Partial Sequence Characterization of *Citrus tristeza virus* Associated with Breaking of the General Resistance to CTV Expressed in *Poncirus trifoliata*

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ABSTRACT. An isolate of *Citrus tristeza virus* (CTV) recovered from citrus trees growing in the Kerikeri district of New Zealand replicated in graft-inoculated seedlings of Rubidoux or Flying Dragon trifoliate orange, hosts which are normally resistant to systemic infection by CTV. Isolate NS25 obtained from New Zealand systemically infected trifoliate orange plants kept at the Exotic Citrus Pathogen Collection at Beltsville Agricultural Research Center, Beltsville, Maryland, USA. An immunocapture-RT-PCR procedure using degenerate primers amplified ~6150 nucleotides of non-contiguous portions of the 1a open reading frame and the 3' end of the genome of NS25 propagated in Madam Vinous sweet orange. Phylogenetic analysis showed that NS25 was a distinct strain of CTV when compared to five complete CTV genomes (T30, T36, VT, Sy568 and NUaga). NS25 was most similar to strain T36. Identification in the international database of a nearly identical homologous genomic fragment from the BAN-2 isolate from India indicated the NS25 genotype may not be restricted to New Zealand.

Widely grown citrus varieties such as sweet orange and grapefruit are generally susceptible to infection by *Citrus tristeza virus* (CTV). Resistance to CTV has been identified in pummelo (8), but, this resistance is not expressed in all pummelo varieties or, in those with resistance, against all isolates of CTV (10). Resistance to CTV also has been demonstrated in other genera in the *Rutaceae* (9), and there has been particular interest in resistance expressed by trifoliate orange. This resistance, associated with a single dominant gene, appeared effective against all tested isolates of CTV, which was a potentially very useful character since this species is also sexually compatible with members of the genus *Citrus* (5). Recently the region of the trifoliate orange genome containing the CTV resistance gene(s) has been identified and interest has increased in introducing CTV resistance from trifoliate orange into important citrus varieties *via* molecular transformation (7, 23). Since CTV can replicate in mesophyll protoplasts from trifoliate orange, the resistance in trifoliate orange is apparently due to a block in either cell-to-cell or long distance movement, or is due to an induced resistance response (2).

Recently an isolate of CTV with the ability to systemically infect trifoliate orange was found in field trees in New Zealand (6). Since the resistance gene from trifoliate orange has been introduced into scion and rootstock varieties of citrus (5), the ability of this isolate of CTV to infect trifoliate orange poses a potential threat to the effectiveness of trifoliate orange derived resistance. Since the resistance-breaking character appeared unique to this isolate, we cloned and sequenced portions of its genome to determine its genetic relatedness to other CTV isolates. We report here the results of this initial study.

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MATERIALS AND METHODS

Maintenance of virus *in planta*. Mexican lime budwood infected with isolates NZ-TD28-NS24 (NS24), NZ-TD25-NS16 (NS16), NZ-KD6-NS25 (NS25), NZ-KD7-NS21 (NS21) and NZ-KD17-NS23 (NS23) was received under permit at the Exotic Citrus Pathogen Collection housed at the U.S. Dept. of Agriculture, Beltsville Agricultural Research Center (BARC), Beltsville, MD, USA, in June, 2002, and was grafted to Madam Vinous sweet orange seedlings for virus propagation. After the grafts healed, the seedlings were trimmed to produce new flush for virus testing. Tissue from Madam Vinous plants which were successfully infected with CTV based on serological testing was used to inoculate seedlings of cultivar "Large Flowered" trifoliate orange.

Sap extracts from inoculated plants were tested for CTV using DASI-ELISA. A rabbit anti-CTV polyclonal antibody was used for trapping (purified IgG at 1 µg/ml) and a mixture of the monoclonal antibodies 3E10 and 11G1 (20) was used as detecting antibodies for general detection. The monoclonal antibody MC13 was also used as a detecting antibody for strain-specific detection (16). Colorimetric detection of bound antibody was done using alkaline phosphatase conjugated to goat anti-mouse IgG and exposure to p-nitrophenyl phosphate substrate (Sigma Corp., St. Louis, MO).

Molecular analysis. Samples desiccated over silica gel were received under permit for serological and molecular analysis at USHRL. Oligonucleotide primers (Table 1) for PCR amplification from genomic regions indicated in Fig. 1 were designed based upon visual assessment of a ClustalX (19) multiple alignment of genome sequences of CTV isolates T36 (13), T30 (1), VT (15) and Sy568 (22). Primers were synthesized by Independent DNA Technologies (Ames, IA)

For amplification, 0.5 g of desiccated stem bark or whole leaf petioles was pulverized in buffer using a Kleco 4200 Pulverizer (Garcia Manufacturing, Visalia, CA). Virions were immunocaptured from sap extracts using anti-CTV rabbit polyclonal IgG attached to magnetic beads coated with goat anti-rabbit antibodies (Dynal, Lake Success, NY). Reverse transcription was performed with random hexamers (Promega, Madison, WI) directly on immunocaptured virions as previously described (12).

Amplification was performed with five microliters of cDNA in a 25 µl reaction volume containing a 1× concentration of reaction buffer (supplied by Promega Corp., Madison WI), 0.2 mM dNTPs, 0.2 µm of each primer and 0.625 units of Taq polymerase (Promega Corp). Specific amplification conditions for primer pairs are listed in Table 1. Molecular cloning was performed using standard protocols and sequencing was performed on an ABI 3730 automated sequencer housed at the USHRL Genomics Facility, Fort Pierce, FL, USA. At least three cDNA clones of each amplified region were used to create a consensus sequence which was termed a contig.

Optimal pairwise alignments were performed with the LALIGN program at the Biology Workbench, San Diego Supercomputer Center, University of California, San Diego. Multiple sequence alignments were performed using ClustalX (19), with visual editing by the program Genedoc (v. 2.6.002). Genetic distances and phylogenetic relationships were calculated with ***MEGA*** version 2.1, available from the Arizona State University, Tempe, Arizona. Database queries were performed using the BLAST (3) server housed at the National Center for Biotechnology Information U.S. National Library of Medicine, 8600 Rockville Pike, Bethesda, MD 20894.

TABLE 1
SEQUENCE, ORIENTATION, GENOMIC LOCATION AND AMPLIFICATION CONDITIONS FOR DEGENERATE PRIMERS USED TO AMPLIFY PORTIONS OF THE GENOME A OF RESISTANCE-BREAKING CTV ISOLATE FROM NEW ZEALAND

					PCR conditions[4]				
Contig[1]	(bp)[2]	Sense	Map units[3]	Primer sequence (5'→3')	Mg^{++} (mM)	94°C	Anneal	72°C	Cycles
ctv3	883	+	16,760-16,779	gtg ctg tgt aca tac aag ct	1.5	30 s	56°C	60 s	30
		-	17,622-17,643	cga ttc gac aat ytc nga aac g					
ctv5	921	+	15,295-15,314	gag gcg cac gcg gtg cga gc	1.5	30 s	38°C	60 s	30
		-	16,197-16,216	tca att tct ttg tyt cgt cg					
ctv9	1073	+	11,795-11,814	tnt ctc ttc tta taa tga gc	2.5	30 s	41°C	60 s	35
		-	12,849-12,868	ctt cct tta aac trg aaa cg					
ctv20	833	+	9724-9744	gtg gar gag caa aty wcy aac	1.5	30 s	45°C	90 s	30
		-	10,537-10,557	cac ytg atc acc aaa gtc ctg					
ctv16	879	+	6465-6485	tga ara acc tyc cvg ttt gtt	4.0	30 s	45°C	90 s	30
		-	7325-7344	ctc tcc aay wcc gaa aac ac					
ctv14	959	+	4829-4849	ggg ygg ggg ttt wtg ydt ggc	1.5	30 s	56°C	60 s	30
		-	5768-5788	ccr tac aag gay tta gca cca					
Block 2	756	+	2472-2492	gay gag cgy gcy tat aar cgt	1.5	30 s	50°C	90 s	30
		-	3228-3248	cat cac ytg ytg rtt ctc act					
L1	747	+	1180-1203	cay ttg tta aag aay tcg tca ct	1.5	30 s	50°C	90 s	30
		-	1904-1927	mac kcg atg gca caa ctc atc aaa					

[1]Please refer to contigs in Fig. 1.
[2]Predicted size of PCR product in base pairs (bp).
[3]Based upon strain T36 genomic sequence (13).
[4]Time in seconds for duplex denaturation at 94°C, temperature for primer template annealing for 60 s, time for primer extension at 72°C and total cycles of amplification.

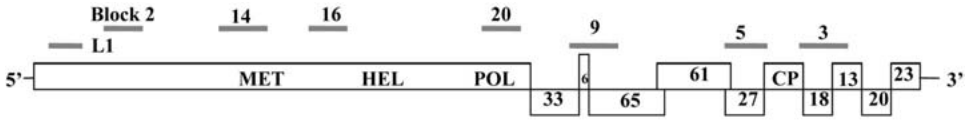

Fig. 1. Schematic view of CTV genome organization. Bars represent positions of indicated contigs amplified in this study. Motifs associated with viral RNA replication and open reading frame assignments are as indicated in Karasev et al. (13).

RESULTS

Serological and molecular detection of NS25. Serological analysis at both BARC and USHRL showed one of two trifoliate orange plants inoculated with NS25 supported replication (Table 2). CTV was not detected by ELISA in trifoliate orange 1 samples, even though CTV was readily detected in the Madam Vinous budwood inoculum grafted to trifoliate orange 1 (Table 2). Positive MC13 values also were obtained for this Madam Vinous sample. CTV was detected by ELISA from trifoliate orange 2, although titers were not equivalent between tests conducted at BARC and USHRL on different samples from the same plant. Samples tested at USHRL from trifoliate orange 2 were negative by MCA13, but overall virus titer was also low. CTV was detected in the Madam Vinous inoculum source grafted to trifoliate orange 2.

Figure 2 shows that CTV DNA sequences of the expected size were amplified with capsid protein gene primers from NS25-inoculated trifoliate orange 2 tissue (lane 2), and the Madam Vinous inoculum source graft (lane 5). An unsuccessful attempt was made to amplify NS25 and a second isolate, NS23, from Madam Vinous budwood grafted to Carrizo citrange rootstock (lanes 3 and 4 respectively). The reasons for the lack of amplification are not known. cDNA from isolate T3 was

TABLE 2
DETECTION OF NEW ZEALAND CTV ISOLATE NS25 BY ELISA IN INOCULATED PLANTS

Sample	Test	MMC[1]	MC13[2]	CTV Status
Trifoliate orange 1[3]	BARC	0.157	n/d	negative
	USHRL	0.053	0.062	negative
Madam Vinous 1	BARC	0.388	n/d	positive
	USHRL	0.505	0.929	positive
Trifoliate orange 2	BARC	0.351	n/d	positive
	USHRL	0.101	0.079	positive
Madam Vinous 2	BARC	0.349	n/d	positive
	USHRL	n/d	n/d	—
Controls[4] T30	USHRL	0.355	0.032	positive
T36	USHRL	0.357	0.274	positive
Healthy	BARC	0.060	—	negative
Madam Vinous	USHRL	0.046	0.031	negative

[1]Mixture of monoclonal antibodies 3E10 and 11B1 used as detecting antibodies for general CTV assay. n/d = test not done.
[2]Isolate-specific monoclonal antibody MC13 used as detecting antibody. n/d = test not done.
[3]Trifoliate orange seedlings were inoculated from sero-positive Madam Vinous sweet orange seedlings.
[4]Florida CTV isolates T30 and T36 used as negative and positive controls respectively for MC13.

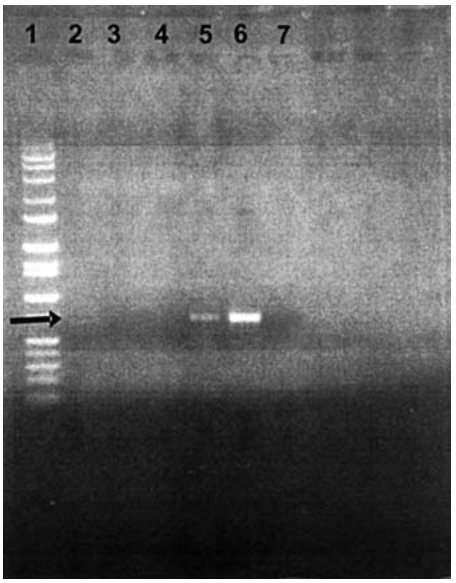

Fig. 2. Gel electrophoresis of CTV isolate NS25 coat protein open reading frame amplified by PCR from indicted tissues. Lane 1. DNA size markers. Lane 2. Trifoliate orange 1. Arrow indicates amplified DNA. Lane 3. Madam vinous on Carrizo citrange. Lane 4. Isolate NS23 from Madam Vinous on Carrizo citrange. Lane 5. Madam Vinous graft on trifoliate orange 1. Lane 6. T3 isolate in Madam Vinous (positive control). Lane 7. Water only (negative control).

used as a positive control and yielded DNA of the expected size (lane 6). A water only control yielded no product as expected (lane 7).

Analysis of degenerate primer amplified sequences. Primer pairs listed in Table 1 amplified products of the expected size from NS25-infected Madam Vinous tissue and these were cloned and sequenced. A minimum of three clones of each amplified region were sequenced and the sequences were aligned to create a consensus sequence from the manually edited alignment. The NS25 cloned sequences represented ~7000 nucleotides (~37%) of the total genome estimated at 19,200 nucleotides (13). Pairwise alignments performed with the complete genomic sequences of isolates T30, T36, VT, Sy568 and NUagA (18) indicated that the degenerate primers amplified the expected homologous regions of the NS25 genome (data not presented). Percent nucleotide sequence identities derived from pairwise alignments of representative NS25 contigs and genomic sequences of the T30, T36, VT, Sy568 and NUagA strains are presented in Table 3. The nucleotide sequence identities of NS25 contigs derived from the 5' terminal portion of the genome (L1, Block 2, ctv14, ctv16 and ctv20) were most similar to the T36 genomic sequence, with 90%, 89%, 90%, 89% and 92% identity respectively. For the same contigs, comparisons with T30, VT, Sy568 and NUaga sequences yielded no identity greater than 82%. For the ctv16 contig, nucleotide sequence identities were 65-66%.

Pairwise comparisons showed the NS25 contigs from the 3' terminal region of the genome (ctv9, ctv5 and ctv3) were more similar to the standard genomes than contigs from the 5' end (Table 3). The range of identities for ctv9 (87-98%), ctv5 (89-96%) and ctv3 (92-97%) was narrower than those observed for contigs from the 5' end. For NS25 ctv9 the highest sequence identity was with strains VT and Nuaga (97% and 98%). It was substantially less with strains T30, T36 and Sy568 (88%, 87% and 88%). For ctv3 nucleotide sequence identities were 97% for VT, 95% for Sy568 and NUaga, and 92% for T30 and T36. Genetic distances between ctv3 contigs indicated that there was less divergence (0.025-0.083) for contigs from the 3' region (Table 4). In contrast, genetic distances for the L1 contig indicated greater nucleotide divergence. The NS25 and T36 sequences were the closest (0.093) and NS 25 was equivalently more distant from the other sequences (0.213-0.240) (Table 5).

A BLAST search using NS25 5' end contigs as query sequences yielded significant matches with isolates T36 (Genbank Accessions AY170468, CTU16304) and Qaha (AY340974). Isolate Qaha is nearly identical to T36 (unpublished data). Contig L1

TABLE 3
PERCENT IDENTITIES OF NS25 CONTIGS DERIVED FROM PAIRWISE ALIGNMENTS WITH GENOMIC SEQUENCES OF FIVE STANDARD CTV STRAINS

	Standard CTV Genomes for Comparison[2]				
NS25 Contig[1]	T30	T36	VT	Sy568	NUaga
L1	77	90	76	77	77
Block 2	73	89	72	72	71
ctv14	72	90	70	72	72
ctv16	66	89	65	65	65
ctv20	81	92	82	78	82
ctv9	88	87	97	88	98
ctv5	96	93	89	90	90
ctv3	92	92	97	95	95

[1]Contig sequences L1, Block2, ctv14, ctv16, ctv20, ctv9, ctv5 and ctv3 are assigned respectively the Genbank accession numbers: DQ074987, DQ074986, DQ074983, DQ074984, DQ074985, DQ074982, DQ074981 and DQ074980.
[2]Shaded values indicate the most closely related standard strain.

also showed significant nucleotide sequence identity to a portion of the genome of Indian isolate BAN-2 (Genbank Accession AY572217) with 98% identity in 287 nucleotides.

Phylogenetic analysis of NS25 sequences. Neighbor-joining and Maximum Parsimony analyses were applied to the sequences of the NS25 contigs. Both methods pro-

TABLE 4
GENETIC (P) DISTANCES OF CTV3 SEQUENCE CONTIGS FROM 3' REGION OF THE GENOME

	VT	NS25	NUaga	Sy568	T30	T36
VT	–	0.025[1]	0.046	0.054	0.081	0.073
NS25	–	–	0.049	0.054	0.081	0.077
NUaga	–	–	–	0.025	0.076	0.082
Sy568	–	–	–	–	0.083	0.080
T3	–	–	–	–	0.079	0.079
T30	–	–	–	–	–	0.067
T36	–	–	–	–	–	–

[1]P-distances were calculated by the program *MEGA* v. 2.1.

TABLE 5
GENETIC (P) DISTANCES OF L1 SEQUENCE CONTIGS FROM 5' REGION OF THE GENOME

	VT	NS25	NUaga	Sy568	T30	T36
VT	–	0.238[1]	0.071	0.043	0.101	0.240
NS25	–	–	0.228	0.228	0.224	0.093
NUaga	–	–	–	0.062	0.088	0.229
Sy568	–	–	–	–	0.089	0.230
T3	–	–	–	–	0.108	0.213
T30	–	–	–	–	–	0.219
T36	–	–	–	–	–	–

[1]P-distances were calculated by the program *MEGA* v. 2.1.

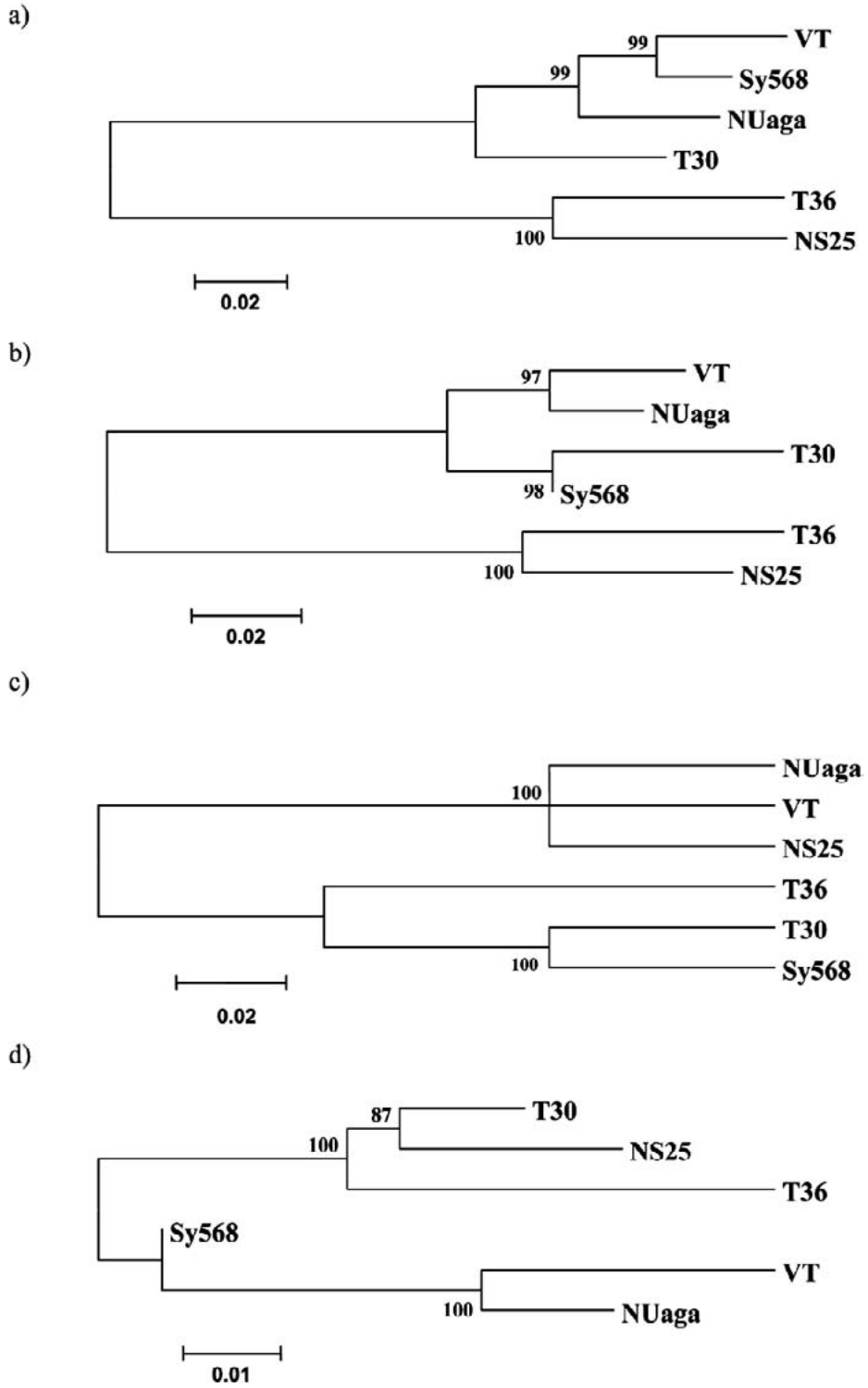

Fig. 3. Phylogenetic relationships between NS25 and standard isolates based on marker sequences. Neighbor-joining trees of: a) L1; b) ctv20; c) ctv9 and d) ctv5 contigs derived using 1000 bootstrap replicates. Branches with less than 70% bootstrap support were collapsed. Scale bar indicates number of substitutions per site.

duced trees with similar topology (data not shown) and the trees derived from Neighbor-joining analysis are presented. Trees are presented as un-rooted, rectangular cladograms with branches having 70% or greater bootstrap replicate support shown. Trees for 5' end contigs L1 and ctv20 were similar with NS25 sequences clustered with strain T36 (Fig. 3a, b). In both trees, strains VT, T30, NUaga and Sy568 clustered together and were clearly separated from NS25 and T36. Results were variable with 3' end contigs. Analysis of ctv9 placed NS25 with VT and NUaga (Fig. 3c), while analysis of ctv5 placed NS25 with T30 and T36 (Fig. 3d).

DISCUSSION

This study yielded molecular and serological evidence supporting earlier reports that NS25 is a strain of CTV that systemically infects trifoliate orange (6). Systemic infection by NS25 indicates that certain populations of CTV are not affected by the resistance gene expressed in trifoliate orange. Since only one of two trifoliate orange plants supported replication of NS25, there may be variability in seedling populations in the expression or effectiveness of this resistance. Although NS25 was found in New Zealand, the 98% sequence identity with the genome fragment of the Indian isolate BAN-2 suggests that this strain may be more widely distributed. No information is available on whether BAN-2 can replicate in trifoliate orange. Further research is needed to determine the global distribution of NS25 and whether sexual hybrids of trifoliate orange also are susceptible to infection by NS25. Previous studies indicated that individuals of trifoliate orange interspecific hybrids could vary in susceptibility to systemic infection by different strains of CTV (10).

The greater genetic distances for the 5' region and lesser distances for the 3' region supported observations from previous studies that the CTV 5' region is more divergent than the 3' region (14, 15, 21). Prior studies conducted to differentiate CTV strains based on nucleotide sequences have emphasized sequence differences in the 5' end (4, 11, 12, 14). Phylogenetic analysis of the NS25 5' contigs indicated that NS25 is more closely related to the T36 strain than the other strains analyzed. The phylogenetic placement of the NS 25 ctv5 contig, which contains sequence of the capsid protein gene, also supported the close sequence relationship between NS25 and T36, and is in agreement with a previous analysis of the capsid protein amino acid sequence (6). The incongruent phylogenetic placement of the NS25 3' contigs suggests possible recombination events in the evolution of NS25. A role for recombination in the evolution of the genomes of strains of CTV has been suggested by earlier studies (17, 21).

NS25 is clearly a new strain of CTV and data indicate its distribution may not be limited to New Zealand. Because of the importance of the *P. trifoliata* resistance gene in citrus scion and rootstock breeding programs, future research on NS25 should focus on its global distribution and the genetic basis of its resistance breaking phenotype.

LITERATURE CITED

1. Albiach-Marti, M. R., M. Mawassi, S. Gowda, T. Satyanarayana, M. E. Hilf, E. C. Almira, M. C. Vives, C. López, J. Guerri, R. Flores, P. M. Moreno, S. M. Garnsey, and W. O. Dawson 2000. Sequences of *Citrus tristeza virus* separated in time and space are essentially identical. J.Virol. 74: 6856-6865.
2. Albiach-Marti, R. M., J. W. Grosser, S. Gowda, M. Mawassi, T. Satyanarayana, S. M. Garnsey, and W. O. Dawson

2004. *Citrus tristeza virus* replicates and forms infectious virions in protoplasts of resistant citrus relatives. Mol. Breeding 14: 117-128.
3. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman
1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410
4. Ayllón, M. A., C. López, J. Navas-Castillo, S. M. Garnsey, J. Guerri, R. Flores, and P. Moreno
2001. Polymorphism of the 5' terminal region of *Citrus tristeza virus* (CTV) RNA: Incidence of three sequence types in isolates of different origin and pathogenicity. Arch. Virol. 146: 27-40
5. Barrett, H. C.
1990. US 119, an intergeneric hybrid citrus scion breeding line. HortScience 25: 1670-1671.
6. Dawson, T. E. and P. A. Mooney
2000. Evidence for trifoliate resistance breaking isolates of citrus tristeza virus in New Zealand. In: *Proc. 14th Conf. IOCV,* 69-76. IOCV, Riverside, CA.
7. Deng, Z., S. Huang, P. Ling, C. Yu, Q. Tao, C. Chen, M. K. Wendell, H. B. Zhang, and F. G. Gmitter
2001. Fine genetic mapping and BAC contig development for the citrus tristeza virus resistance gene locus in *Poncirus trifoliata* (Raf.). Mol. Genet. Genomics 265: 739-747.
8. Fang, D. Q. and M. L. Roose
1999. A novel gene conferring citrus tristeza virus resistance in *Citrus maxima* (Burm.) Merrill. HortScience 34: 334-335.
9. Garnsey, S. M., H. C. Barrett and D. J. Hutchinson
1987. Identification of citrus tristeza virus resistance in citrus relatives and its potential applications. Phytophylactica 19: 187-191.
10. Garnsey, S. M., H. J. Su, and M. C. Tsai
1996. Differential susceptibility of pummelo and Swingle citrumelo to isolates of citrus tristeza virus. In: *Proc. 13th Conf. IOCV,* 138-146. IOCV, Riverside, CA.
11. Hilf, M. E., A. V. Karasev, M. R. Albiach-Martí, W. O. Dawson, and S. M. Garnsey
1999. Two paths of sequence divergence in the citrus tristeza virus complex. Phytopathology 89: 336-342
12. Hilf, M. E. and S. M. Garnsey
2000. Characterization and classification of citrus tristeza virus isolates by amplification of multiple molecular markers. In: *Proc. 14th Conf. IOCV*, 18-27. IOCV, Riverside, CA
13. Karasev, A. V., V. P. Boyko, S. Gowda, O. Nikolaeva, M. E. Hilf, E. V. Koonin, C. L. Niblett, K. Cline, D. J. Gumpf, R. F. Lee, D. J Lewandowski, and W. O. Dawson
1995. Complete sequence of the citrus tristeza virus RNA genome. Virology 208: 511-520.
14. López C., M. A. Ayllón, J. Navas-Castillo, J. Guerri, P. Moreno, and R. Flores
1998. Sequence polymorphism in the 5' and 3' terminal regions of citrus tristeza virus RNA. Phytopathology 88: 685-691.
15. Mawassi, M., E. Mietkiewska, R. Gofman, G. Yang, and M. Bar-Joseph
1996. Unusual sequence relationships between two isolates of citrus tristeza virus. J. Gen. Virol. 77: 2359-2364.
16. Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee
1990. A monoclonal antibody that discriminates strains of citrus tristeza virus. Phytopathology 80: 224-228.
17. Rubio, L., M. A. Ayllón, P. Kong, A. Fernández, M. Polek, J. Guerri, P. Moreno, and B. Falk
2001. Genetic variation of *Citrus tristeza virus* isolates from California and Spain: evidence for mixed infections and recombination. J. Virol. 75: 8054-8062.
18. Suastika, C., T. Natsuake, T. Hirotsugu, T. Kano, H. Ieki, and S. Okuda
2001. Nucleotide sequence of citrus tristeza virus seedling yellows isolate. J. Gen. Plant Pathol. 67: 73-77.
19. Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins
1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24: 4876-4882.
20. Tsai, M. C., H. J. Su, and S. M. Garnsey
1993. Comparative study on stem-pitting strains of CTV in Asian countries. In: *Proc. 12th Conf. IOCV*, 16-19. IOCV, Riverside, CA.
21. Vives, M. C., L. Rubio, C. López, J. Navas-Castillo, M. R. Albiach-Marti, W. O. Dawson, J. Guerri, R. Flores, and P. Moreno
1999. The complete genome sequence of the major component of a mild citrus tristeza virus isolate. *J. Gen. Virol*. 80: 811-816.
22. Yang, Z-N., D. M. Mathews, J. A. Dodds, and T. E. Mirkov
1999. Molecular characterization of an isolate of citrus tristeza virus that causes severe symptoms in sweet orange. Virus Genes 19: 131-142.
23. Yang, Z. N., X. R. Ye, J. Molina, M. L. Roose and T. E. Mirkov
2003. Sequence analysis of a 282-kb region surrounding the *Citrus tristeza virus* resistance gene (Ctv) locus in *Poncirus trifoliata*. Plant Physiol. 131: 482-492.