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DIVERSITY OF CITRUS TRISTEZA VIRUS STRAINS IN CHLEF VALLEY (ALGERIA)

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ABSTRACT

Citrus crop in Chlef valley showed many cases of decline and other typical symptoms of tristeza disease in old and young trees grafted on various rootstock and seedlings acquired from multiple origins in the valley. In order to decipher the reasons of the this citrus quick decline syndrome, a large scale survey of citrus tristeza virus (CTV) and its aphid vectors was carried out from the spring of 2016 to the autumn of 2019 in order to evaluate its current situation in term of distribution and strains. Samples collected from 93 orchards located in 21 localities were tested by DTBIA/DAS-ELISA. The analyses have confirmed the presence of CTV in 54 samples through the study area. Some of the CTV sources were chosen for further molecular genotype characterization associated with the CTV isolates now spreading in the Chlef area. Characterization with multiple molecular markers M.M.M.s and CP gene sequencing showed the presence of the T30 and VT genotypes. This result allowed confirmation of the presence of a virulent strain belonging to the VT genotype. The other CTV isolates were close to those recorded in Mitidja region, with nucleotide identity of 98,6 to 99,1% with the worldwide T30 mild CTV isolates. This early finding of a strain belonging to the severe VT genotype beside to efficient aphid vectors is an issue for Algerian citrus producers and needs rapid actions to be taken by the National phytosanitary services, extending the surveillance to other citrus production regions and uprooting the infected trees.

Keywords: *Citrus, Virulent strain, CTV, Chlef valley, Algeria.*

INTRODUCTION

Citrus orchards in Algeria cover an area of approximately 71 000 ha and are located mainly along the coastal zones (MADRP, 2018). Sadly, citrus cultivation has been seriously damaged by outbreaks of Citrus tristeza virus (CTV) in the Mitidja region, the main citrus cultivation area in Algeria, reaching an infection rate of 17.6% (Larbi *et al.,* 2015), with no management actions were taken. CTV,

the causal agent of the most important viral diseases of citrus, belongs to the genus Closterovirus in the plant virus family Closteroviridae (Karasev *et al.*, 1995). It has a positive-sense, single-stranded RNA genome of about 19.3 kb and contains 12 open reading frames (Moreno *et al.*, 2008). CTV is transmissible by grafting and aphids and occurs as diverse strains that range from asymptomatic to severely virulent. CTV can also cause extreme stem pitting in sweet orange and grapefruit regardless of the rootstock (Yokomi *et al.*, 2018). The present study aimed to genotyping CTV isolates present in the Chlef valley.

MATERIALS AND METHODS

Following the outcome of a multidisciplinary investigation on a recurrent quick decline symptoms on citrus trees of different species and varieties associated with CTV infection in several citrus trees located in the Chlef Valley (Ali Arous *et al.*, 2017), the second most important area of citrus cultivation in Algeria, (MADRP, 2016) (Fig. 1), a first survey to assess the status of this epidemic disease was carried out. The survey was performed every year during the blossom period from March to May in 2016, 2017, 2018 and 2019 on approximately 2000 citrus trees belonging to more than 100 commercial orchards. The area sampled was representative of the main citrus growing areas of the Chlef Valley (Fig. 1).

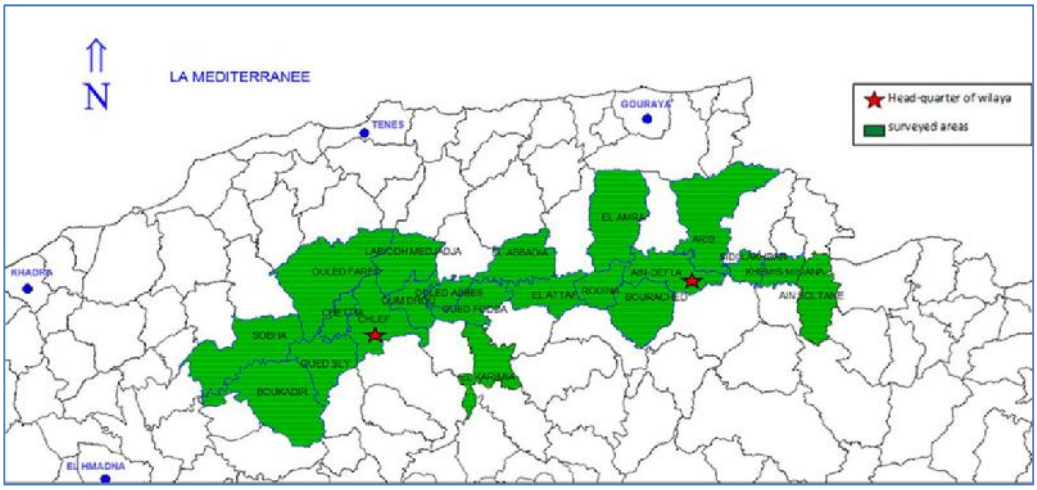

Figure 1. Geographical location of the study area (Chlef Valley).

The survey was performed in accordance with the hierarchical method of Gottwald and Hughes (2000) or randomly on the diagonals of the fields. The collected samples (new shoots, leaf petioles and pedicels of flowers) were printed (fresh section prepared) onto a nitrocellulose membrane and processed by direct tis- sue blot immunoassay (DTBIA) analysis (Garnsey *et al.*, 1993; Djelouah and D'Onghia, 2001), using PlantPrint Diagnostics SL© kit (IVIA, Valencia, ES) and the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

technique (Bar-Joseph *et al.*, 1989; Cambra *et al.*, 2000), using monoclonal antibodies (Citrus Tristeza Virus diagnostic SEDIAG®, France). Based on defined parameters (origin, symptoms, age, root- stock and location), five local sources of CTV (SY-1-ALG, NN-2-ALG, PC-3-ALG, WN-4-ALG and PL-6-ALG), were inoculated and maintained in the screenhouse for further molecular investigations. The purpose of this step was to determine the genotype associated with the CTV isolates in the Chlef area. Total RNAs were extracted from leaves using the RNeasy Plant MiniKit (Qiagen, DE). First-strand cDNA was synthesized from 2 lg of total RNA using M-MLV Rev- erse Transcriptase (Promega, Madison, WI, USA). PCR was carried out following the protocol described by Hilf & Garnsey (2000), using a GoTaq Hot Start Polymerase kit (Pro-mega) according to the manufacturer's protocol. The primer pairs used were targeting the universal coat protein gene (T36CP25) and four specific multiple molecular markers (MMMs), T30POL, T36POL, VTPOL and T3K17, associated, respectively, with the standard isolates T30, T36, VT and T3 (Hilf *et al*., 2005).

RESULTS AND DISCUSSION

Fifty-four trees were found to be infected with CTV, which corresponds to an infection rate of 3.21%. Overall, thirteen mandarin trees were contaminated by Tristeza virus, the infection rate (3.86%) was highest than in sweet oranges (3.16%), the survey highlight that the occurrence of CTV was significant, about 24% of mandarin orchards and 14.5% of sweet orange groves were CTV infected (Fig 2). According to Kitajima *et al.* (1974) et Muller *et al*. (1974), *Citrus sinensis* (L), *Citrus reticulata* (Blanco) et *Citrus paradisi* (Macf) are the principal host of CTV.

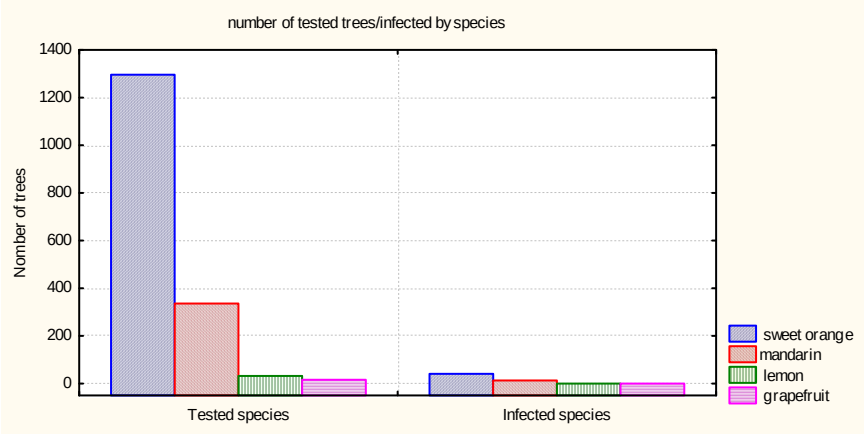

Figure 2. Infected trees by species

Most CTV infected trees were symptomless (Fig. 3, B), but some plants were showing clear symptoms of tristeza disease, particularly in a private orchard in the Abiodh Medjadja locality where severe forms of leaf chlorosis and yellowing were

recorded on four young trees of mandarin, *Citrus reticulata* (Blanco), grafted on *Citrus macrophylla* (Wester) produced by a nursery located in the Mitidja region (Fig. 3, A). It is important to highlight that the trees infected with CTV manifested severe vein corking symptoms before declining. Moreover, heavy stunting and chlorosis were observed on 12-year-old sweet orange trees, *Citrus sinensis* (L. Osbeck), grafted on *C. macrophylla* rootstock, at the Abiodh Medjadja and Oued Sly localities. The plant material in both orchards was imported from Spain in 2006.

Figure 3. A) yellowing and leaf crocking year old mandarin (declined months later); B) symptomless clementine tree of 60 years old; C) Quick decline of 20 years old Navel tree

Amplification was observed with the five selected isolates when the broad-spectrum T36CP primers were used, confirming the results obtained by DTBIA and DAS-ELISA. The characterization with MMMs showed the presence of the T30 and VT genotypes. Two isolates, WN-5-ALG from a 15-year-old sweet orange tree grafted on sour orange rootstock and PL-6-ALG from a 50-year-old sweet orange *C. sinensis* (L) grafted on a sour orange root-stock *Citrus aurantium* (L), reacted only with T30POL, indicating the presence of the mild T30 genotype. The VT genotype was retrieved from sample NN-2-ALG, from an imported sweet orange tree grafted on *C. macrophylla*(Wester) and sample SY-1-ALG collected from a young mandarin tree, *C. reticulata* (Blanco), grafted on *C. macrophylla* and produced by a local nursery. Both reacted only with the VTPOL primer, which is a specific marker of the VT strain. PC-3-ALG was designated a mixed infection of T30 and VT genotypes, since this isolate yielded both VTPOL and T30POL markers.

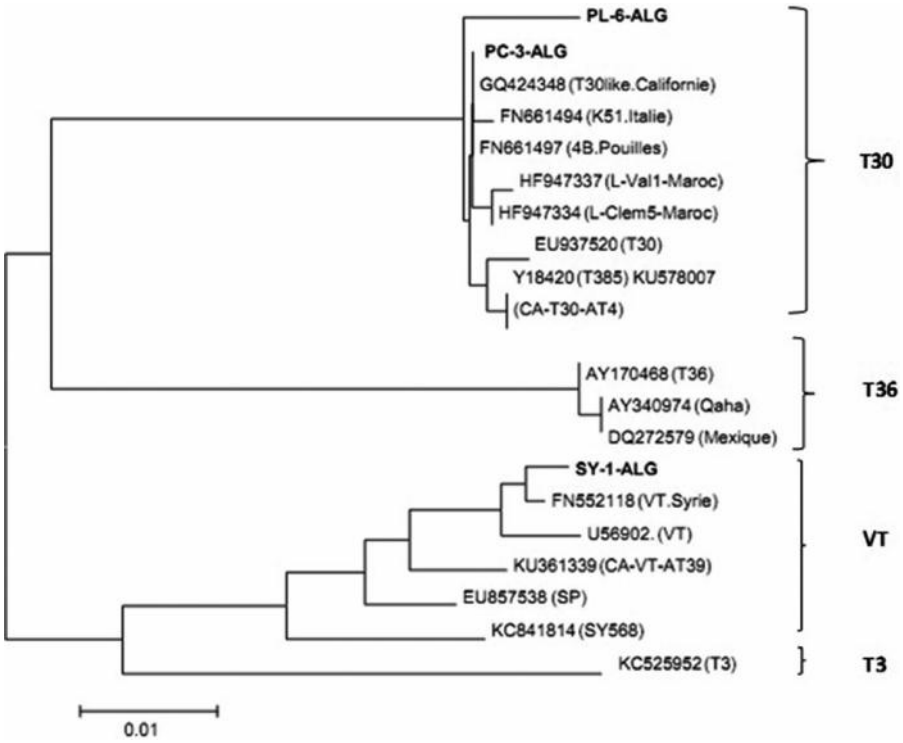

Figure 4. Phylogenetic tree generated by the neighbour-joining method from the alignment of the nucleotide sequences of the CPg25 marker of the selected local isolates PL-6- ALG, PC-3-ALG and SY-1-ALG with other strain references available in the NCBI database. Evolutionary analysis was conducted in Mega 10.

Following the outcome of the MMMs analysis, the PCR products of three sources (SY-1-ALG, PC-3-ALG and PL-6-ALG) were cloned into a pUC18 vector plasmid (Agilent Technologies), and then four clones of each amplicon were sequenced. One of each clone was deposited in NCBI Gen- Bank with accession numbers MK049162–MK049164. Based on the CP gene sequence, CTV isolates PL-6-ALG and PC-3-ALG shared 99.1% nucleotide identity with the moderate T30 strains; however, SY-1-ALG was clustered with the group of virulent VT strains (Fig.4). This work allowed confirmation of the presence of virulent strain belonging to the VT genotype represented by the local isolate SY-1-ALG isolated in a private orchard located in the study area. The other CTV isolates were, as expected, similar to those isolated from the Mitidja region, which show 99% nucleotide identity with the Spanish mild CTV isolate T385 (Larbi *et al.*, 2015).

CONCLUSIONS

The study allowed categorizing local CTV isolates in two principal groups, the Mediterranean T30 mild CTV group and the exotic severe VT group. In addition to the Spanish mild CTV isolate T385 like isolate characterized few years ago in

Mitidja valley. This is the first report of a VT-like CTV isolate infecting citrus in Algeria. Virulent strains induce rapid decline, stunting and/or seedling yellowing and extreme stem pitting in sweet orange and grapefruit regardless of rootstock. This early finding is of utmost importance as it allows infected trees to be eradicated before natural spread to adjacent fields by aphid vectors can occur; for this purpose, rapid action needs to be taken by the national phytosanitary services, extending the investigations to other regions and eradicating the infected trees.

REFERENCES

Ali Arous S., Guenaoui Y., Djelouah K. (2017). Current status of citrus tristeza virus (CTV) and its vectors in Chlef valley (Algeria). Book of proceeding of the VIII International Scientific Agriculture Symposium, Jahorina, Bosnia and Herzegovina, pp. 1064-1069.

Bar-Joseph M., Marcus R., Lee R.F. (1989). The continuous challenge of Citrus tristeza virus control. Annual Review of Phytopathology, 27, 292–316.

Cambra M., Gorris M..T., Marroquin C., Roman MP., Olmos A., Martınez M.C., Hermoso de Mandoza A., Lopez A., Navarro L. (2000). Incidence and epidemiology of citrus tristeza virus in the Valencian Community of Spain. Virus Research, 71, 75–85.

Djelouah K., D'Onghia A.M. (2001). Detection of Citrus Psorosis virus (CPsV) and Citrus tristeza virus (CTV) by direct tissue blot immunoassay. In: Proceeding for the Mediterranean Network on Certification of Citrus, Options Mediterraeennes B 33, CIHEAM publications, Bari, pp. 108–113.

Garnsey S.M., Permar T.A., Cambra M., Henderson C.T. (1993). Direct tissue blot immunoassay (DTBIA) for detection of Citrus tristeza virus (CTV). In: Proc. 12th Conf. Int. Org. Citrus Virol., IOCV, Riverside (US). pp. 39–50.

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

Hilf M.E., Garnsey S.M. (2000). Characterization and classification of Citrus tristeza virus isolates by amplification of multiple molecular markers. In: 14th Conf. Int. Organ., Riverside, CA. Citrus Virol, pp.18–27.

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.

Karasev A.V., Boyko V.P., Gowda S., Nikolaeva O.V., Hilf M.E., Koonin E.V., Niblett C..L., Cline K., Gumpf DG., Lee R.F. (1995). Complete sequence of *Citrus Tristeza virus* RNA genome. Virology, 208, 511–520.

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.

MADRP (2016). Agricultural Statistics, Productions and surfaces, Serial B, 2016. Ministry of agriculture, rural development and fishing of Algeria. 77p.

Moreno P., Ambros S., Albiach-Marti M., Guerri J., Pena L. (2008). Citrus tristeza virus: a pathogen that changed the course of the citrus industry. Molecular Plant Pathology, 9, 251–268.

Yokomi R., Selvaraj V., Maheshwari Y., Chiumenti M., Saponari M., Giampetruzzi A., Weng Z., Xiong Z., Hajeri S. (2018). Molecular and biological characterization of a novel mild strain of citrus tristeza virus in California. Archives of Virology 163, 1795–1804.