INFLUENCE OF THE QUANTITY AND VARIABILITY OF *CITRUS TRISTEZA VIRUS* ON TRANSMISSIBILITY BY SINGLE *TOXOPTERA CITRICIDA*

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SUMMARY

Citrus tristeza virus (CTV) is transmitted in a semi-persistent manner by several aphid species, among which *Toxoptera citricida* is most efficient. After 24 h acquisition access period, eight CTV isolates were detected in single *T. citricida* with frequency of 63.3-91.1% and 71.1-91.1% by nested RT-PCR and real-time RT-PCR, respectively. More copies of CTV-targets were detected in single viruliferous *T. citricida* individuals that acquired high transmissibility CTV isolates. Analysis by single strand conformation polymorphism (SSCP) of viruliferous *T. citricida* individuals that had acquired CTV isolates ST9, GS14, CT11, HH12, and of CTV-infected receptor plants showed that most of the CTV-infected receptor plants and individual of viruliferous aphids carried one CTV haplotype, and that CTV in the receptor plants showed less haplotype diversity than that in aphids.

Key words: CTV, aphid transmissibility, quantity and variability of CTV, epidemiology, SSCP.

INTRODUCTION

Citrus tristeza virus (CTV), the agent of one of the most destructive citrus diseases in the world, induces a variety of symptoms among which stem pitting, decline and small fruits (Bar-Joseph *et al*., 1989). Economic losses caused by CTV have been recorded in some areas of China (Zhao *et al*., 1979; Zhou, 1997), and the virus has become more harmful to Chinese citrus production in recent years (Xu *et al*., 2006; Zhou *et al*., 2007). Tolerant rootsocks are extensively used to control tristeza decline syndrome, and mild strain cross protection (MSCP) has played an important role in controlling stem pitting in grapefruit and sweet orange (Lin *et al*., 2002; Powell *et al*., 2003).

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CTV is disseminated by grafting and some aphid species in a semi-persistent manner. *Toxoptera citricida* (Kirkaldy) is the most efficient vector (Bar-Joseph *et al*., 1989). It can spread different CTV strains, including those occasionally transmitted by other aphid species (Rocha-Pena *et al*., 1995). *T. citricida* used to be widely distributed in southern China, southeast Asia, South America, and Africa (Bar-Joseph *et al*., 1989), but has recently spread to Caribbean, Central America and Florida, breaking down cross protection in grapefruit and sweet orange (Powell *et al*., 2003).

As to molecular interactions between CTV and aphid vectors, Cambra *et al*. (2000) found that there was a correlation between RT-PCR detection of CTV in single *Aphis gossypii* individuals and transmission rate to Mexican lime seedling. Thereafter, Satyanarayana *et al.* (2001) suggested that CTV quantification in the aphids is important to assess its epidemiology. Moreno *et al*. (2009) found that the amount of *Plum pox virus* (PPV) plays a role in aphid transmissibility. The availability of more sensitive virus detection techniques in plant and vectors, now facilitates the study of relationships between virus titre in the aphids and transmission efficiency.

In this study, real-time RT-PCR was used to estimate if the number of CTV targets in single *T. citricida* individuals had a bearing on transmission efficiency, and the level of CTV variability during single aphid transmission was analyzed by single strand conformation polymorphism (SSCP).

MATERIALS AND METHODS

Maintenance of CTV. CTV isolates used in this investigation are part of a collection kept at the Citrus Research Institute in Chongqing. Isolates SS7, HH12, GS14 and HB1 are severe and cause stem pitting in Symons sweet orange (*Citrus sinensis*), Feng-huang pummelo (*C. grandis*) and Duncan grapefruit (*C. paradisi*), whereas CT11, ST9, HH3 and LJ1 are mild isolates that do not induce apparent symptoms in the above hosts. All these isolates were graft-inoculated onto Jincheng sweet orange (*C. sinensis*) seedlings in the greenhouse at 15 to 27°C. Virus infection was con-

firmed 90 days post inoculation by direct tissue blot immunoassay (DTBIA) (Garnsey *et al*., 1993).

Single aphid transmission. CTV-free *T. citricida* colonies were established as reported by Broadbent *et al*. (1996) and maintained on young flushes of healthy Jincheng sweet orange seedlings in insectaries. CTV-infected Jincheng seedlings were used as donor plants to feed 100 to 200 CTV-free apterae adult aphids. After 24 h acquisition period, the aphids were placed singly on young flushes of CTV-free Jincheng seedlings for 24 h. Receptor plants were then transferred into an insect-proof greenhouse at 18 to 25°C.

Thirty apterae adult aphids were used to transmit each CTV isolate in single aphid transmission tests. Transmissions were repeated three times to insure consistency in transmission rate. Four months later, the receptor plants were tested by DTBIA (Garnsey *et al*., 1993).

RNA extraction. Total RNA was extracted from donor plants and individual aphids which had fed on CTV-infected donor plants for 24 h by Trizol reagent (Invitrogen, USA). RNA extracts were resuspended in 25 µl of RNase-free water and treated with RNase-free DNase (TaKaRa, Japan). After single aphid transmission of isolates ST9, GS14, HH12 and CT11, total RNAs from infected receptor plants were extracted as reported by Zhou *et al*. (2001). All RNA extracts were stored at -80°C.

Detection and characterization of a conserved 3' UTR fragment from CTV isolates in aphids and receptor plants. Nested PR-PCR amplification was done according to Olmos *et al*. (1999). Nested PR-PCR products of CTV RNA from single aphids that had acquired isolates ST9, GS14, HH12, CT11 and from CTV-infected receptor plants were examined by SSCP analysis in 8% polyacrylamide gel at 4°C and 200 V for 3 h, as described (D'Urso *et al*., 2000).

Sequence analysis. Twenty nested RT-PCR products of each particular SSCP pattern were transformed into the vector PMD-18T (TaKaRa, Japan) and cloned into *E. coli* JM-109. Five clones of each RT-PCR products were custom sequenced (Shenggong, China)

Standard curves. The cDNA used as template for *in vitro* transcription was obtained by RT-PCR with primer PM198R and PM261F that includes the T7 promoter sequence at its 5' terminus (Ruiz-Ruiz *et al*., 2007). RT-PCR products were transcribed *in vitro* with T7 RNA polymerase (Promega, USA), and the transcripts were purified with Transcript RNA Clean Up Kit (TaKaRa, Japan). The concentration of transcripts was estimated twice with a NanoDrop ND-1000 UV Spectrophotometer (Thermo Scientific, USA). Ten-fold serial dilutions were prepared using RNA extracts (10 ng/µl) from healthy citrus, and stored at -80°C. Dilutions from 10^9 to 10^2 were employed to generate standard curves.

CTV detection by real-time RT-PCR. Real-time RT-PCR with and without reverse transcriptase were run in parallel, to ensure the absence of DNA template in transcript preparations. The RNA extracts from *T. citricida* and CTV donor plants were tested in the iCycler iQ platform (Bio-Rad, USA) with primers PM261F/PM198R targeting conserved sequence in CTV ORFs 1b (Ruiz-Ruiz *et al*., 2007).

Statistic analysis. The percentages of viruliferous *T. citricida* that acquired different CTV isolates were statistically analyzed using the generalized linear model (McCullagh *et al*., 1989). Differences among quantitative CTV levels in single *T. citricida* individuals were calculated with the one-way ANOVA method of the SPSS13.0 package (Bertolini *et al*., 2008).

Table 1. Detection of CTV in the receptor plants after single aphid transmission.

Isolates	Pathogenicity	Transmissibility	Mean transmissibility (%)	Isolates	Pathogenicity	Transmissibility	Mean transmissibility (%)
SS7	Severe	7/30[a]	22.2	CT11	Mild	11/30	37.8
		8/30				11/30	
		5/30				12/30	
HH12		15/30	47.8	ST9		2/30	8.89
		15/30				3/30	
		13/30				3/30	
HB1		9/30	28.9	HH3		7/30	27.8
		10/30				9/30	
		7/30				9/30	
GS14		0/30	2.22	LJ1		0/30	1.11
		1/30				0/30	
		1/30				1/30	

[a]Numerator = number of plants infected; denominator = number of test plants used.

Table 2. Detection of CTV in the donor plants by real-time RT-PCR.

Transmissibility	High					Intermediate	Low	
CTV isolate	HH12	CT11	HB1	HH3	SS7	ST9	GS14	LJ1
Ct ± S.D[a]	20.6±0.8	21.3±0.6	22.1±0.8	21.8±0.5	21.9±0.4	21.5±0.9	19.9±0.3	21.7±1.0
Mean number of CTV targets	621,420,766	542,414,268	474,226,829	497,625,047	487,255,109	520,862,377	692,576,873	497,825,509

[a] Average threshold cycle and standard deviation.

RESULTS

Single aphid transmission. The single aphid transmission rate of different CTV isolates ranged from 1.1 to 47.7% (Table 1). The average transmission rate of severe and mild CTV isolates was 25.3% and 18.9%, respectively, but this difference was not statistically significant (P=0.892). Based on the transmissibility by *T. citricida*, CTV isolates were classified into three groups with low (0-5%), intermediate (6-15%) and high (over 16%) trasmission rate (Yokomi *et al.*, 2010). Most of our CTV isolates fell in group three (high transmissibility).

CTV detection in aphids by nested RT-PCR. After 24 h acquisition period, CTV targets were detected in 533 out of 720 aphids (74.0%). No significant difference was found between transmissibility rate by *T. citricida* and percentage of viruliferous *T. citricida* that acquired the different isolates (P=0.127). Isolate GS14 had highest transmission rate (91.1% of the aphids were viruliferous), whereas with 63.3% of viruliferous aphids, isolate HH12 proved to be the least transmissible (Table 2).

Detection and quantification of CTV in donor plants and single aphid. To estimate the amount of CTV in donor plants, these were analyzed by real time RT-PCR, and the results showed the estimate number of CTV targets in donor plants ranged from 474,226,829 to 682,576,873 (Table 2). Detection rates obtained by real-time RT-PCR ranged from 71.1% to 91.1% (Table 3). When CTV isolates with high, intermediate and low transmissibility were acquired by *T. citricida*, the mean value was 2,984,953, 993,593 and 1,207,566 copies of CTV-targets in a single viruliferous aphid, respectively (Table 3, Fig. 1). According to the mean number of CTV-targets quantified, significant differences were observed when CTV isolates with high transmissibility were compared with those with intermediate (P=0.0257) and low transmissibility (P=0.0412). However, when low and intermediate transmissibility groups were compared, no significant difference was observed in the number of acquired CTV targets (P = 0.783).

SSCP analysis. Sequence analysis of five clones from each nested RT-PCR product showed that no more than four nucleotide difference was found among the same

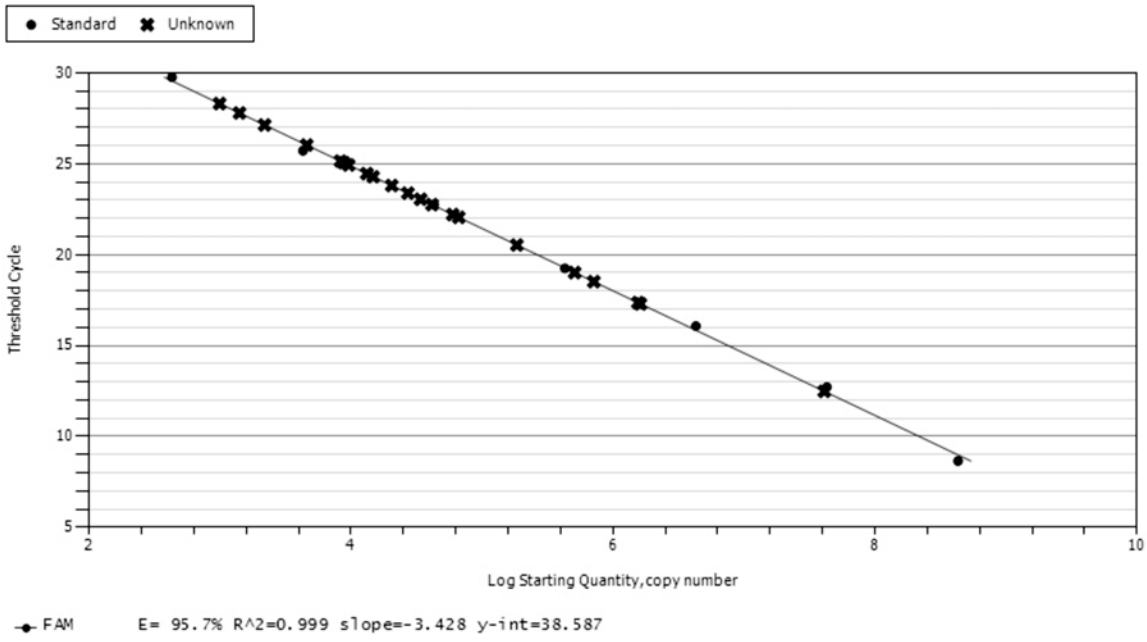

Fig. 1. Quantification range of the real-time assay based on the standard curve obtained with eight repetitions of 10-fold serial dilutions of CTV control transcripts.

Table 3. Detection and quantitation of CTV in *T. citricida* by nested RT-PCR and real-time RT-PCR.

Transmissibility	Isolate	Detection by nested RT-PCR		Detection by real-time RT-PCR		
		Number of positive aphids[a]	Detection rate (Mean ± SE[b])	Number of positive aphids[a]	Ct ± S.D[c]	Number of CTV targets (mean± SE[d])
High	HH12	18/30	0.7471±0.1415a	20/30	25.4±1.1	2,984,953±1,019,170a
		17/30		21/30		
		22/30		23/30		
	CT11	27/30		27/30	25.5±0.7	
		15/30		22/30		
		25/30		25/30		
	HB1	26/30		26/30	26.3±0.3	
		22/30		23/30		
		25/30		25/30		
	HH3	25/30		24/30	25.7±0.8	
		18/30		22/30		
		22/30		23/30		
	SS7	17/30		23/30	26.8±0.5	
		22/30		25/30		
		26/30		24/30		
Intermediate	ST9	11/30	0.6567±0.2483a	22/30	28.5±1.1	993,593±278,637b
		24/30		21/30		
		24/30		25/30		
Low	GS14	28/30	0.8283±0.0998a	28/30	27.9±0.9	1,207,566±468,849b
		27/30		27/30		
		27/30		27/30		
	LJ1	24/30		22/30	28.5±0.7	
		20/30		25/30		
		23/30		24/30		

[a] Numerator: number of viruliferous aphids; denominator: number of test aphids used.
[b] Means followed by different letters are significantly different using the generalized linear mode statistical analysis.
[c] Average threshold cycle and standard deviation.
[d] Means followed by different letters are significantly different using a one-way ANOVA after transforming the response variable by the natural logarithm statistical analysis.

Table 4. SSCP analysis of CTV isolates.

Haplotypes of CTV isolates	ST9 Aphids[a]	Plants[b]	GS14 Aphids	Plants	HH12 Aphids	Plants	CT11 Aphids	Plants
ST9I	20	8						
ST9II	3	0						
ST9III	29	0						
ST9I+III	7	0						
GS14I			11	1				
GS14II			14	0				
GS14III			13	0				
GS14I+II			21	1				
GS14I+III			23	0				
HH12I					34	30		
HH12II					5	3		
HH12III					3	0		
HH12I+II					10	8		
HH12I+III					5	2		
CT11I							45	34
CT11II							22	0

[a] Number of CTV haplotype in a single viruliferous aphid.
[b] Number of CTV haplotype in the receptor plants after single aphid transmission.

SSCP pattern, suggesting that the CTV isolates with the same SSCP patterns belong to the same haplotype. CTV from receptor plants showed less haplotype diversity than CTV from aphids, since most of the receptor plants and single viruliferous aphid contained one haplotype. Compared with the severe isolates, mild isolates ST9 and CT111 could be more easily separated. When high transmissibility CTV isolates were moved by *T. citricida*, aplotypes HH12I and CT11I were more redily transmitted to the receptor plants than others (Table 4, Fig. 2).

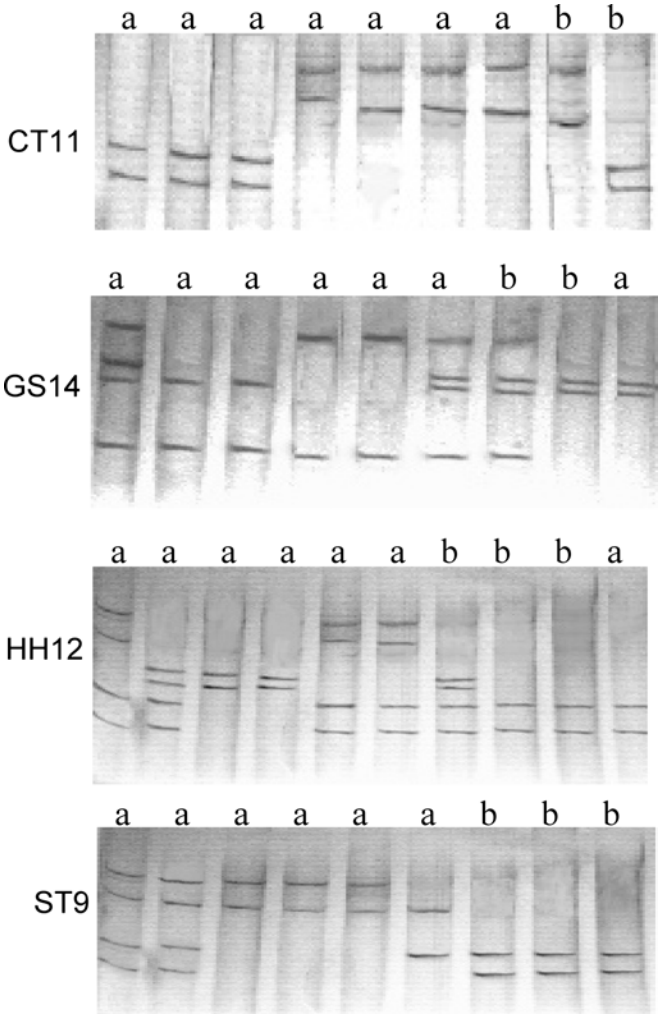

Fig. 2. SSCP patterns of virus variants of CTV isolates CT11, GS14, HH12 and ST9. a: SSCP patterns of virus variants carried by single *T. citricida*; b: SSCP patterns of CTV aphid-transmitted sub-isolates in the receptor plants.

DISCUSSION

In this study, although there was a clear trend towards a higher transmissibility of severe CTV isolates, differences between transmission rates of severe and mild isolates were not statistically significant. However, severe CTV isolates could spread quickly in the field (Sharma, 1989), thus sub-isolates of aphid transmission need to be further studied by indexing.

Previous studies on CTV, *Potato leaf roll virus* (PLRV) and *Barley yellow dwarf virus* (BYDV) indicated that the percentage of viruliferous migrant aphids is one of the major factors influencing virus epidemiology (Singh *et al*., 1995; Cambra *et al*., 2000; Fabre *et al*., 2003).

In this study, all aphids were analyzed by techniques, such as nested and real-time RT-PCR, that allow CTV detection also in samples testing negative by other detection methods (Olmos *et al*., 1999; Bertolini *et al*., 2008). The results showed that more than 71.1% of *T. citricida* individuals were viruliferous after 24 h acquisition access period and that there was no significant difference between the transmission rate by *T. citricida* and the percentage of viruliferous aphids that had acquired different CTV isolates. Cambra *et al*. (2000) reported that, although there was a correlation between CTV detection in *A. gossypii* individuals by PCR and its transmission to Mexican lime seedling, CTV isolates were still detected consistently in *T. citricida*, *A. spiraecola*, *T. aurantii*, *A. nerii and Hyalopterus pruni*, regardless of their transmissibility. Furthermore, Moreno *et al*. (2009) showed that 88.5% of tested aphids inoculated *Plum pox virus* (PPV) to receptor plants, whereas PPV infection rate was only 20%. These results indicate that it is the amount of virions involved in aphid transmission rather than the percentage of viruliferous aphids that has a bearing on transmission efficiency.

Escriu *et al*. (2000) reported that the efficiency of transmission of *Cauliflower mosaic virus* (CMV) depended on the virus titre in the plants, and a correlation between virus accumulation and transmissibility with a possible plateau was positive when virus concentration reached 500 µg/g of leaf or higher. Previous studies indicated that an aphid could transmit more efficiently when it fed on CTV-infected Mexican lime, because the CTV titre in this host is higher than that in other citrus plants (Marroquın *et al*., 2004). In this study, the possibile interference from the abundant subgenomic RNAs and defective RNAs in assessing CTV titre was excluded by targeting ORF1b (Ruiz-Ruiz *et al*., 2007). However, no obvious relationship between transmissibility and CTV accumulation in the donor plant was found and the amount of CTV in different donor plants was similar.

Moreno *et al*. (2009) reported that the amount of PPV inoculated in a plant by a single aphid could influence the frequency of infection. For CTV, however, little information is available on the influence of the number of virus particles acquired by a single aphid on the efficiency of transmission (Cambra *et al*., 1981; Saponari *et al*., 2008). In our study, CTV isolates with high transmissibility were present with a much higher number of particles in single *T. citricida* individuals than isolates with a lower transmissibility, suggesting that the quantity of CTV copies acquired by the aphid determines transmission efficiency. This finding may have potential value for explaining the epidemiological behaviour of CTV in regions where annual epidemics are caused by migrant viruliferous aphids. Furthermore, in other studies, the number of virions required for effective infection and the aphid behaviour were both involved in virus acquisition and inoculation processes as previously reported (Soosaar *et al*., 2005; Moreno *et al*., 2009).

Nolasco *et al.* (2008) found that almost all CTV haplotypes from a donor plants were present in the aphids, some of which carried more than one haplotype, although 54% of the aphids carried similar haplotypes

with very low nucleotide diversity. Because of its conservation, the CTV 3' UTR region is useful for assessing changes in viral populations. So in this study, nested RT-PCR amplicons of the 3' UTR region were used by SSCP and sequencing for further analysis of the genetic structures of CTV populations during aphid transmission. Results indicated that some aphids carried a viral content distinct from that of other aphids, and that the majority of aphids acquired one haplotype of CTV.

We were unable to detect some CTV haplotypes in receptor plants. This may taken as an indication that CTV isolates contain some non-transmissible components, or that the transmission rate of some components is too low to be detected with the number of replications used in our study (Bar-Joseph *et al*., 1989; Rocha-Pena *et al*., 1995). A further step toward improving real-time RT-PCR with specific primers or probes may be used to detect the different CTV haplotypes, so as to provide a better understanding of the relationship between viral genotype and its transmission.

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REFERENCES

Bar-Joseph M., Marcus R., Lee R.F., 1989. The continuous challenge of *Citrus tristeza virus* control. *Annual Review of Phytopathology* **27**: 291-316.

Bertolini E., Moreno A., Capote N., Olmos A., de Luis A., Vidal E., Pérez-Panadés J., Cambra M., 2008. Quantitative detection of *Citrus tristeza virus* in plant tissues and single aphids by real-time RT-PCR. *European Journal of Plant Pathology* **120**: 177-188.

Broadbent P., Brlansky R.H., Indsto J., 1996. Biological characterization of Australian isolates of *Citrus tristeza virus* and separation of subisolates by single aphid transmission. *Plant Disease* **80**: 329-333.

Cambra M., de Mendoza H.A., Moreno P., Navarro L., 1981. Use of enzyme linked immunosorbent assay (ELISA) for detection of *Citrus tristeza virus* (CTV) in different aphid species. *Proceedings of 4th International Society of Citriculture, Japan 1981*: 444-448.

Cambra M., Gorris M.T., Marroquín C., Román M.P., Olmos A., Martínez M.C., Mendoza A.H., López A., Navarro L., 2000. Incidence and epidemiology of *Citrus tristeza virus* in the Valencian community of Spain. *Virus Research* **71**: 85-95.

D'Urso F., Ayllon M.A., Rubio L., Sambade A., Hermoso de Mendoza A., Guerri J., Moreno P., 2000. Contribution of uneven distribution of genomic RNA variants of *Citrus tristeza virus* (CTV) within the plant to changes in the viral population following aphid transmission. *Plant Pathology* **49**: 288-294.

Escriu F., Perry K.L., García-Arenal F., 2000. Transmissibility of *Cucumber mosaic virus* by *Aphis gossypii* correlates with viral accumulation and affected by the presence of its satellite RNA. *Phytopathology* **90**: 1068-1072.

Fabre F., Kervarrec C., Mieuzet L., Riault G., Vialatte A., Jacquot E., 2003. Improvement of *Barley yellow dwarf virus*-PAV detection in single aphids using a fluorescent real time RT-PCR. *Journal of Virological Methods* **110**: 51-60

Garnsey S.M., Permar T.A., Camber M., Henderson C.T., 1993. Direct tissue blot immunoassay (DTBIA) for dection of *Citrus tristeza virus* (CTV). In: de Graca J.V., Moreno P., Yokomi R.K. (eds). *Proceedings of the 12th Conference of the International Organization of Citrus Virologist*, *Riverside 1993*: 39-50.

Lin Y.J., Rundell P.A., Powell C.A., 2002. In situ immunoassay (ISIA) of field grapefruit trees inoculated with mild isolates of *Citrus tristeza virus* indicates mixed infections with severe isolates. *Plant Disease* **86**: 458-461.

Marroquın C., Olmos A., Gorris M.T., Bertolini E., Martinez M.C., Carbonell E.A., de Mendoza A.H., Cambra M., 2004. Estimation of the number of aphids carrying *Citrus tristeza virus* that visit adult citrus trees. *Virus Research* **100**: 101-108.

McCullagh P., Nelder J.A., 1989. Generalized Linear Models. Chapman & Hall, London, UK.

Moreno A., Fereres A., Cambra M., 2009. Quantitative estimation of *Plum pox virus* targets acquired and transmitted by a single *Myzus persicae*. *Archives of Virology* **154**: 1391-1399.

Nolasco G., Fonseca F., Silva G., 2008. Occurrence of genetic bottlenecks during *Citrus tristeza virus* acquisition by *Toxoptera citricida* under field conditions. *Archives of Virology* **153**: 259-271.

Olmos A., Cambra M., Esteban O., Gorris M.T., Terrada E., 1999. New device and method for capture, reverse transcription and nested PCR in a single closed tube. *Nucleic Acids Research* **27**: 1564-1565.

Powell C.A., Pelosi R.R., Rundell P.A., Cohen M., 2003. Breakdown of cross-protection of grapefruit from decline-inducing isolates of CTV following introduction of the brown citrus aphid. *Plant Disease* **87**: 1116-1118.

Rocha-Pena M.A., Niblett C.L., Lee R.F., Ochoa-Corona F.M., Lastra R., Garnsey S.M., Yokomi R.K., 1995. *Citrus tristeza virus* and its aphid vector *Toxoptera citricida*: threats to citrus production in the Caribbean and Central and North American. *Plant Disease* **79**: 437-445.

Ruiz-Ruiz S., Moreno P., Guerri J., Ambrós S., 2007. A real-time RT-PCR assay for detection and absolute quantitation

of *Citrus tristeza virus* in different plant tissues. *Journal of Virological Methods* **145**: 96-105.

Saponari M., Manjunath K., Yokomi R.K., 2008. Quantitative detection of *Citrus tristeza virus* in citrus and aphids by real-time reverse transcription-PCR (TaqMan®). *Journal of Virological Methods* **147**: 43-53.

Satyanarayana T., Bar-Joseph M., Mawassi M., Albiach-Martí M.R., Ayllón M.A., Gowda S., Hilf M.E., Moreno P., Garnsey S.M., Dawson W.O., 2001. Amplification of *Citrus tristeza virus* from a cDNAclone and infection of citrus trees. *Virology* **280**: 87-96.

Sharma S.R., 1989. Factors affecting vector transmission of citrus tristeza virus in South, Africa. *Zentralblatt Fur Mikrobiologie* **144**: 283-294.

Singh R.P., Kurz J., Boiteau G., Bernard G., 1995. Detection of potato leafroll virus in single aphids by the reverse transcription polymerase chain reaction and its potential epidemiological application. *Journal of Virological Methods* **55**: 133-143.

Soosaar L.M., Burch-Smith T.M., Dinesh-Kumar S.P., 2005. Mechanisms of plant resistance to viruses. *Nature Reviews* **3**: 789-798.

Xu X.F., Zhou C.Y., Song Z., Yang F.Y, 2006. Preliminary studies on CPG/Hinf-RFLP groups of *Citrus tristza virus* infected sweet orange in China. *Agricultural Sciences in China* **5**: 39-44.

Yokomi R.K., Polek M., Gumpf D.J., 2010. Transmission and spread of *Citrus tristeza virus* in Central California. In: Karasev A.V., Hilf M.E. (eds). *Citrus Tristeza Virus* Complex and Tristeza Diseases. pp.151-165. APS Press, St. Paul, MN, USA.

Zhao X.Y., Jiang Y.H., Zhang Q.B., Qiu Z.S., Su W.F., 1979. The distribution of seedling yellow tristeza virus and its reaction on six kinds of sour orange rootstocks. *Acta Phytopathologica Sinica* **9**: 61-63.

Zhou C.Y., 1997. Occurrence guidelines and outlook of *Citrus tristeza virus* in China. *Proceedings of the first Chinese Symposium on plant virus and viral diseases control researches, China 1997*: 182-187.

Zhou C.Y., Deborah H., Rachael C., Barkley P., Bowyer J., 2001. A micro and rapid nucleotide acid extraction method of *Citrus tristeza virus* for amplification by RT-PCR. *Journal of Fujian Agricultural University* **30**: 200.

Zhou Y., Zhou C.Y., Song Z., Liu K.H., Yang F.Y., 2007. Characterization of *Citrus tristeza virus* isolates by indicators and by molecular biology methods. *Agricultural Sciences in China* **5**: 101-105.

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