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A brief review of plant diseases caused by *Cactus virus X*

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ABSTRACT

Plant pathogens, including viruses, have a known negative impact on plant growth and development. Here, plant diseases caused by cactus virus X (CVX) are reviewed. CVX infects a range of plant species within the *Cactaceae* family. When expressed, symptoms include chlorosis, necrosis, and morphological alterations, such as the twisting of arms, but at times plants can be asymptomatic. CVX may be transmitted through contaminated grafting or cutting tools and can also occur as mixed infections with other viruses, including zygocactus virus X and pitaya virus X. CVX may also play a role in altering the plant's physiology. The virus can be detected by molecular assays, including polymerase chain reaction (PCR), and transmitted to indicator plants. Sanitation in both nurseries and fields is critical in controlling the spread of CVX. This paper highlights CVX as a potential production threat to some *Cactaceae* family members, particularly the now popular *Hylocereus* species or dragon fruits.

1. Introduction

Since discovering the first plant virus in 1892, numerous studies have followed in establishing plant virology. Along with many other members of higher plants, comprehensive studies in Cactaceae have been reported. Early researchers were curious about this plant family due to their strange shape when cultivated in Europe. Due to the peak of interest in studying cacti, reports of virus infection infecting these plant species have also flourished.

Cactus virus X (CVX) has been extensively studied since it was first described by Amelunxen (1958), who recognized rod-shaped particles in *Opuntia monacantha* in Germany. CVX is currently classified in the genus *Potexvirus*, family *Alphaflexiviridae*, and has a positive-sense single-stranded RNA ((+) ssRNA) unipartite genome and total genome size of 6.5 kb. The virions of CVX are rod-shaped, filaments are 520 nm long and 13 nm wide (Liao et al., 2003; Kim et al., 2016).

This paper provides a review of the current understanding of plant diseases caused by CVX, including host range and transmission, symptomatology, co-infection with other viruses, infected plants' physiology, and insights into plant disease management.

2. Host range and symptoms

Cactus virus X has been detected in many cacti plants, including *Austrocylindropuntia*, *Cereus*, *Echinocereus*, *Echinopsis*, *Epiphyllum*, *Eriocereus, Ferrocactus*, *Hylocereus*, Lobivia, *Mammillaria*, *Notacactus*, *Opuntia*, *Pereskia*, *Platyopuntia*, *Saguaro*, *Schlumbergera*, and *Zygocactus* (Park et al., 2018; Weber, 1953; Koenig and Lesemann, 1983; Liou et al., 2001; Kim et al., 2016; Aragao et al., 1993). The typical external symptom is systemic mottling. However, this symptom is unreliable for diagnosis since infection by other potexviruses can also induce such symptoms.

Symptoms caused by CVX infection can vary, and some plants may appear asymptomatic. Some plants show severe symptoms (Fig. 1) aside from systemic mottling. Mottling is usually accompanied by morphological alterations observed during plant growth and development. A CVX strain in California identified by Attathom et al. (1978) induced distorted aereoles, deformed spines, and necrosis in *Ferrocactus acanthodes*. Moreover, CVX has also been reported by Chen and Tzeng (1996) to cause chlorosis and reduced growth of *Oputina ficus-indica* in Taiwan. Izaguirre-Mayoral and Marys (1995) observed superficial sunken necrotic lesions in cacti *Nopaea cochenillifera* and *Acanthocereus tetragonus* infected with CVX in Venezuela. Mottling, necrosis, and chlorosis also occur in *Hylocereus* spp. infected with CVX. Masanto et al. (2017) assessed the presence of necrotic spots manifesting in *H. undatus* and *H. polyrhizus* orchards in Malaysia as a secondary infection. Irregular chlorotic halos and spots with red-brown margins were observed in *H. polyrhizus* in China by Peng et al. (2016) and *H. undatus* in Taiwan, Korea, and Florida (Liou et al., 2001; Kim et al., 2016; Gazis and Boudel 2018). Twisting of arms was also reported by Matthews (n.d.) in

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Hylocereus spp. and in 2011, Kim et al. observed the same arm twisting of *H. trigonus,* which also had a rough surface and stunted growth. In contrast to these deformations, Maliarenko and Mudrak (2013) argues that viruses morphologically similar to CVX infecting cacti species *Mammillaria elongate, Echinopsis chamaecereus, Echinocereus pectinatus*, *Eriocereus jusbertii*, and *Echinopsis macrogona* play no role in plant fasciation in the *Cactaceae* family.

3. Co-infection of CVX with other cacti viruses

CVX can co-infect hosts with other viruses. Li et al. (2015) reported the mixed infection of CVX in *H. undatus* with other potexviruses, namely zygocactus virus X and pitaya virus X. Park et al. (2018) detected CVX with both potexviruses and tobomoviruses co-infecting *Notocactus leninghausii f. cristatus* in Korea – cactus mild mottle virus, pitaya virus X, rattail cactus necrosis-associated virus, Schlumbergera virus X, and zygocactus virus X. Both studies elaborated on the possibility of their stems or cladodes appearing asymptomatic and external symptoms limited only to systemic mottling with no other morphological deformities. This observation was supported by Milcic and Udjbinac (1961), who suggested that viruses can infect cultivated cacti without showing any externals symptoms.

4. CVX and host plant physiology

Reports have suggested that CVX may play a role in stress regulation involving the plant pigment betalain in Cactaceae (Chessin, 2000). The same red-brown spots were observed in *Epiphyllum truncatum* and *E. bridgesii* Weber (1953). Severe cases in which reddening of the whole stem was observed in CVX infected *Hylocereus* species (Matthews, n.d.). Weber and Kenda (1953) studied deformations in the stomatal structures of infected *Opuntia, Epiphyllum,* and *Rhipsalis*. Protein spindles, assumed to be virus bodies, in the epidermal cells contributed to the presence of numerous abnormal stunted twin stomata.

CVX infection can also affect the titrable acidity and crassulacean acid metabolism (CAM) of infected plants. *Nopaea cochenillifera* infected with CVX had reduced titrable acidity when exposed to high irradiance (Izaguirre-Mayoral and Marys, 1995). On the other hand, CAM activity was significantly reduced in infected *Acanthocereus tetragonus* regardless of the irradiance conditions.

5. Transmission of CVX

As there are no known insect or animal vectors aiding the spread of CVX infection, mechanical means like grafting (or plant to plant contact) are only the known transmission process. There is no evidence of seed transmission yet. However, there is a possibility in the pollen transmission of CVX, as suggested by the study conducted by Milbrath and Nelson (1972). The authors noted that the development of symptoms usually starts during the start of the flowering stage. They speculated that pollen from infected plants is infectious. Thus, the spread of the virus may be done during pollination. Pollen transmission has yet to be assessed and confirmed in other species of the cacti family.

6. Detection of CVX

Detection of CVX in both symptomatic and asymptomatic plants can be achieved by polymerase chain reaction (PCR) assays. Amplicons of 735 and 584 base pairs, respectively, for PCR primer Potex-1RC/Potex-5 and Potex-2RC/Potex-5 are amplified by PCR. These degenerate primers were developed based on the partial viral replicase-encoding region (ORF1) of potexvirus (Van der Vlugt et al., 2002). PCR primer pair

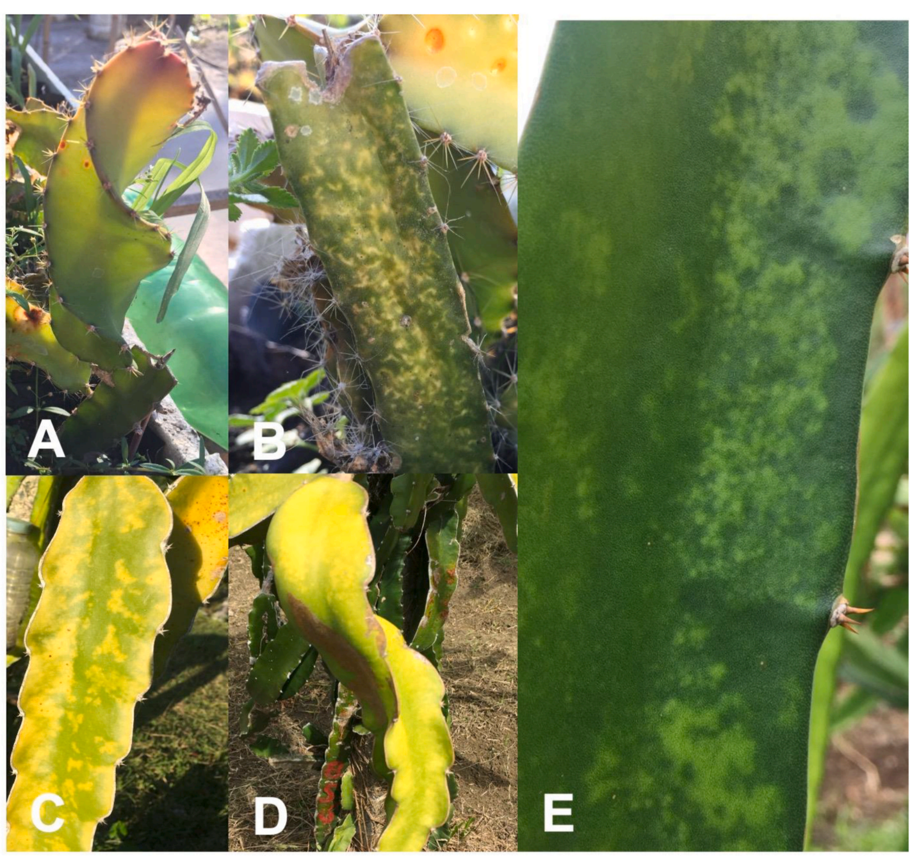

Fig. 1. Reported symptoms of CVX-infection in *Hylocereus* species. Infected plant show twisting with reddening (A), mottling with aereole and spine deformation (B), chlorosis (C), chlorosis with stem twisting and aereole/spine deformation (D), and mottling (E).

CVX-D-F/CVX-D-R (Zhang et al., 2016) is used to detect CVX with a resultant 650 bp PCR amplicons. The primer pair can be used to detect the virus by reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Zhang et al., 2016).

Indicator plants can also be used to detect the presence of CVX through sap inoculation. CVX causes systemic infections of chlorotic spots in *Celosia argentea* and local necrotic lesions in *Chenopodium amaranticolor*, *C. quinoa,* and *C. murale* (Liao et al., 2001). Symptoms are often observed 20 days post-inoculation.

7. Disease control options

Curative procedures are known for managing CVX infected plants. The recommended strategy in preventing CVX spread in cacti orchards is the proper sanitation of pruning materials used in grafting or disposal of the whole infected plant since sap contains high virion content. Lobo et al. (2014) recommend decontaminating tools with bleach and other known surface disinfectants. Nonetheless, early detection of the infected plant is key to the rapid implementation of field sanitation by removing infected plants.

Stem cuttings are usually used as the source of planting materials for dragon fruits. As there is no evidence for seed transmission of CVX, seed-derived cuttings could be used as a source of planting materials. However, establishment from seed would take longer than the cuttings, and good sanitation practices would be important in maintaining this virus-free status.

Further, Kim et al. (2011) managed to develop a CVX-resistant *Hylocereus trigonus* with CVX coat protein gene RNAi (CVXcp-RNAi). In their study, cut stems of transgenic and non-transgenic *H. trigonus* were dipped in the CVX stock solution. Their study shows the morphological differences between the two *H. trigonus*— the transgenics grew long and normal. However, the non-transgenic grew short segments and had a rough surface. CVX was detected in the non-transgenic plants but not in the transgenic plants based on electron microscopy. Moreover, they have detected the CVXcp-RNAi transgene in the initial and fourth, and fifth-generation transgenic *H. trigonus* through genomic PCR (Kim et al., 2012).

8. Outlook and prospects

Members of the cacti family have been popularized globally. They are part of the flourishing international trades in ornamental and crop plants. A good example is the now popular dragon fruits (*Hylocereus* species). However, along with this trade comes a significant threat offered by CVX in these plants. CVX is easily transmitted through mechanical means, and cacti propagation often involves grafting. CVX has been considered a growing production threat to the budding dragon fruit industry worldwide (Lobo et al., 2014; Balendres and Bengoa, 2019).

Moreover, the diagnosis of CVX presence is challenging as some hosts remain asymptomatic despite being infected in the field. Nevertheless, PCR assays, including RT-LAMP, are available, and indicator host plants can also be used to detect the virus. Aside from proper sanitation of pruning material, epidemiological studies (e.g., factors influencing disease spread and effect of fertilizer applications) and biotechnology applications (e.g., cell selection technique) will significantly contribute to establishing preventive and curative management strategies regarding virus infections in plants, as seen by the works on transgenic plants (Kim et al., 2011). However, consumers' acceptance of edible fruits developed through genetic engineering may be challenging, particularly in Europe, where genetically modified organisms remain restricted. Thus, other possible control measures for diseases caused by CVX are still needed, such as breeding for CVX-resistance through conventional or non-conventional (not based on genetic engineering) means (e.g., mutation breeding or somatic cell selection).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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