Variability of ‘Candidatus Phytoplasma solani’ Affecting Grapevine in Iran

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Abstract—“Bois noir” (BN) is one of the most widespread diseases of grapevine in European and Mediterranean countries. In recent years, incidence of BN throughout Iranian vineyards has increased, with high losses in production recorded in some part of Iran. The genetic diversity of ‘Candidatus Phytoplasma solani’ strains was assessed by molecular typing analysis in Vitis vinifera in important viticultural areas of Iran from 2015 to 2017. Red and white berry cultivars were observed during the surveys, and leaf samples were collected from grapevines showing reddening or yellowing of the leaves, leaf rolling, flower drying, shrivelled bunches and small black pustules along the canes. Using 16S rDNA amplification followed by sequencing, ‘Ca. P. solani’ was detected in most of the symptomatic plants. Molecular typing analysis was carried out on the tuf and vmp1 genes. Most of the ‘Ca. P. solani’ strains resulted tuf-type b, which is known to be associated with bindweed (Convolvulus arvensis). More detailed characterisation was carried out by RFLP analysis of vmp1 gene, which distinguished five genetic variants.

Key words—grapevine, phytoplasma, “stolbur”, tuf, Vitis vinifera, vmp1 gene

I. INTRODUCTION

“Bois noir” (BN) is a widespread grapevine yellows disease all over Europe, for the Mediterranean area and also in the Middle East (Maixner 2011; Foissac et al. 2013). The associated agent is ‘Candidatus Phytoplasma solani’ (Quaglino et al. 2013), which can infect a wide range of vegetable crops, causing severe economic losses. Grapevine is one of the most important crops, but in the life cycle of ‘Candidatus Phytoplasma solani’ it is a dead-end host, as it becomes infected during erroneous feeding of the polyphagous cixiidae Hyalesthes obsoletus. The natural life-cycle of ‘Ca. P. solani’ infected grapevines might be associated with different reservoir plants and vector populations (Langer and Maixner 2004). To clarify the epidemiology of the disease, the characterisation of non-ribosomal genes such as tuf, vmp1 and stamp is usually studied (Langer and Maixner 2004; Pacifico et al. 2009; Fabre et al. 2011; Murolo and Romanazzi 2015).

The aim of this study was to examine the variability among selected “stolbur” phytoplasma strains infecting Iranian grapevine using multilocus sequence typing (MLST).

II. MATERIALS AND METHODS

Sampling was carried out in July-September 2015 to 2017 in the main grapevine-growing regions of Iran. Total DNA was extracted using the phytoplasma enrichment procedure of Angelini et al. (2001), from midrib and petioles of symptomatic plants. The samples were amplified using the universal primers P1/P7 followed by R16F2n/R2, and the fragment of about 1,200 bp was sequenced in both directions.

The tuf gene characterisation was carried out on samples identified as ‘Ca. P. solani’ by sequence analysis. The tuf gene fragments of approximately 940 bp were amplified by nested PCR using the ftufAY/rtufAY primer pair followed by ftufAY/rtufAY primers (Schneider et al. 1997). Amplicons were digested with HpaII (Langer and Maixner 2004) and analysed by electrophoresis in 2.5% agarose gels.

Finally, the vmp1 gene fragments were amplified using nested PCR with the StolH10F1/R1 primers, followed by the TYPH10F/R primer pair, as described by Fialova et al. (2009). Fragments of approximately 1.1 to 1.5 kbp were digested with Rsal and separated by electrophoresis in 2.5% agarose gels. Representative samples were selected for sequence analysis of amplicons generated by the primer pair TYPH10F/TYPH10R at GENEWIZ Genomics sequencing service (Genewiz UK, Takeley, UK; https://www.genewiz.com/). Nucleotide sequences were aligned using Clustal X (ver. 1.83) (Thompson et al. 1997). Mega 5 (http://www.megasoftware.net/index.html) (Tamura et al. 2013) was used to calculate the phylogenetic relationships.

III. RESULTS

The symptoms on the leaves started to appear from the end of July and become more evident as the vegetative season advanced. The plants showed crispy, brittle, downwards rolling leaves, reddening (orange to purple) in red varieties, and yellowing (golden to chlorotic) in white varieties (Fig. 1). Discoloration always also involved the main veins, sectorial discoloration of the blades occurred in some varieties, and flower abortion, berry withering and shrivelling bunches were also seen. Most of the sequences obtained from the symptomatic samples analysed using
the universal primers followed by sequence analysis showed high nucleotide homology with ‘Ca. P. solani’.

From the molecular characterisation of the *tuf* gene, amplification was successfully obtained from all of the samples. Restriction analysis with the *HpaII* enzyme indicated that all the samples belonged to *tuf*-type b, according to Langer and Maixner (2004). In the *vmp1* gene typing, restriction analysis with *RsaI* of the fragments obtained in all the samples showed the presence of five profiles. For the sequence analysis of the *vmp1* fragment, there was good correlation between the RFLP molecular typing and the clusters in the phylogenetic tree.

**IV. DISCUSSION**

“Bois noir” is one of the most widespread diseases of grapevines. In recent years, there has been increased incidence of BN throughout Iranian vineyards, with high losses of production in some part of the country (Salehi et al. 2014; Mirchenari et al. 2015; Zamharir et al. 2017). In this study, the molecular characterisation of ‘Ca. P. solani’ in grapevine according the *tuf* and *vmp1* genes was carried out. From the *tuf* gene characterisation, *tuf*-type b was detected in the grapevines analysed, and in *C. arvensis* in the vineyards. This finding clearly indicates that the abundant presence of *C. arvensis* has a major role in BN epidemiology in the Iranian vineyards surveyed.

To gain insight into the genetic diversity among these ‘Ca. P. solani’ strains in Iran, more detailed characterisation was carried out on the *vmp1* gene, which codes membrane proteins putatively involved in the recognition and interaction of ‘Ca. P. solani’ with its hosts (Cimerman et al. 2009; Fabre et al. 2011). Based on *RsaI*-RFLP digestions of the *vmp1* gene, the presence of high variability, with five *vmp1*-types in these grapevine samples was demonstrated. Nucleotide sequence analysis was performed on *vmp1*, with the V10 profiles prevalent among the ‘Ca. P. solani’ strains analysed. The discovery of the role of *C. arvensis* as a source plant opens a new research area for the epidemiology of BN in vineyard ecosystems in Iran. Development of BN is directly related to the preferred or alternative host plants for the vector and the pathogen. Therefore, control strategies should be developed to manage these host plants, to reduce the source of the phytoplasma and also the abundance of the insect vector. Additionally, future studies on the life cycle of the insects associated with *C. arvensis* should provide valuable information that can be incorporated into control measures.

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