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Geographical and genetic diversity of '*Candidatus Phytoplasma solani*' isolates in Croatian grape growing regions

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INTRODUCTION

'*Candidatus Phytoplasma solani*' (CPs), a phytoplasma endemic to the Euro-Mediterranean basin is associated with several plant diseases, including the grapevine yellows disease „bois noir“ (BN) (Quaglino *et al.* 2013). CPs spreads through highly complex disease cycle comprising several, possibly intermixed epidemiological networks which include diverse insect vectors and a broad range of host plant species. *Hyalesthes obsoletus* is considered to be the principal vector of CPs associated with BN. It feeds on various herbaceous plants but is most commonly associated with *Convolvulus arvensis* and *Urtica dioica* as hosts for its nymphs and adults (Langer and Maixner, 2004). A vector role in BN transmission has been proven also for *Reptalus panzeri* (Cvrković *et al.* 2014) and *Dictyophara europea* (Cvrković *et al.* 2022). The epidemiology of CPs is highly correlated with distribution of the host plants - tentative inoculum source in vineyards from which the insect vectors acquire phytoplasma. As different CPs strains have been shown to have different ecological reservoirs and pathways for spread, the genetic characterization of CPs strains is essential. The variability, prevalence, and distribution of the CPs genotypes from various hosts involved in the BN pathosystems was assessed by MLST approach to better document the BN situation in the Croatian vineyards.

MATERIALS AND METHODS

In the frame of a national survey programme, from 2009 to 2020, overall number of 3336 samples of grapevine, 89 weed and other plant samples, along with 267 of known and presumed CPs vectors from the suborder Auchenorrhyncha were collected from all Croatian grape-growing regions and tested for the presence of CPs. Total nucleic acids were extracted according to the previously described CTAB extraction protocol from grapevine or other plants (Plavec *et al.* 2019) or with the commercial kit OmniPrep™ (G-Biosciences, St. Louis, MO, USA) from individual insects. Samples were then analysed by triplex real-time PCR assay according to Pelletier *et al.* (2009). From most CPs foci in the country representative isolates were chosen and further analysed by MLST including *tuf*, *secY*, *vmpI* and *stamp* genes (Schneider *et al.* 1997; Fialova *et al.* 2009; Cimerman *et al.* 2009; Fabre *et al.* 2011). Amplicons were sequenced in both directions followed by editing and assembly with Geneious (<http://www.geneious.com/>) and alignment with ClustalX 2.0. Phylogenetic analyses were conducted by MEGA 7 and nucleotide sequences attributed to sequence type by their comparison with sequences deposited in GenBank following the previously designed nomenclature (Foissac *et al.* 2013).

RESULTS AND DISCUSSION

The presence of CPs was confirmed in grapevine, *Ailanthus altissima*, *Robinia pseudoacacia* and *Polygonum aviculare* as well as in insects *H. obsoletus*, *D. europaea* and *Cixius wagneri*. The MLST analyses performed on 78 plant samples and 20 insect samples revealed two new genotypes for *stamp* and *vmpI* genes, designated as ST59 and V28, respectively. *Stamp*, with its presumed important role in the interactions with insect vector was found to be a highly discriminative marker with 13 genotypes detected. Overall, analysis identified 28 different CPs MLST genotypes. The prevalent MLST genotype in grapevine CPsSqt21 (S6/ST6/V18/tuf-b2) found across central and north-western Croatia

was affiliated to *H. obsoletus* and *U. dioica*. The other two most frequent genotypes were the *U. dioica*-associated CPsSqt28 (S39/ST46/V3/tuf-a) and the *C. arvensis*-associated CPsSqt2 (S1/ST9/V4/tuf-b1). Especially interesting was CPsSqt11 (S4/ST4/V28/tuf-b1) shared by two hosts, *A. altissima* and *C. wagneri*, which encompasses a new *vmp1* genotype (V28). Moreover, three positive *C. wagneri* specimens found in different regions shared the same *vmp1* genotype. Nevertheless, our results with prevalent MLST genotypes are in accordance with regional distribution of CPs strains in neighbouring countries (Mehle *et al.* 2022). Although the *H. obsoletus* is a principal vector of CPs in Croatia, our survey revealed that its population did not correlate with the distribution and high number of CPs positive plants. Even though this finding could be attributed to the limited insect sample number or locally determined interactions of hosts and vectors that favour adaptive mutations, the substantial number of MLST genotypes found indicates the co-existence of several independent epidemiological cycles. This is certainly a consequence of a unique geographical position of Croatia, bridging the different eco-climatic areas of central and south-eastern Europe.

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