Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

EUPHRESCO III-Special Issue on Plant Health Priorities
RESEARCH PAPERS

New citrus tristeza virus strains (*Closterovirus tristezae*) detected in the Chlef Valley, Algeria

Nabil Abdellah BENTURQUIA[1,2], Malika MEZIANE[2,*], Khaled DJELOUAH[3], Samir ALI-AROUS[2]

[1] *Natural Bio-resources Laboratory, Hassiba Benbouali University of Chlef, Algeria*
[2] *Research Laboratory of Crop Production and Protection, Hassiba Benbouali University of Chle, Algeria*
[3] *International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (Bari), Italy*
*Corresponding author. Email: m.meziane@univ-chlef.dz

Summary. Citrus production is economically important in the Chlef Valley, Algeria, but faces several challenges from diseases that threaten yields, fruit quality, and long-term sustainability of orchards. During recent decades, several citrus orchards have shown decline and severe symptoms, mostly associated with tristeza and caused by the virulent VT strain reported in 2019. To update current distribution and strains of tristeza pathogens and potential aphid vectors, an additional survey was conducted in the Chlef Valley from 2021 to 2024. Samples collected from different citrus areas were serologically assessed, showing an increase in the infection rates from 3.2% in 2019 to 4.24% in 2024. The molecular genotype characterization using strain specific RT-PCRs confirmed the presence of the pre-existing T30 and VT genotypes, and two new strains, S1 and T3, were also detected. This study has confirmed the widespread dissemination of the virulent VT genotype of CTV, and has detected additional CTV strains. Discovery of new strains and the further spread of the severe VT genotype, combined with detection of the aphid vector *Aphis gossypii* Glover, poses significant threats to the citrus industry in Algeria. This situation requires proactive measures by the National Phytosanitary Services, including extending surveillance to other citrus Algerian production regions.

Keywords. Citrus, CTV, New strains, M.M.Ms, Algeria.

OPEN ACCESS

Citation: Benturquia, N. A., Meziane, M., Djelouah, K., & Ali-Arous, S. (2026). New citrus tristeza virus strains (*Closterovirus tristezae*) detected in the Chlef Valley, Algeria. *Phytopathologia Mediterranea* 65 (EUPHRESCO III - Special Issue): 1-6. doi: 10.36253/phyto-17068

Accepted: June 1, 2026

Published: June 10, 2026

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Nihal Buzkan, Kahramanmaraş Sütçü Imam University, Turkey.

ORCID:
NAB: 0000-0003-4290-1555
MM: 0000-0002-4642-4584
KD: 0000-0002-4806-122X
SA-A: 0000-0002-4183-308X

INTRODUCTION

Citrus production continues to face significant threats from citrus tristeza virus (CTV) *(Closterovirus tristezae),* which is the most destructive viral pathogen of citrus (Folimonova and Sun, 2022). In Algeria, citrus crops have considerable economic and cultural significance in many areas in the country's coastal zones (MADRP, 2023). However, citriculture encounters ongoing challenges from diseases that threaten yields, fruit quality, and the long-term sustainability of orchards. During recent decades, orchards throughout the main citrus producing areas in the Mitidja and Chlef Valleys have shown increases

in decline syndromes and severe symptoms associated with CTV, affecting old and young orchards (Larbi *et al.,* 2015; Ali-Arous *et al.,* 2019). This situation prompted a large-scale survey conducted from 2016 to 2018 across Algeria's main citrus-growing areas, which confirmed the presence of a virulent VT genotype strain of CTV in a citrus orchard located in the Chlef Valley. This detection was followed by eradication action to sanitize the area (Ali-Arous *et al.,* 2019). The survey also found other CTV isolates with 99% nucleotide identity with T30 mild CTV. The virus was widely distributed with an overall infection rate of 3.2% (Ali-Arous *et al.,* 2019).

The causal agent of citrus tristeza is a closterovirus with a 2,000 × 11 nm, non-segmented positive-sense RNA genome, which transmitted through infected propagation plant material and by aphid vectors (*Hemiptera*, *Aphididae*) in a semi-persistent manner (Albiach-Marti, 2013). CTV has the largest genome of RNA viruses infecting plants, has extensive strain diversity, and is pathogenic to a wide range of hosts. The virus is currently classified into at least eight strains (genotypes), of which T36, T30, T3, T68, VT, RB, HA16-5 and S1 are well documented.

CTV strains can induce different symptoms depending on the citrus host species (Sun *et al.,* 2024). The RB strain is the only variant able to infect *Poncirus trifoliata* (Yokomi *et al.,* 2017). Depending on the hosts and viral strains, CTV may cause different symptoms, including seedling yellows, rapid host decline, and stem pitting (Licciardello *et al.,* 2025), leading to gradual losses of productivity and fruit quality (Yokomi *et al.,* 2018).

To reassess the current distribution of CTV strains in the Chlef Valley, which is the major citrus production region of Algeria, and to additionally survey for exotic virus strains or strains not previously detected, a new survey was conducted from 2021 to 2024. This aimed to provide information to support sustainable citrus management, and to strengthen plant health certification and quarantine programmes in Algeria.

MATERIALS AND METHODS

A field survey was carried out each spring from 2021 to 2024, to assess the current CTV status in the Chlef Valley, including distribution and incidence of the virus. Sampling for CTV was not dependent on symptom expression, and followed either a random design along field diagonals or hierarchical sampling as described by Gottwald and Hughes (2000). Samples consisted of young shoots, and petioles and pedicels of fully expanded leaves and flowers.

In total, 1,743 citrus plants were sampled from 112 commercial orchards and one private nursery, all located within the Chlef Valley. Samples were tested by double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) (Bar-Joseph *et al*., 1979; Cambra *et al*., 2000), using CTV-DAS-ELISA Complete Kit (Bioreba®).

Based on the origin of the plant propagation material, geographical distribution and symptoms, six CTV-positive samples were selected for virus strain determinations. Nucleic acids extractions were carried out using a semi-automated magnetic bead method and the Maxwell® RSC 48 instrument, following the protocol guidelines provided in the Maxwell® RSC Plant RNA Kit (Promega®). RNA extracts were stored at -20°C. First-strand cDNA was synthesized from 2 µg of total RNA using M-MLV Reverse Transcriptase (Promega®). Confirmation of CTV infections was obtained using a one-step TaqMan real-time RT-PCR method (Saponari *et al*., 2008). A partial gene fragment of the major coat protein CP (101 bp) was amplified using GoTaq® Probe qPCR Master Mix, GoScript™ RT for one-Step RT-qPCR (Promega®). This is a universal CTV detection assay using primers P25F/P25R and a CY-5 labelled probe (Table 1), with the following cycling parameters; one cycle at 45°C for 5 min, followed by 2 min at 95°C, and then 40 cycles each at 95°C for 15 seconds and 59°C for 30 s. Strain detection was achieved using eight strain-specific PCR assays for the strains T36, T30, T3, T68, VT, RB, HA16-5, or S1 (Table 1), following previously established protocols (Roy *et al*., 2010; Cook *et al*., 2016; Yokomi *et al*., 2018). The amplified products were then analyzed in 1% agarose gels stained with GelGreen (0.02 µL mL^{-1}).

In parallel, monitoring of indigenous potential aphid vectors biotypes occurring in citrus was carried out in the Chlef Valley area during the 2021 to 2024 period. Aphids were collected from infested plant organs, using fine brushes coated with Arabic gum to facilitate adhesion and handling. Collected specimens were then prepared and observed under a microscope for dichotomic identification using the guide of Blackman and Estop (2000).

RESULTS AND DISCUSSION

CTV was detected during the 4-year survey of different areas, and citrus orchards of different ages and variety/rootstock combinations in the Chlef Valley (Figure 1). Different symptoms were observed that could possibly be associated with CTV, including tree stunting, defoliation, and decline, leaf chlorosis and vein corking. Serological DAS-ELISA detected CTV in 74 of 1,743 sam-

Table 1. Primers used for CTV strain characterization and their respective annealing temperatures (Ta).

CTV strain	Primer	Sequence (5′-3′)	Amplicon size (bp)	Position	Ta (°C)	Reference
P25	Sense	AGCRGTTAAGAGTTCATCATTRC	101	16.376	59	Saponari *et al*., 2008
	Anti-sense	TCRGTCCAAAGTTTGTCAGA		16.399		
	CTV-G (TaqMan)	MGB-CRCCACGGGYATAACGTACACTCGG (CY-5)		16.412–16.437		
T36	Sense	GGT GTA AGG AAG CGT GTG TCG CAT TTA	537	5,641	66	Cook *et al*., 2016
	Anti-sense	ACC TGC ACC GTC TAA CAA CAT CAT CG		6,152		
HA16-5	Sense	TAG GAA GGG TCA CTG CCC TGA CA	610	2,128	56	Cook *et al*., 2016
	Anti-sense	GTA AGT ATC TAA AAC CAG GAG		2,717		
VT	Sense	TTT GAA AAT GGT GAT GAT TTC GCC GTC A	302	1,945–1,972	60	Roy *et al*., 2010
	Anti-sense	GAC ACC GGA ACT GCY TGA ACA GAA T		2,222–2,246		
T30	Sense	TGT TGC GAA ACT AGT TGA CCC TAC TG	206	588–613	60	Roy *et al*., 2010
	Anti-sense	TAG TGG GCA GAG TGC CAA AAG AGA T		769–793		
T3	Sense	GTT ATC ACG CCT AAA GTT TGG TAC CAC T	409	4,846–4,873	60	Roy *et al*., 2010
	Anti-sense	CAT GAC ATC GAA GAT AGC CGA AGC		5,231–5,254		
T68	Sense	GTTAAGAAGGATCACCATCTTGACGTTGA	510	2,124–2,152	59	Roy *et al*., 2010
	Anti-sense	AAAATGCACTGTAACAAGACCCGACTC		2,607–2,633		
RB	Sense	AAG YTA CTT GCA CAA GTT GTC ACC ATC TTA	596	12248- 12277	60	Roy *et al*., 2010
	Anti-sense	TGG TCG ATT GAT ACT GTT TCA CTA ATC CCA T		12874-12844		
S1	Sense	AGGCCGGTAGCGATTCATCCTCTAGCTC	273	4,686-4,713	60	Yokomi *et al*., 2018
	Anti-sense	AAGACAATCTCCCAGACGGTGACGA		4,935-4,959		

ples, which represents an average incidence of the virus of 4.24%, and indicates an increase from the previous survey conducted in 2019 (Ali-Arous *et al.,* 2019). New infection foci were detected in Oued fodda and Ouled fares localities in the Chlef Valley, where CTV was previously undetected.

These new results indicate that CTV is present in areas of the Chlef Valley, particularly where Sour orange is still the main rootstock, and that there are new incursions of the virus that were likely to be introduced by aphids. A previous study conducted in the Chlef Valley showed that the CTV T30 strain was efficiently transmitted by *A. gossypii*, and that the more abundant aphid in the region, *A. spiraecola* Patch was less efficient for transmitting this strain under experimental conditions (Ali-Arous, 2020).

Six of 74 CTV-positive samples identified with DAS-ELISA were selected and confirmed positive with a universal CTV RT-PCR assay, and were further assessed for strain composition. These CTV samples were either single-strain infections or were infected with two strains (Table 2). Detection of VT and T30 was consistent with previous findings (Ali-Arous *et al*., 2019). However, strains T3 and S1 were recorded for the first time in Algerian citrus orchards. Strains T68, T36, RB and HA16-5 were not detected in the six samples assessed.

Three CTV sources (samples Sdz1, Sdz4 and Sdz5) were positive for VT as single infections. Sample Sdz2 was positive for both VT and the previously undetected strain S1. Sample Sdz6 contained a mixed infection of CTV strains T30 and S1. Sample Sdz3 was positive for strains T30 and T3, which were also previously undetected in Algeria. None of the analyzed isolates tested positive for the CTV strains T68, T36, RB or HA16-5.

Detection of VT in some samples was associated with symptoms that could be ascribed to CTV, as was one sample containing T3. Sample Sdz1 was from a stunted 22-year-old Navel sweet orange tree (*Citrus sinensis* L. Osbeck) grafted onto *Citrus macrophylla* (Wester) rootstock, on a farm located in Abiodh Medjadja Province (Figure 3). Sample Sdz4 was from Oued Fodda Province, from a symptomless *Citrus macrophylla* seedling tree in a nursery. Sdz5, the third VT positive sample, was detected in a 5-year-old mandarin tree (*Citrus reticulata* Blanco) grafted on sour orange rootstock (*Citrus aurantium* L.), and this tree had severe leaf yellowing and decline. CTV isolate Sdz2 with co-infection by VT and S1 genotypes, had severe vein corking symptoms (Figure 3). Sdz6 was

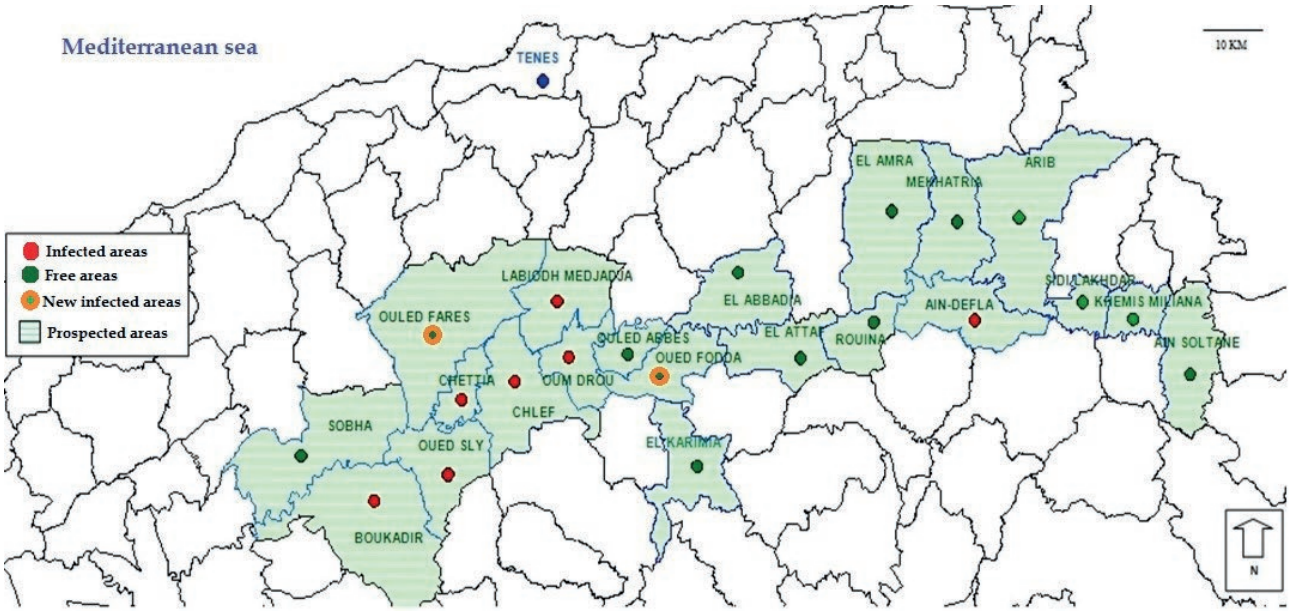

Figure 1. Distribution of CTV in Chlef Valley of Algeria, as determined in a large-scale survey carried out during 2021 to 2024.

Figure 2. The most dominant potential aphid species vectors observed in the surveyed Chlef Valley of Algeria during the spring of 2025. (A) curled orange Navel leaves infested by *Aphis spiraecola*. (B) colony of *A. gossypii* on the underside of a Navelina orange leaf.

a mixed infection of T30 and S1 genotypes, and was recovered from a symptomless clementine tree located in an orchard of the study area (Figure 3). Sdz3 had co-infection of T30 and T3, and was associated with severe dwarfing of a 22-year-old sweet orange tree on a macrophylla rootstock in the Oued Sly locality.

Strains of CTV that were previously unreported in Algeria were detected from the survey carried out in this study. These included strains S1 and T3 of the virus. Strain S1 was detected in California, as mixed infections with either T30 or VT.S1, and was shown to be a mild strain based on biological indexing (Sun *et al.*, 2024). Strain T3 was shown to induce seedling yellows and stunting (Yokomi *et al.,* 2011; Yokomi *et al.*, 2018). Strain S1 was therefore found for the first time in Chlef Valley, along with the pre-existing T30 mild strains PC-3-ALG and PL-6-ALG (respectively, NCBI accession numbers MK049163 and MK049163) (Ali-Arous

Table 2. Genotype assignment to six CTV isolates from the Chlef Valley based on M.M.Ms analyses.

Origin	Samples	T36	VT	T3	T30	T68	RB	S1	HA16-5
Chlef Valley	Sdz1	-	+	-	-	-	-	-	-
	Sdz2	-	+	-	-	-	-	+	-
	Sdz3	-	-	+	+	-	-	-	-
	Sdz4	-	+	-	-	-	-	-	-
	Sdz5	-	+	-	-	-	-	-	-
	Sdz6	-	-	-	+	-	-	+	-

et al., 2019). Strain T3 is the second potentially virulent strain detected in the study area, following VT (SY-1-ALG (MK049163), as reported by Ali-Arous *et al.* (2019). Strain T3 was identified in new citrus orchards since its first detection in 2019, highlighting the likely spread of this virulent strain through the Chlef Valley.

This second and extensive CTV survey in Chlef Valley has shown the first detection of strains S1 and T3 of the virus, and indicates spread of CTV to new locations, including those where strains VT and T3 were detected. For decades, moderate local CTV strains were successfully managed with CTV-tolerant rootstocks, including Troyer and Carrizo citrange, rather than sour orange. However, the exotic severe strains VT and T3 which can induce host plant stem pitting regardless of rootstock were subsequently detected. In addition, introduction of the RB strain of CTV remains a threat, which was recently reported in Morocco (Afechtal *et al*., 2018), and this will make disease management difficult.

This situation is likely due to failure of CTV eradication efforts of the plant protection authorities during recent years. For an effective management strategy, the local citrus industry should adopt adequate aphid control and appropriate horticultural practices, by using certified plant propagation material for tree production. Additionally, extension activities should encourage rigorous quarantine measures which should be applied to contain the dissemination of virulent CTV strains in Chlef Valley to other citrus production zones in Algeria.

ACKNOWLEDGEMENTS

This research was funded by the Algerian Ministry of Higher Education and Scientific Research (MESRS), and the Directorate-General of Scientific Research and Technological Development (DGRSDT).

LITERATURE CITED

Afechtal M., D'onghia A.M., Coccusa G.E.M., Djelouah K., 2018. First report of the Citrus tristeza virus resistance-breaking strain in Morocco. *Journal of Plant Pathology* 100: 351. https://doi.org/10.1007/s42161-018-0059-1

Albiach-Marti MR (2013) The complex genetics of citrus tristeza virus. In: *Current Issues In Molecular Virology - Viral Genetics and Biotechnological Applications* (Romanowski V, ed.), InTech pp. 1–26.

Ali-Arous S., 2020. *Rôle des Pucerons dans la Propagation du Virus de Tristeza (CTV) Responsable du Dépérissement Rapide des Agrumes dans le Moyen Chlef et Identification des Biotypes Vecteurs des Souches Virulentes*. PhD thesis: agricultural production and sus-

Figure 3. Three citrus trees that tested positive for CTV. A) Severe dwarfing associated with sample Sdz1 (infected with CTV strain VT). B) A young stunted and chlorotic Clementine tree, associated with sample Sdz2 (infected with strains VT and S1). C) Symptomless Clementine tree associated of sample Sdz6, infected with strains T30 and S1.

tainable agricultural development. Abdelhamid Benbadis University of Mostaganem (Algeria). 82 pp.

Ali Arous S., Guenaoui Y., Drais M.I., Djelouah K., 2019. First detection of a virulent strain of Citrus tristeza virus (Closteroviridae) in a citrus orchard of Chlef Valley (Algeria). *Bulletin OEPP/EPPO Bulletin* 49 (2): 321–326. https://doi.org/10.1111/epp.12563

Bar-Joseph M., Garnsey S.M., Gonsalves D., Moscovitz M., Purcifull D.E.., Clark M.F., Loebenstein G., 1979. The use of enzyme-linked immunosorbent assay for detection of Citrus tristeza virus. *Phytopathology* 69(2): 190–194. https://doi.org/10.1094/phyto-69-190

Blackman RL., Eastop VF., 2000. *Aphids on the World's Crops. An Identification and Information Guide*. 2nd edition. Vol. 1, John Wiley and Sons, Chichester, 466 pp.

Cambra M., Gorris M.T., Marroquın C., Roman M.P., Olmos A., Navarro L., 2000. Incidence and epidemiology of citrus tristeza virus in the Valencian Community of Spain. *Virus Research* 71(1-2): 85–95. doi: 10.1016/s0168-1702(00)00190-8

Cook G., van Vuuren S.P., Breytenbach J.H., Burger J.T., Maree H.J., 2016. Expanded strainspecific RT-PCR assay for differential detection of currently known Citrus tristeza virus strains: A useful screening tool. *Journal of Phytopathology* 164(10): 847–851. https://doi.org/10.1111/jph.12454

Folimonova S.Y., Sun Y.D., 2022. Citrus Tristeza Virus: From Pathogen to Panacea. *Annual Review of Virology* 9(1): 417–435. https://doi.org/10.146/annurev-virology-100520-114412

Gottwald T.R., Hughes G., 2000. A new survey method for Citrus tristeza virus disease assessment. In: *Proceedings 14th Conference of IOCV* (Brazil, 1998), IOCV, Riverside (US), 77–78.

Hilf M.E., Mavrodieva V.A., Garnsey S.M., 2005. Genetic marker analysis of a global collection of isolates of Citrus tristeza virus: characterization and distribution of CTV genotypes and association with symptoms. *Phytopathology* 95: 909–917. https://doi.org/10.1094/PHYTO-95-0909

Larbi D., Belkahla H., Djelouah K., D'Onghia A.M., 2015. Monitoring citrus tristeza virus (CTV) in Algeria and characterization of local isolates. *Acta Horticulturae* 1065: 767–771. https://doi.org/10.17660/ActaHortic.2015.1065.96

Licciardello G., Scuderi G., Russo M., Bazzano M., Paradiso G., Bar-Joseph M., Catara, A.F., 2025. Progress in Our Understanding of the Cross-Protection Mechanism of CTV-VT No-SY Isolates Against Homologous SY Isolates. *Pathogens* 14: 701. https://doi.org/10.3390/pathogens14070701

MADRP, 2023. Statistique Agricole, superficies et productions, SERIE B, 2021. Ministry of agriculture, rural development and fishing of Algeria, 77 pp.

Roy A., Ananthakrishnan G., Hartung J.S., Brlansky R.H., 2010. Development and application of a multiplex reverse-transcription polymerase chain reaction assay for screening a global collection of Citrus tristeza virus isolates. *Phytopathology* 100(10): 1077–1088. https://doi.org/10.1094/phyto-04-10-0102

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(1): 43–53. https://doi.org/10.1016/j.jviromet.2007.07.026

Sun Y., Yokomi R.K., Folimonova S.Y., 2024. Citrus tristeza virus: a century-long challenge for the world's citrus industries. *Annals of Applied Biology* 185 (3): 304–322. https://doi.org/10.1111/aab.12939

Yokomi R., Saponari M., Metheny P., Vidalakis G., 2011. Genetic Differentiation and Biology of Citrus tristeza virus Populations Spreading in California. *International Organization of Citrus Virologists Conference Proceedings* 1957–2010. 18. 10.5070/C58T2153M4.

Yokomi R.K., Selvara V., Maheshwari Y., Saponari M., Giampetruzzi A., Hajeri S., 2017. Identification and Characterization of Citrus tristeza virus Isolates Breaking Resistance in Trifoliate Orange in California. *Phytopathology* 107: 901–908. https://doi.org/10.1094/PHYTO-01-17-0007-R

Yokomi R., Selvaraj V., Maheshwari Y., Chiumenti M., Saponari M., Subhas Hajeri., 2018. Molecular and biological characterization of a novel mild strain of citrus tristeza virus in California. *Archives of Virology* 163: 1795–1804. https://doi.org/10.1007/s00705-018-3799-5.