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A Review On *Citrus tristeza virus* (CTV) And Its Management Approaches

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ABSTRACT

Citrus tristeza virus (CTV), one of the casual agents of citrus decline, is responsible for the death of millions of citrus trees and reduced production and productivity of citrus orchards worldwide. CTV epidemics has been recorded from several parts of the world where mainly steam pitting (SP) and quick decline (QD) strains of CTV have induced severe disease reactions. Identification and characterization of CTV isolates primarily has been focused on the biological assaying in indicator plants, serology-based ELISA and molecular PCR tests. Controlling the presence and spread of CTV where it is absent or establishment is limited heavily relies upon preventive measures, quarantine and legislations. Cross protection is an appealing technique especially for controlling CTV – Stem Pitting strains and use of CTV resistant rootstocks largely prevent infection by CTV – Quick Decline strains. More reliable and effective way to control CTV is breeding for resistant or tolerant cultivars. Advances in molecular biology have lead scientists to find out genes and map genetic loci of CTV resistant citrus and related species that could be exploited in breeding. However incorporation of resistant ability offered by a handful of citrus and its related species into the susceptible cultivars containing other desirable agronomical traits is challenging through classical plant breeding approaches. The following review work is based on Citrus tristza virus and its management practices.

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Background

Citrus (*Citrus spp*; *Rutaceae*) is a popular fruit tree cultivated worldwide from households to commercial level. The genus *Citrus* is believed to be originate in Southeast Asia where *C. maxima* (*grandis*), *C. medica*, *C. reticulata*, and *C. balimii* are known to be its' parent species (Dugo and Giacomo, 2004). According to (FAO 2020), the total citrus production worldwide in 2019 was 143,755.6 thousand tonnes where China, Brazil and the United States of America were the top citrus producing countries in the world. Citrus fruits are known for containing rich sources of nutrients, such as glucose, sucrose, fructose, vitamin A, vitamin B, vitamin C, dietary fiber, carotenoids, flavonoids, limonoids and much more providing diverse health benefits (Liu et al., 2012; Khan et al., 2021). Furthermore, several researches has supported that citrus waste generated after the processing of fruit can be utilized in biofuel generation (Mahato, 2021; Aghizadeh-Alisaraei, 2017). Due to its wide use and popularity, citrus is the third most important fruit crop after apple and banana with more than 100 million tonnes of production and cultivation area spread over 7.2 million hectares (Savita et al., 2012).

Like any other cultivated crop, *Citrus* is also prone to attack by a wide range of pests and diseases hampering its production and productivity. Major citrus diseases worldwide are known to be caused by 30 viruses, 2 phytoplasmas, 1 spiroplasma, 3 viroid, 11 fungi, 2 nematodes and 3 bacterial agents and a few others with unknown etiology (Alhawat and Pant, 2003). The citrus decline is a holistic term used to denote various biotic and abiotic factors that lead to reduced production and productivity of citrus. Citrus tristeza virus (CTV) is one of the casual agents of citrus decline (Alhawat and Pant, 2003). CTV epidemics and its involvement in the decline of citrus orchards have been documented in several parts of the world, such as: Brazil, Argentina, California, Venezuela, Spain, Israel, Cuba, Mexico, the Dominican Republic, California, Florida and several other countries (Moreno et al., 2007; Atta et al., 2012) where the virus has caused the death of millions of trees. This review work intends to provide a general overview of the pathogen itself and information on some of the management approaches useful for preventing its spread to an epidemic scale.

Introduction to pathogen

Citrus tristeza virus (CTV): Genus: *Closterovirus*, Family: *Closteroviridae* (Lefkowitz et al., 2017) is one of the economically and epidemiologically important plant viruses in the world. It is also one of the most challenging viruses to handle due to its large RNA genome size, the fragile nature of virions having a shape of long flexuous thread like elements and a narrow host range limited to slow growing Citrus species in which the virus primarily infects phloem-associated cells (Folimonova, 2020). Morphologically CTV is found to be thread like particles having 2000 nm in length and 10-12 nm in diameter when studied under an electron microscope (Bar-Joseph et al., 2002). The complete sequence of CTV viral genome (CTV-T36 Florida isolate) has revealed that the genome is a single stranded positive sense RNA, 19,296 nucleotides in length. It contains 12 open reading frames (ORFs) that potentially code for 17 protein products and 2 untranslated regions (UTRs) that are 107 and 273 nucleotides long in their 5' and 3' terminals respectively (Karasev, 1995; Moreno et al., 2007).

Symptom Development

The natural hosts of CTV include *Citrus spp* and *Fortunella spp*, however, the virus can be transmitted experimentally through mechanical inoculation and aphid vectors to other citrus relatives and a few non-citrus species as well (Moreno et al., 2007). CTV is known to induce two economically significant diseases; stem pitting of grapefruit, pomelo, lime and some sweet orange varieties regardless of the rootstock used and quick decline of some *Citrus* scions, mainly sweet oranges grafted on sour orange *C. aurantium* rootstock (Bar-Joseph et al., 2002; Folimonova, 2013). The decline is the result of virus induced phloem necrosis in sour orange rootstock bark underneath the bud union with the scion. Due to such necrosis, carbohydrate movement to the root system from the canopy is disrupted, after a certain period plant stops growing new fibrous roots due to the limiting supply of energy reserve, which is followed by subsequent degeneration of existing fibrous root system (Garnsey et al., 1998). Infected plants may exhibit thin foliage dull green to yellow in color, leaf shedding, twig dieback, chlorotic leaves and pale colored fruits that have a low market value (Moreno et al., 2007). In the case of stem pitting, the virus interferes with normal differentiation of the cambial cells in different areas of the stem leading to the pit like structures in the trunk, branches, xylem cells and several other parts where the virus has been present. Plants infected by steam pitting strains also show symptoms like stunting, reduction of the radial growth, yellowish small leaves, reduced fruit size and quality (Garnsey et al., 1998; Moreno et al., 2007). Researchers have indicated that different loci in the viral genome are responsible for inducing decline and steam pitting syndromes (Garnsey et al., 1991). An additional symptom of seedling yellows is also observed in some young sour oranges, grapefruit and lemons, mainly in experimental condition (Bar-Joseph et al., 2002). Seedling yellows response has been characterized by a severe reduction of seedling size and severe chlorosis of the foliage in inoculated plants (Roistacher et al., 2010). Seedling yellows have been primarily considered to be associated with a more severe form of decline or steam pitting causing CTV strains, however, some stem pitting and decline isolates do not produce seedling yellows (Garnsey et al., 1998). Along with viral strains involved in infection, other parameters especially rise/fall in temperature and coinfection with other viruses or pathogens could alter symptoms development in the host plants (Garnsey et al., 1991).

CTV transmissibility

CTV is a phloem limited virus, transmitted by aphids (Hempitera*: Aphididae*) and mechanically by graft propagation of virus infected plant tissues (Albiach-Marti, 2013). Among different types of grafting, bark and leaf piece grafting are found to be the most effective for CTV transmission (Balaraman and Ramakrishnan., 1979). The aphid transmission happens in a semi-persistent manner and the most effective vector worldwide is *Toxoptera citricida* (Albiach-Marti, 2013; Marroquin *et al*., 2004; Balaraman and Ramakrishnan, 1979). However, in regions where *T. citricida* is absent, *Aphis gossypii* has been found as a main vector of CTV (Marroquín *et al*., 2004). Other aphid species, such as *Aphis spiraecola*, *Toxoptora aurantii*, *Myzus persicae*, *Aphis craccivora* and *Uroleucon jaceae* are also found to playing a role in CTV dispersion; however, they are experimentally less efficient for CTV transmission than *Toxoptera citricida* and *Aphis gossypii (*Marroquín et al., 2004). Apart from aphids, dodder (*Cuscuta spp*) has been found to carry and transmit CTV (Hosford, 1967). Transmission probably involves an association of dodder and host cells, and directional movement of nutrients. It has long been known that dodder's haustoria establishes cellular connections with the host plant that could result in transmission of virus as well (Hosford, 1967). CTV was not found to be transmitted by seeds in Acid limes (*Citrus aurantifolia*) in an experimental condition (Balaraman and Ramakrishnan., 1979). However, in a work done by Davino *et al*., (1991) for Indexing of seeds, CTV was detected in seeds of different citrus species in varying concentration based on enzyme-linked immunosorbent assay (ELISA) and serologically specific electron microscopy (SSEM). In that experiment, the virus wasn't detected in seeds of trifoliate orange but detected in a high concentration in Marsh grapefruit and Shamouti sweet orange sample seeds, however whether that would lead to the seed transmission is not clearly understood. In cases of many *Citrus* species and cultivars, seed transmission of CTV is still largely unknown (Garnsey et al., 1998).

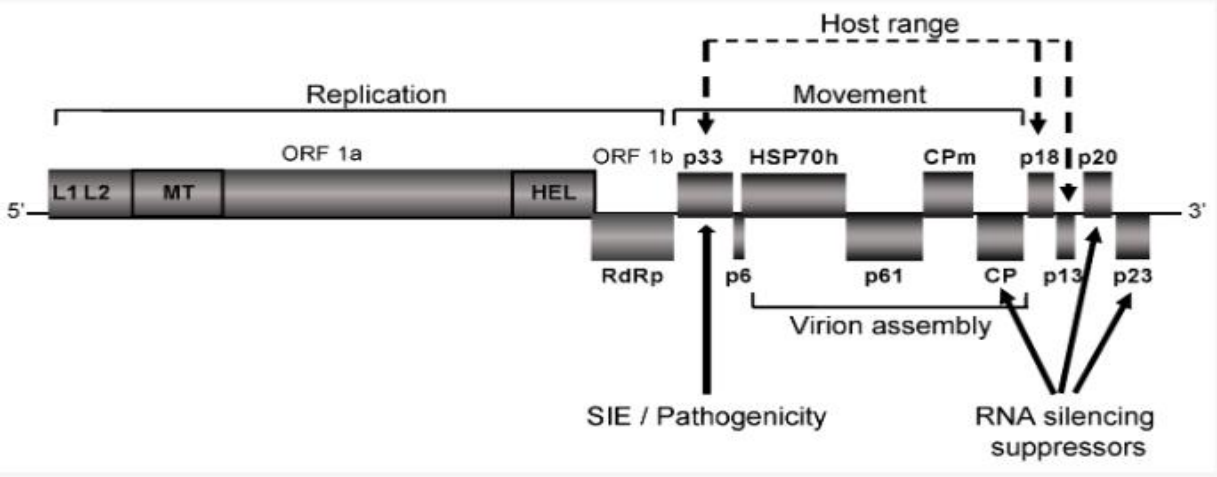

Figure 1. Genome organization of *Citrus tristeza virus* (Folimonova et al., 2020)
ORF- Open Reading Frame. L1, L2- papain-like Leader protease domains. MT- Methyltranferase-like domain. HEL- Helicase-like domain. RdRp- an RNA dependent RNA polymerase. HSP70h, HSP70 homolog. CPm- minor Coat protein. SIE- Superinfection exclusion.

Figure 2. A CTV infected citrus tree in field showing steam-pitting symptoms (Folimonova et al., 2020)
Pits on the scion of a citrus tree trunk induced by CTV (above) vs the corresponding piece of bark peeled from the CTV infected tree trunk.

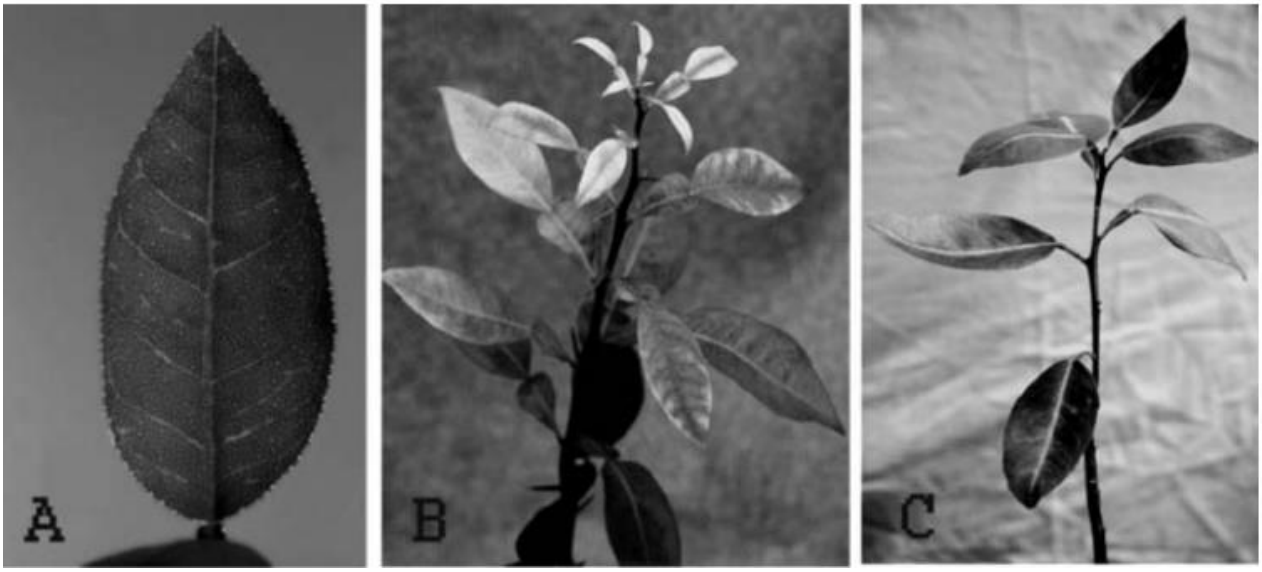

Figure 3. Variety of symptoms induced by CTV in different indicator plants (Garnsey et al., 2005)
(A) Mexican lime infected by CTV is showing vein clearing (B) Sour orange infected by CTV is showing seedling yellows(chlorosis and reduced leaf size) (C) Sweet orange grafted on sour orange infected by CTV is showing mid vein chlorosis symptoms (Garnsey et al., 2005)

Detection of CTV

Detection of CTV can be done through several different means, each method having its own features and specifications. Biological indexing is a way to detect and characterize CTV in which the virus inoculation in indicator plants is achieved via graft, mechanical or vector transmission and the assay results are evaluated based on general and distinctive visual symptoms on indicator plants; by the use of electron microscopy or light microscopy to look at the virus inoculation bodies and gum deposits within plant cells; and also by using various serological and molecular techniques (Roistacher, 1998). Mexican lime, sour orange, Duncan grapefruit, Madam Vinous sweet orange and a graft result of sweet orange on sour orange rootstock are commonly used as indicator plants for biological assaying (Garnsey, 1987). Quick diagnosis of CTV isolates, especially while testing suspected samples in bulk, can be performed by using serological ELISA tests. Several variations of ELISA have been used for this purpose (Rocha-Peña and Lee, 1991; Cambra et al., 1991). Both polyclonal and monoclonal antibodies for ELISA testing have been developed and used for detection however monoclonal antibodies found to be more useful for differentiating severe isolates of CTV (Cambra et al., 1991; Permar et al., 1990). Direct immunoprinting ELISA using specific monoclonal or recombinant antibodies does not require a sophisticated laboratory, or technical expertise and can be performed without the need for an excess amount of extract preparation (Cambra et al., 2000). Direct tissue blot immunoassay (DTBIA) is another way in which CTV infected tissues of a plant are stained and the location of the virus can be recognized under 10X microscope (Garnsey et al., 1993). Field-based diagnosis of CTV can be achieved by using a relatively modern fluorescence resonance energy transfer-based biosensor in which the equipment is conjugated with a specific antibody against the coat protein of CTV (Shojaei et al, 2016). Meanwhile, more advanced molecular characterization of CTV isolates can be carried out by using Real time RT-PCR and TaqMan RT-PCR following their corresponding protocols (Rosa et al., 2007). PCR primers designed based on the coat protein coding regions of the CTV genome templates are found to be useful for the amplification of genes of interest of CTV isolates (Huang et al., 2004). European diagnostic protocols (DIAGPRO) suggest graft inoculation of Mexican lime, sweet orange and grapefruit seedlings, DAS and DASI-ELISA, tissue print-ELISA, immunocapture RT-PCR and immunocapture nested RT-PCR in a single closed tube (conventional, print and squash capture formats) methods to be used for the detection of CTV in adult trees (Cambra et al., 2002).

Management of CTV

Control of CTV spread where the incidence is limited or absent heavily relies on the effectiveness of exclusion, quarantine and budwood certification programs. It is important to give thorough emphasis on the import of *Citrus* propagating materials to be free from CTV (Garnsey et al., 1998). In order to prevent propagation of CTV-infected trees, regular indexing of the primary budwood source trees and budwood increasing blocks under the protected facilities is essential (Garnsey et al., 1998). Usually, the indexing follows after having pathogen free budwood obtained by shoot-tip grafting *in-vitro* with or without thermotherapy (Roistacher, 1998). Similarly, controlling CTV insect vectors by following a comprehensive integrated pest management scheme is an important preventive measure. A Plethora of literature dealing with the management of aphid species by utilizing biological, physical and chemical techniques in a variety of crops from different parts of the world can be reviewed (Vásquez et al., 2006; Michaud, 2000; Michaud and Belliure, 2001; Zhang 1992; Zhang *et al*., 2016; Zhang et al., 2020; NajarRodríguez et al., 2007; Foster et al., 2012; Chen et al., 2020; Fiedler and Sosnowska, 2007).

Suppression of the virulent strain of CTV with the preinoculation of its mild strain in the form of cross protection technique has been practiced in the areas where CTV is endemic, mild strain has been identified and found as a technically and economically viable alternative (Garnsey et al., 1998). Cross protection, however, is not a permanent solution as it doesn't guarantee that the mild strain of CTV introduced to the plant before the virulent strain become established will forever be able to suppress the latter one. Furthermore, it is reported that superinfection exclusion occurs between the isolates of the same strain of the virus, but not between the isolates of different strains. For instance, *Citrus* plants initially infected with (CTV T-36 mild strain) in an experimental condition showed protection against fluorescent-tagged more severe strain (CTV T-36 virulent strain) but not against other heterogeneous strains. Similarly, in many cases, the mild strain of CTV was found to be effective to prevent infection from a more severe strain of the virus on only those cultivars in which mild strain was found in the first place, but not on other cultivars (Folimonova, 2013; Folimonova et al., 2010; Fulton, 1986). Cross protection is one of the primary ways to save *Citrus* trees from a more severe form of stem pitting strains of CTV (Roistacher et al., 2010). Folimonova (2013) provided a number of objectives to make cross protection successful against CTV infection; (I) Identification of the genotype of severe isolate that needs to be taken control of, (II) Finding the mild isolate of the same genotype, (III) If the mild isolate is not found, recombinant DNA technologies could be used to artificially manipulate the genome of the severe isolate, (IV) The introduction of mild isolate should exclude the later infection by severe one to make cross protection effective.

In small orchards where limited CTV infection has been observed and the rate of infection is found to be low, individual trees can be replaced as they decline and the profitability of the orchard can be maintained to a certain extent. There is a theoretical possibility to save trees grafted on sour orange by inarching CTV tolerant seedling rootstocks above the bud union of the scion. However, the practicability of these techniques has several technical and economical limitations (Garnsey et al., 1998). There is also a possibility of strain specific detection and eradication of CTV, which is to develop and utilize strain selective probes to distinguish between mild and severe strains and then remove trees carrying the severe form of the virus.

Similarly, precise molecular level detection of CTV isolates based on the molecular markers that differentiate the viral genome responsible for the expression of decline and steam pitting can be utilized in CTV eradication programs. However, the documented cases of molecular strain specific CTV detection have to be realized in a wider realm (Garnsey et al., 1998).

Breeding for CTV tolerant cultivars

The long term and more effective way to manage plant viral diseases is breeding for resistant varieties. Developing virus resistant varieties, in general, require searching for genotypic resistance in plant genetic resources often in the centers of origin and areas of diversification of cultivated plants where the selection pressure against pests and pathogens tends to be higher. Later these plants are screened for resistance, studied for the inheritance of resistance and some additional works would involve studying the specificity and mode of action of the resistant genes (Leppik, 1970; Khetarpal et al., 1998).

The *citrus* genus has very limited genetic resistance to CTV strains that can be utilized in breeding programs. Scions that are resistant to CTV are not many and for rootstocks, there are relatives of Citrus, such as, *Poncirus trifoliata*, *Severinia buxifolia*, and *Swinglea glutinosa* that provide resistance to most of the CTV strains if not all. *Swinglea glutinosa* is sexually incompatible and *Severinia buxifolia* is very difficult to hybridize with Citrus. Therefore, for the most part, *Poncirus trifoliata* has been extensively used as a resistant rootstock against diverse strains of CTV because it is sexually compatible and the resistant ability can be transferred to the citrus hybrid rootstocks via sexual crossing (Albiach-Marti et al., 2004; Garnsey et al., 1998). Swingle citrumelo (*C. paradisi* × *P. trifoliata*), Troyer and Carrizo citranges (*C. sinensis* × *P. trifoliata*) and hybrids resulting from crossing between *C. jambhiri, C. volkameriana, C. limonia* and *P. trifoliata* are found to be tolerant against different strains of CTV (Albiach-Marti et al., 2004; Garnsey *et al*., 1998). Trifoliate orange and its hybrid rootstocks are primarily used against the quick decline strains of CTV (Roistacher *et al*., 2010). *C. latifolia, C. grandis* and some mandarins are generally considered as somewhat tolerant to stem pitting CTV strains; however, their resistant mechanism is not universal and certainly not applicable to all of the steam pitting strains of CTV (Garnsey *et al*., 1998). Similarly, some of the *C. maxima* (Chandler pummelo) are also known to have resistant characteristics to CTV (Yoshida, 1985; Jeong et al., 2018). The segregation of CTV immunity occurring in the first-generation hybrids of trifoliate orange, and the usefulness of swingle citrumeloes as a CTV tolerant along with tolerant to phytophthora foot rot, citrus nematode and cold temperature have been reported by (Castle and Wutscher, 1988). However, some other CTV-tolerant rootstocks such as, rough lemon, *C. volkameriana*, rangpur lime, citranges and trifoliate oranges in many cases are found to be susceptible to citrus blight and phytophthora diseases, which might limit their usefulness where blight and phytophthora causing pathogens are prevalent (Garnsey et al., 1998). Asins et al., (1999) in their paper has mentioned that obtaining phylogenetic data of *Citrus* and related species to search for new genotypes of CTV resistance along with those expressing desirable agronomical qualities is crucial to the citrus breeding program. For instance, based on phylogenetic data of *Citrus* and related species, Asins et al. have pointed out *Fortunella crassifolia* to be more closely related to *Citrus* than *P. trifoliata*. *F. crassifolia* shows resistance to CTV and yields edible fruits, thus, they have highlighted its importance in scion breeding program because *P. trifoliata* does not yield edible fruits and is limited to rootstock improvement (Asins et al., 1999, Mestre et al., 1997). More studies are needed to understand the inheritance of resistance, genetic location, functioning and efficiency of *Fortunella crassifolia* against different isolates of CTV (Asins et al., 1999).

Regarding molecular breeding, a study was done by Gmitter et al., (1996) to map out CTV resistant markers in intergeneric back cross families that were segregating for CTV resistance, found 8 RAPD (Random Amplified

Polymorphic DNA) markers surrounding the region of the CTV-resistance (*Ctv*) gene. Yoshida (1985) has also mentioned that a single dominant (Ctv) gene controls CTV resistant gene in trifoliate orange. Another novel dominant gene (*Ctv2*) resistant to CTV has been found by (Fang and Roose, 1999) specifically in Chandler' pumelo (*C. maxima*) and this gene is said to be independently assorted from *Ctv*. Two segregating populations for CTV tolerance derived from *Poncirus trifoliata* var. flying dragon by self pollination and pollination to *Citrus medica* var. etrog when inoculated with CTV isolates and tested under bulk segregation analysis has resulted in 7 RAPD genes linked to the CTV resistance that seems to be located between chromosome cW18 and cK16 (Mestre et al., 1997).

Different studies, such as the ones done by (Gmitter *et al*., 1996; Deng et al., 1997; Mestre et al., 1997) involve the identification of dominant RAPD markers and their incorporation into linkage study of the CTV resistant gene region in *P. trifoliata*, however, it is hard to construct high resolution map around the genetic region and use these dominant markers for map based cloning approach (Fang et al., 1998). Fang et al., (1998) in their research have provided 11 closest PCR-based DNA markers, 10 of which were cloned and converted to co-dominant markers for the study of segregation patterns and linkage study. As a result, Fang et al. found that two of those markers (RfC19 and RfZ16) implied the existence of resistant gene clusters in (*Ctv*) region of all of the tested trifoliate oranges segregations. Further study of the *Ctv* locus of *Citrus* and its relatives by generating BAC (Bacterial Artificial Chromosome) clones have found seven resistant genes (*R1* to *R7*) whose composition encodes coiled-coil-nucleotidebinding site-leucine-rich repeat (CC-NBS-LRR) receptors (Yang et al., 2003). Suggesting that one of the *R* genes might be a *Ctv* gene conferring CTV resistant characteristics (Yang et al., 2003; Jeong et al., 2018). CC-

NBS-LRR is one of the largest gene families in the plant genome and is also known as a gene family having the largest known plant disease resistant genes (Torregrosa et al., 2008). However, the complete set of these *R* genes was only found in *P. trifoliata* and its derivative cultivars which further explains the withstanding resistance shown by *P. trifoliata* against different strains of CTV (Garnsey

et al., 1987; Mestre et al., 1997; Jeong et al., 2018). The multiplex PCR set markers developed and used by (Jeong et al., 2018) to detect the composition of seven *R* genes in the genetic locus of 155 citrus genetic resources and Korean landraces could be a beneficial tool for improving scion or rootstock cultivars in a citrus breeding program.

Challenges of developing CTV resistant cultivars

There are numerous of challenges for developing CTV resistant cultivars by following classical plant breeding approaches. Citrus, being a perennial fruit tree have a larger plant size, take a long time to develop and attain maturity and have a complex reproductive biology. Classical plant breeding involves the identification and evaluation of diverse resistant sources followed by screening and genetic testing, which becomes time consuming and laborious process in citrus breeding (Mestre et al., 1997; Atta et al., 2012). Furthermore, another challenge in the citrus breeding program is the genetic erosion of *Citrus* (Jeong et al., 2018). CTV strains might take several months if not a year to induce observable symptoms in their host plants. Some CTV isolates, especially the milder ones, could latently remain in their hosts without producing any symptoms until exposed to the more susceptible hosts and or a suitable climatic condition. CTV isolates have a wide genetic diversity due to segregation induced by constant selection pressure in its narrow host range (Moreno et al., 1995). Mutations, recombination, genetic drift and gene flow are some of the events that are known to be responsible for diversifying the CTV population (Moreno et al., 2007) these factors further challenge the durability of resistance offered by limited *Citrus* species and cultivars. Regarding resistant breaking events for instance, (Harper et al., 2010; Dawson and Mooney, 2000) have provided some cases of it. It is known that the resistance attributes of *P. trifoliata* can be lost in first- or second-generation trifoliate hybrids (Garnsey et al., 1987). These and several other factors make it difficult for classical plant breeding programs dealing with the development of CTV resistant cultivars to be effective and efficient in the long run. Thus, scientists have been focusing their research on finding alternative ways, such as genetic engineering and transgenic plant breeding to combat CTV infection in citrus cultivars (Cervera et al., 2010; Soler et al., 2011; Muniz et al,. 2012).

Conclusive remarks

Citrus spp is an important fruit crop cultivated worldwide. *Citrus tristeza virus* (CTV) is one of the major plant viruses of Citrus trees inducing varying degrees of disease severity in infected plants. Two main strains of CTV, namely, Quick Decline and Steam Pitting causing isolates are responsible for the destruction of Citrus at an epidemic scale. The quick decline mainly occurs to sweet orange scion grafted on sour orange rootstock, whereas steam pitting can occur in grapefruit, pomelo, lime and other sweet orange varieties regardless of rootstock used. CTV can be transmitted by infected planting materials, aphid species and dodder. Detecting the correct strain of CTV is an important step to devise an appropriate management strategy against it. Biological assaying in indicator plants, serology-based ELISA and molecular-based PCR techniques have been commonly used for its detection and characterization. Management of CTV is a challenging task, therefore development of integrated pest and disease management scheme tailored with CTV and *Citrus* orchard should be prioritized. The primary way to prevent CTV before it is introduced or where the incidence cases are low is to use certified budwood for propagation, control CTV transmitting vectors and follow quarantine and legislative measures. Trifoliate orange and its derivative cultivars are used as a resistant rootstock against Quick Decline isolates of CTV. Similarly, cross protection technique could be useful against Stem Pitting isolates of CTV. Cross protection should be used if the disease (CTV) is endemic, its mild isolate has been identified and other methods are not being useful for controlling its spread. A long-term strategy for CTV management is breeding for resistant or tolerant cultivars. It is known that a single dominant gene is responsible for CTV resistant characteristics in *P. trifoliata*. Many researches have been done in the area of molecular mapping of CTV resistant genetic loci and their interaction with resistance phenomenon. However, designing and implementing classical citrus breeding program against CTV has its own challenges. With that, transgenic plant breeding approach could be an appealing alternative for combating CTV infection.

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