Evidence of a Low Rate of Seed Transmission of Citrus tatter leaf virus in Citrus

J. D. Tanner, M. Kunta, J. V. da Graça, M. Skaria and S. D. Nelson

Texas A &M University-Kingsville Citrus Center, Weslaco TX 78596, USA

ABSTRACT. Citrus tatter leaf virus (CTLV) (*Apple stem grooving virus*) is mechanically-transmitted in citrus, causing bud-union crease in trees budded on trifoliate orange and its hybrids, but is symptomless in most scions. Seed transmission of a strain of CTLV has been reported in *Lilium longiflora* and *Chenopodium quinoa*. In order to test whether CTLV is seed transmitted in citrus, seed was collected from four adjacent CTLV-infected citrus trees of different species, namely Clementine mandarin, Meyer lemon, Eureka lemon and Meiwa kumquat. The resulting 355 seedlings and the four parent trees were tested for CTLV presence by RT-PCR using three primer sets. The four parents and two of the 136 Eureka lemon seedlings were found to be CTLV positive. This is the first report of CTLV seed transmission in citrus. Cloning and sequencing of the coat protein gene amplified using primer set TL1 showed that the sequence from the seedlings had an 89.7% homology with the parent tree, but 100% homology with the Meyer lemon and Meiwa kumquat trees, suggesting a filtering effect during transmission through the Eureka lemon parent as reported elsewhere.

Key words: Citrus tatter leaf virus, seed transmission

Citrus tatter leaf virus (CTLV) is a bud transmissible *Capillovirus* that causes tissue incompatibility between scion and trifoliate hybrid rootstocks. CTLV is considered an isolate or a close relative of *Apple stem grooving virus* (ASGV) (6, 12), the type member of the *Capillovirus* genus in the *Betaflexiviridae* family. The virus is known to infect apple, pear, apricot, cherry, lily and citrus, as well as many herbaceous plants experimentally such as *Chenopodium quinoa, Vigna unguiculata,* and *Nicotiana occidentalis* (4, 7).

CTLV is predominantly transmitted by mechanical means with no reports of an insect vector. Transmission studies using aphids failed to produce any positive results (11). Seed transmission of CTLV was detected in lily (*Lilium longiflorum*) and *Chenopodium quinoa* (4). Seed transmission was reported to be 1.8% in lily and 60% in *C. quinoa*.

The only reported attempt to detect seed transmission of CTLV in citrus was by Nishio et al. (8) who were unable to detect it in kumquat. There are only a few known cases of reported virus seed transmission in citrus. *Citrus leaf blotch virus* (CLBV) is reported to have a 2.5% rate of seed transmission in citrange, kumquat and sour orange (3), and an unidentified oak leaf pattern inducing agent was reported to be seed transmitted in Carrizo citrange (1).

To study the possibility of seed transmission of CTLV in citrus, four adjacent CTLV- positive field grown Clementine mandarin (*C. reticulata*), Meyer lemon (*C. limon*), Eureka lemon (*C. limon*) and Meiwa kumquat (*C. japonica*) trees were selected as the seed source trees; these trees were previously shown by biological indexing to be infected (2). To confirm this, young fully expanded leaves were collected form all four trees, total RNA was isolated using the RNeasy Plant Mini kit (Qiagen, Valencia CA). RNA samples were stored at -80^{o}C until they were used in reverse transcription-polymerase chain reaction

(RT-PCR). One micro gram of total RNA was reverse transcribed using SuperScript® II RT enzyme (Invitrogen, Carlsbad, CA). One micro liter of the cDNA was amplified in a total reaction volume of 25 µL consisting of 2.5 µL 10x PCR buffer, 0.1 mM dNTPs, 1.5mM magnesium chloride ($MgCl_2$), 0.2 µM gene specific primers [CTL5607F and CTL6407R (5) or TL1F and TL1R (9) or CTLV F3 and CTLV R3 (10)], and 1 U Platinum *Taq* DNA Polymerase (Invitrogen). PCR amplification was performed at 94°C for 2 min followed by 35 cycles of heating to 94°C for 30 s, 56°C for 30 s and 68°C for 1 min. The PCR products were analyzed by electrophoresis on a 1% agarose gel. All four trees were positive for CTLV (Fig.1).

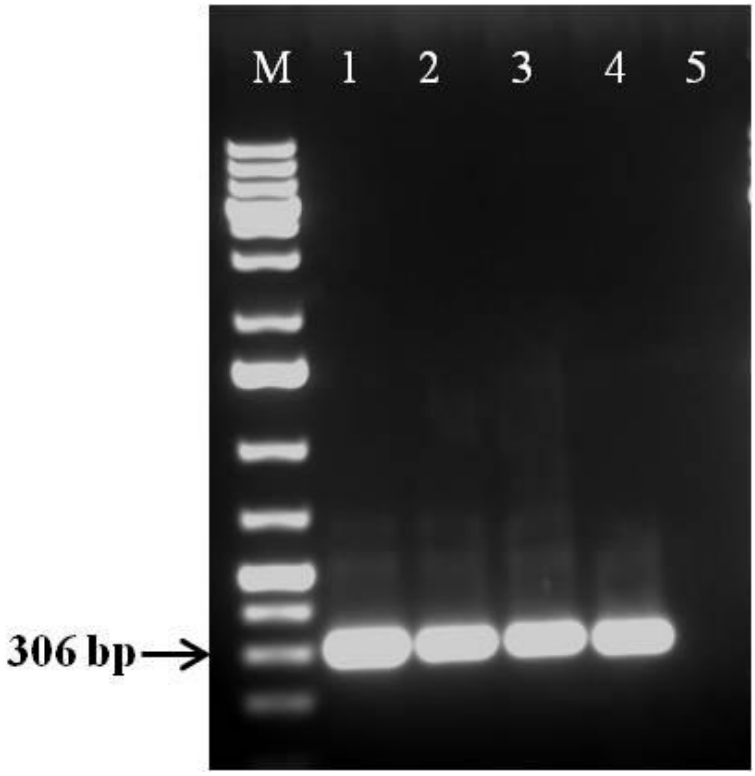

Fig. 1. Agarose gel electrophoresis of RT-PCR products amplified from seed source trees using Citrus tatter leaf virus TL1 primers. Arrow indicates the expected 306 bp PCR product. M: 1 kb plus DNA ladder (Fermentas). Lanes 1, 2, 3 and 4: Leaves collected from Clementine mandarin, Meyer lemon, Eureka lemon, and Meiwa kumquat. Lane 5: Non-template control (Blank).

Of the three primer sets used, TL1 primers were found to be the easiest to read because of the absence of non-specific bands and the presence of bright bands corresponding to the expected amplification of 306 bp in CTLV positive samples, and therefore were used as the primary screening primer for the remainder of this project. Any

CTLV positives that were identified using TL1 primer set were then re-tested using the other two primer sets to confirm positive results.

Seeds were collected from the four CTLV positive field trees, and sown in the flat beds with sterile peat moss based potting mix containing perlite. Leaves from each of the 355 seedlings obtained were collected, and tested for CTLV presence by RT-PCR as described above. Two out of 136 Eureka lemon seedlings were positive for CTLV (Fig. 2). This translates into a transmission rate of 1.47%. Of the other 219 samples tested, no additional positives were detected; 93 Clementine mandarin, 86 Meiwa kumquat, and 40 Meyer lemons seedlings. Careful review of the sequence data revealed similarities among all the PCR fragments originated from the four seed source trees.

Fig. 2. Agarose gel showing RT-PCR screening of Eureka lemon seedlings for the presence of CTLV using TL1 primers. Arrow indicates the expected 306 bp PCR product. M: 50 bp DNA ladder. Lane 1: Leaves from Eureka lemon seed source tree. Lane 2 and 3: Leaves from Eureka lemon seedlings that showed a positive result.

The sequence information obtained using TL1 primer set amplifying partial nucleotide sequence encoding CTLV coat protein from the Eureka lemon seedlings compared to the nucleotide sequence from the infected parent Eureka lemon tree using CLUSTAL W showed a homology of only 89.7%. However, when the nucleotide sequence obtained from the Eureka lemon seedlings was compared with those from the Meyer lemon and Meiwa kumquat field trees, the homology of the DNA sequences exactly matched (100% homology). This brings up some interesting questions. The DNA sequence of CTLV coat protein coming from the Eureka lemon parent tree is distinct from all other DNA sequences that were found including DNA sequences from its own offspring. Out of the 263 bp nucleotide sequence amplified with the TL1 primers, the Eureka lemon parent tree sequence had 23 bp that were different. The Eureka lemon seedling DNA sequence suggests that the strain of CTLV also found in the Meyer lemon and Meiwa kumquat parent trees is the dominant strain found in the sample set tested. If the Eureka parent tree was mechanically infected from one of its neighbors, it is possible that a suppressed variant became dominant in the parent Eureka, but the original form regained dominance in the seedlings. It has been demonstrated that sequential passage of ASGV/CTLV through host plants can

change the composition of sequence variants compared to original isolates (6).

Results of our tests indicate the occurrence of a low rate of seed transmission of CTLV in Eureka lemon. The non-detection of CTLV in the seedlings of the other species should not be interpreted to mean seed transmission is impossible in varieties other than Eureka lemon but to say that it has yet to be observed and reported. It is interesting to note, however, that Nishio et al. (8) were also unable to detect seed transmission in kumquat. Testing a larger number of seedlings might determine if there is a host effect. Further work to determine whether CTLV is seed transmissible in important rootstock varieties is needed to ensure that certified virus-free budwood is not grafted onto infected rootstock seedlings in the nursery.

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