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# MOLECULAR CHARACTERIZATION OF CORSICAN ISOLATES OF CITRUS TRISTEZA VIRUS



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## INTRODUCTION

Tristeza disease, caused by the *Citrus tristeza virus* (CTV), is the most important viral disease for almost all citrus species. Syndromes and symptoms expression depend on the interactions of factors involved in the pathosystem, host-strain-rootstock-climate-vector. Several strains of CTV have been studied all over the world. Two Corsican isolates have been previously characterized, K strain from Marumi kumquat, known to be symptomless on Mexican lime, and Cal-1 from calamondin inducing stem pitting. No sequence data are published or available in GenBank (1).



## FIELD OBSERVATIONS

Two new strains of CTV were recently found in Corsica during routine survey in citrus orchards. The isolates, named as LA5 and CO3, were detected in two different orchards of 40 year old Clementine on sour orange trees that did not show evident decay. A mild decline, observed in few trees of both field, was related by the growers to *Phytophthora* spp. infections, a common pathogen in the area. No other symptoms ascribable to tristeza, such as honeycombing or stem-pitting, were registered in the trees.

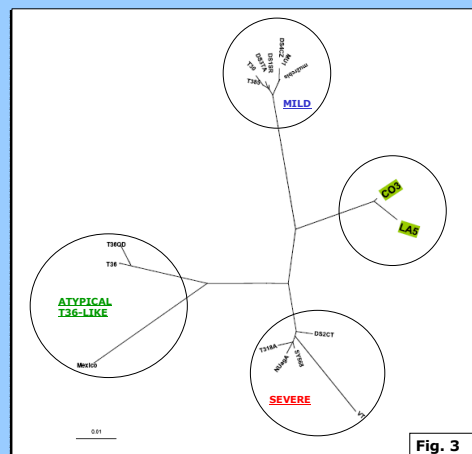
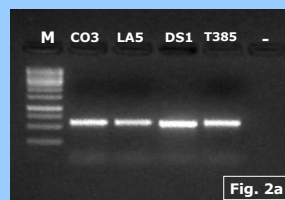
## MOLECULAR CHARACTERIZATION

After the preliminary detection by DAS-ELISA, the two isolates CO3 and LA5 were identified and characterized by molecular tools. Total RNA were extracted by using TRIZOL<sup>®</sup> (Invitrogen) method from 100 mg of leaf tissue. Two µl of suspension were used for RT-PCR with P20 gene specific primers (CP gene). The Spanish T385 and Italian DS1 isolates were used in the reaction as positive control (2). PCR fragments (540 bp) were characterized by SSCP analysis, cloned in an apCR2.1-TOPO vector Kit (TOPO-TA Cloning, Invitrogen) and sequenced in both directions. The sequences obtained were aligned with those of related isolates using the CLUSTAL W program and then analyzed for phylogenetic relationship using the MEGA based neighbour-joining method with a 1,000 replicate bootstrap value.

## RESULTS AND DISCUSSION

CTV presence was confirmed both in CO3 and LA5 samples by RT-PCR. The obtained amplicons showed expected size of 540 nt. (Fig. 2a) and evidenced two different SSCP profile (Fig. 2b). Sequences analysis pointed out high similarity between CO3 and LA5 (98%) and evidenced their high similarity with the isolate NZRB-G90 from New Zeland (GenBank Acc. N. FJ525432), 98% and 97% respectively.

Compared to previously described groups of CTV isolates (mild, severe, atypical), phylogenetic analysis has defined a new cluster including both LA5 and CO3 (Fig. 3). Given the absence in the literature and in GenBank of sequence data of the K strain is now impossible to correlate the new Corsican isolates with the asymptomatic one. This comparison is however necessary in order to hypothesize phylogenetic relationships or patterns of disease spread in the field.



### References

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