu et al. 2014). An analysis of the diversity in 49 cDNA sequences in CVd-I-LSS (CBLVd variant) isolates showed that the Pakistan population was more diverse than the populations from Japan or China. Phylogenetic analyses resulted in the clustering of the predominant examined sequences into three main clades. Only the sequences from the Pakistan isolates were present in all three clades, implying that Pakistan may be the original source of CVd-I-LSS (CBLVd variant). Cultivar import records and the close phylogenetic relationship between CVd-I-LSS (CBLVd variant) from China and Japan suggested that the viroid isolated in China originated in Japan. The CVd-VI nucleotide sequences in nine citrus cultivars from four citrus-growing regions in China were 94.2%-97% similar to the nucleotide sequence of CVd-VI (CVd-OS) in Japan (Cao et al. 2017).

Literature describing the occurrence of citrus viroids in Korea is relatively inaccessible, but one published report described the detection of CV-Ib (CBLVd) and CV-III in Korea (Lee 2017).

3. Symptoms induced by citrus viroids

The determination of the symptoms induced by individual citrus viroids in Japan was hindered by the fact most of the examined citrus trees were infected with multiple citrus viroids (Ito et al. 2002a). However, it became clear that multiple infections of citrus viroids induced symptoms that were as severe as those caused by CEVd in Arizona 861-S1 Etrog citron trees (Ito et al. 2002b). Furthermore, in previous investigations, some field-grown citrus trees with severe bark-scaling symptoms, which are characteristic of the exocortis disease in trifoliate orange rootstocks, contained only citrus viroids, indicating that certain exocortis-like diseases in Japan are likely caused by some combination of citrus viroids (Ito et al. 2002b, Muramoto 2001). Similarly, field experiments in Corsica, France demonstrated that bark-cracking symptoms on trifoliate orange rootstocks are associated with CVd-IV and HSVd (Vernière et al. 2004). Another study showed that certain combinations of citrus viroids can induce exocortis-like scaling symptoms on trifoliate orange rootstock in the absence of CEVd (Vernière et al. 2006).

4. Detection

(1) PCR-related detection

A multiplex RT-PCR analysis detected CVd-Ib (CBLVd variant), CVd-II (HSVd variant), CVd-III, CVd-IV, and CVd-VI (CVd-OS) in citrus plants grown in Japan (Ito et al. 2002a). The multiplex RT-PCR assay was also designed to distinguish between CVd-I-LSS (CBLVd variant) and CVd-Ib (CBLVd variant) (Ito et al. 2002a). In another study, RT-PCR was also used for detecting CVd-V from 44 of 275 field citrus trees in Japan (Ito & Ohta 2010). The RT-PCR-based detection of CVd-VII has not been performed in Japan. In a recent study, CVd-VII was detected by an RT-PCR assay using primers VIIF1 and VIIR3, which are homologous and complementary to nucleotide positions 233-252 and 218-235, respectively, of the type variant LD4 in New South Wales, Australia (Chambers et al. 2018). A more sensitive quantitative RT-PCR diagnostic assay was recently developed and validated to detect CVd-VII in citrus plants (Chambers et al. 2022).

In China, a one-step multiplex RT-PCR assay was designed to simultaneously detect CVd-Ib (CBLVd variant), CVd-II (HSVd variant), CVd-III, and CVd-IV in addition to CEVd (Wang et al. 2009). The method, although it does not detect CVd-VI (CVd-OS), is simpler than the two-step multiplex RT-PCR assay reported by Ito et al. (2002a).

5. Control

Similar to the control of CEVd, preventing infections is the only way to control citrus viroids. Citrus viroid-free mother trees have been generated from the original trees infected with CVd-II (HSVd) and CVd-VI by shoot-tip grafting *in vitro* (Nakajima et al. 2017). Other citrus viroids, including CVd-I-LSS (CBLVd variant), CVd-IIb (HSVd variant), and CVd-III, have successfully been eliminated via shoot-tip grafting *in vitro* (Ohta 2016). The elimination of CV-Ib (CBLVd variant), CVd-IV, CVd-V, and CVd-VII has never been attempted in Japan presumably because they generally do not infect important mother trees.

Obviously, it is also important to prevent the transmission of citrus viroids through sap during the pruning of field-grown trees. The mechanical transmission of HSVd-plum was prevented by immersing tools in a 0.5% sodium hypochlorite solution (Shimomura & Kusano 1997). However, there has been no scientific report in Japan regarding the effective sanitation of citricultural tools to remove citrus viroids.

Citrus vein enation virus

1. Occurrence

(1) Occurrence in Japan

Vein enation in citrus species caused by CVEV was first observed by Wallace and Drake (1953). They subsequently reported that CVEV induces the development of woody galls on rough lemon following a graft-inoculation (Wallace & Drake 1960). Graft-transmission tests on sour orange in the late 1950s indicated that some satsuma mandarin lines as well as Eureka lemon and Washington navel orange grown in Japan might be infected with CVEV (Tanaka & Yamada 1961). Among the citrus species grown in Japan, yuzu plants have the most conspicuous disease symptoms (Iwanami et al. 1992).

(2) Occurrence outside of Japan

Characteristic small vein enations induced by CVEV were previously observed on 11 citrus varieties and hybrids in orchards in Huangyan, Zhejiang province, China (Chen et al. 1996). Additional field surveys for CVEV are lacking, likely because this virus does not severely affect citrus production (Huang et al. 2015). However, in a recent RT-PCR-based study, 52 of 55 symptomatic samples and 6 of 10 asymptomatic samples collected in Sichuan province tested positive for CVEV (Wu et al. 2019). Moreover, a survey conducted at 203 groves in the southern part of the Korean peninsula and on Jeju Island detected CVEV in 136 groves (67%), in which 85.4% and 77.8% of the yuzu and satsuma mandarin trees were infected, respectively (Kim et al. 2019). In the Philippines, graft-transmission experiments elucidated the occurrence of CVEV infections resulting in vein enation and woody galls on rough lemon (Ochasan et al. 1996).

2. Biological and molecular biological properties

In the USA, CVEV has been transmitted via the brown citrus aphid and the green peach aphid [*Myzus persicae* (Sulz.)]. In Japan, CVEV is readily transmitted by the brown citrus aphid (Iwanami et al. 1992), but it is unknown whether other aphids can serve as vectors. Woody gall-like symptoms were observed in a rough lemon tree in the experimental field of the National Fruit Tree Research Station at Okitsu, Shizuoka, Japan, but their association with CVEV was unclear (Tanaka 1981b). These findings were in contrast to what was demonstrated in the Philippines, where vein enation was rare and woody galls were relatively common in fields and graft-transmission tests revealed the simultaneous development of vein enation and woody galls (Ochasan et al. 1996). One unique feature of CVEV is that it may provide cross protection against citrus tristeza virus infections (Koizumi & Sasaki 1980).

Deep sequencing of the genetic material in Etrog citron tissue infected with Spanish isolate VE-1 revealed that the CVEV genome consists of a single-stranded (+)-sense RNA sequence, which includes 5,983 nucleotides and five open reading frames (Vives et al. 2013). Phylogenetic analyses conducted on the basis of RNA polymerase and coat protein amino acid signatures strongly suggested that CVEV belongs to the genus *Enamovirus* (Vives et al. 2013). The complete genomic sequence of a Chinese isolate (CVEV-XZG) was subsequently determined using amplicons obtained by PCR amplifications using the primer sets reported by Vives et al. (2013). The XZG genome sequence was similar to that of VE-1 (Huang et al. 2015). Moreover, the genomic sequences of five Japanese CVEV isolates were determined by deep sequencing (Nakazono-Nagaoka et al. 2017). The genomes of the Japanese isolates were similar to that of the Spanish isolate VE-1 (98.0%-99.8% sequence similarity).

3. Taxonomy and strains

(1) Taxonomy

CVEV belongs to the genus *Enamovirus* in the family *Solemoviridae* (ICTV 2021c).

(2) Strains

As described above, CVEV infections of rough lemon in the USA and the Philippines induced the vein enation in leaves and the development of galls on trunks, but wood galls have never been observed in response to an infection by Japanese CVEV isolates. It is possible that some CVEV isolates readily induce the production of woody galls, whereas others do not, but this will need to be experimentally verified.

Despite the overall similarities in the genomes of Spanish, Chinese, and Japanese CVEV isolates (Huang et al. 2015, Nakazono-Nagaoka et al. 2017), there are four amino acid changes that are present in only 10 Japanese isolates (Nakazono-Nagaoka et al. 2017), suggestive of a local differentiation.

4. Detection

(1) ELISA and immunochromatography test

The development of serological methods for detecting CVEV has been delayed because of the challenges associated with preparing specific antibodies. Although a DAS-ELISA for CVEV has never been used in Japan, a DAS-ELISA using antibodies targeting cereal yellow dwarf virus RPV and RMV successfully detected CVEV-infected tissues in South Africa (Clark & da Graça 2000). There are no reports describing the utility of an ELISA or other serological assays using homologous antibodies against CVEV.

(2) PCR-related detection

The highly conserved genomic RNA sequences of CVEV field isolates (Nagaoga-Nakazono et al. 2017) enabled the development of PCR-related methods for detecting CVEV. Indeed, genomic RNA fragments from 10 field isolates were readily amplified by RT-PCR (Nagaoga-Nakazono et al. 2017). A quantitative RT-PCR assay developed in China (Wang et al. 2016) was 100 times more sensitive than the conventional RT-PCR method. Interestingly, the calculated virus titer was highest in the roots, followed by the bark and leaves (Wang et al. 2016).

5. Control

CVEV induces only a few leaf vein enations and minimally affects the fruit production of most citrus cultivars. For this reason, aggressive CVEV control measures are unnecessary in Japan. Woody galls induced by CVEV might be a serious problem if rough lemon is used as a rootstock, which is uncommon in Japan and the surrounding countries. Thus, CVEV-infected plants do not need to be quarantined in Japan. Similarly, the Korean quarantine agency recently decided to exclude CVEV-infected materials from quarantine because CVEV is already widely distributed in the main citrus-producing areas in the country (Kim et al. 2019).

Citrus leaf blotch virus

Citrus leaf blotch virus (CLBV) was first detected in "Nagami" kumquat (*Fortunella margarita* Swingle) clone SRA-153 from Corsica, France (Vives et al. 2002). CLBV has filamentous particles (~900 × 14 nm), a single-stranded (+)-sense genomic RNA sequence comprising 8,747 nucleotides, and a coat protein (~41 kDa) (Vives et al. 2002). The genomic RNA sequence contains three open reading frames as well as 5′ and 3′ untranslated regions (73 and 541 nucleotides, respectively) (Vives et al. 2002). On the basis of its biological and molecular properties, CLBV was classified in a new virus genus (Vives et al. 2002). It was subsequently identified as the sole species in the genus *Citrivirus* in the subfamily *Trivirinae* in the family *Betaflexiviridae* (ICTV 2021c). CLBV was detected in four satsuma mandarin cultivars from Japan (Vives et al. 2002). These results suggest that CLBV can spread through citrus plants grown in Japan. However, there has been no comprehensive survey of CLBV infections in Japan and the occurrence of CLBV in citrus species remains uninvestigated. To date, CLBV has been detected only in a noncitrus ornamental plant species, *Nandina domestica* (Kimitani et al. 2021), and in an herb, *Rehmannia glutinosa* (Uehara-Ichiki et al. 2018), in Japan.

In China, CLBV has been found not only in lemon (Li et al. 2018) but also in sweet cherry (Wang et al. 2016) and kiwifruit (Liu et al. 2019). Surprisingly, the average CLBV incidence rate was as high as 28.53% in kiwifruit grown in Shaanxi province (Liu et al. 2019). The detection of CLBV in sweet cherry and kiwifruit in China, in *N. domestica* in Japan, and in peony (*Paeonia lactiflora*) in the USA (Gress et al. 2017) implies that CLBV can infect a wide range of plant families, and citrus is only one of its hosts.

In 1968, a pathogen from a Cleopatra mandarin (CRC 270) plant grown in Florida, USA, induced chlorotic blotching in Dweet tangor [a hybrid between a tangerine (*Citrus reticulata* Blanco) and an orange] after grafting (Roistacher & Blue 1968). The suspected causal agent was named Dweet mottle virus (Roistacher & Blue 1968), but it was later revealed that dweet mottle was caused by CLBV (Vives et al. 2005).

Earlier electron microscopy-based examinations of satsuma mandarin trees with leaves exhibiting yellow mottling in Kanagawa Prefecture in Japan detected the presence of rod-shaped virus-like particles (Garnsey 2000a, Ushiyama et al. 1980). The virus was named citrus yellow mottling virus and its virions were mostly 690-740 nm long (Ushiyama et al. 1980). However, the author of this review once observed many filamentous particles ~900 × 14 nm in size after the mechanical transmission to *Nicotiana benthamiana* from replicates of the satsuma mandarin trees analyzed by Ushiyama et al. (the author's unpublished data). This finding indicates that the citrus yellow mottling virus named by Ushiyama et al. might be identical to CLBV. Unfortunately, the replicates were lost before they could be further tested, and no additional diseased trees were found in the fields in Kanagawa Prefecture, presumably because the virus is not a major pathogen in this region. Whether citrus yellow mottling virus is identical to CLBV remains unknown.

Other viruses

Some citrus viruses that have been detected outside of Japan are not present or are very rare in Japan. Accordingly, they are minimal threats to the Japanese agricultural industry and economy. However, because some of these viruses can cause serious diseases, they must be prevented from invading Japan.

Citrus psorosis virus (CPsV) occurs worldwide and causes annual losses of ~5% of field trees as well as the progressive decline of trees through its effects on conductive tissues (Achachi et al. 2014). CPsV causes a serious disease that has primarily affected orange and mandarin trees in Argentina and Uruguay (Francesco et al. 2015). There is no report on the occurrence of CPsV in commercial fields in Japan, but it was previously detected in two "Shiranui" trees, [*C. unshiu* (Swingle) Marcow. × *C. sinensis* (L.) Osbeck] × *C. reticulata* Blanco, in the experimental field of the National Agricultural Research Organization. Neither tree exhibited the bark-scaling symptom typical of CPsV infections (Ito et al. 2011). In countries surrounding Japan, CPsV has been detected in China (Zhou 2018).

Citrus variegation virus (CVV), citrus crinkly leaf virus (CCLV), and citrus leaf rugose virus (CLRV) are related viruses belonging to the genus *Ilarvirus* in the family *Bromoviridae*. These citrus viruses infect most citrus cultivars, but generally result in few or mild symptoms (Garnsey 2000b). Both CVV and CLRV have been identified in the USA, whereas CVV has been reported in the Mediterranean region and in Latin America (Moreita et al. 2011). The occurrence of CLRV in Japan was confirmed on the basis of an electron microscopy analysis and biological indexing of Mexican lime (Namba et al. 1980), but there have been no further epidemiological and molecular characterizations, likely because of the very limited economic impact of the virus. There is currently no report on the occurrence of CVV, CCLV, or CLRV in countries and regions near Japan (e.g., China, Korea, and Taiwan).

Other major citrus viruses include the following: citrus leprosis viruses, which occur mainly in Florida, USA, and some parts of Central and South America (Roy et al. 2015); citrus yellow mosaic virus, which occurs in India (Vadlamudi et al. 2021); Indian citrus ringspot virus, which seriously affects "Kinnow" mandarin production in India (Kokane et al. 2021); citrus yellow vein clearing virus, which was first detected in Pakistan, but it is now present in India, Turkey, and China (Loconsole et al. 2012a); citrus chlorotic dwarf-associated virus, which was initially identified in Turkey (Loconsole et al. 2012b) and then in other countries, including China and Thailand (Zhou et al. 2017, 2020); citrus concave gum-associated virus and citrus virus A, both of which mainly occur in the Mediterranean region (Minutolo et al. 2020); and citrus sudden death-associated virus, which was first reported in Brazil (Maccheroni et al. 2020). There are no confirmed reports of the presence of these viruses in the citrus-growing regions in Japan. Concave gum-like disease was detected once in Japan according to the biological indexing of Dweet tangor (Ieki et al. 1993), but this will need to be verified using more specific identification methods (e.g., sequencing the genome of the causal agent).

Conclusion

CEVd and all known citrus viroids but CVd-VII have been reported in Japan. CVd-I-LSS (CBLVd variant), CVd-II (HSVd variant), CVd-III, CVd-V, and CVd-VI (CVd-OS) were apparently common, whereas CEVd, CVd-Ib, and CVd-IV were less common in Japan. Incidence of these viroids is similar in China, and probably in Korea. Damage induced by CEVd and some combinations of citrus viroids has been shown, and it is strongly recommended that budwoods that are free from these pathogens should be prepared in introducing new cultivars intra-and internationally. Techniques to prepare and check viroids-free materials that has been developed for the past 30 years should be fully utilized to implement such measures.

CVEV occurs only occasionally, and the incidence of CLBV and CLRV is extremely rare. Further, foliar

enation induced by CVEV is economically negligible. For these reasons, it is considered that no quarantine measures are necessary for these viruses. Woody gall induced on rough lemon by CVEV can be a serious problem if rough lemon or similarly susceptible cultivars are cultivated in Japan in the future.

Some major citrus viruses that seriously affects citrus production outside of Japan have not yet been reported in Japan. However, similar symptoms have been observed occasionally, and care should be incessantly taken. Continuous efficient monitoring taking advantage of sensitive and rapid molecular detection techniques are required to promote citrus production in and around Japan.

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