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Epidemiology

Looking for wild plant reservoirs and potential insect vectors for '*Candidatus* Phytoplasma solani' in "bois noir"-affected vineyards in Bekaa valley-Lebanon

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Abstract

"Bois noir" is a frequent grapevine yellows in the vineyards of Bekaa valley in Lebanon and is associated with '*Candidatus* Phytoplasma solani'. Genotyping through sequencing of the *tuf* gene and the variable gene *stamp* mainly revealed the presence of '*Ca.* P. solani' strains of genotype tuf-b1/ST14. The high incidence of the disease in two vineyard plots cultivar Chardonnay and the aggregation of "bois noir" cases suggested the establishment in the vineyard itself of reservoir plants and insect vectors. Survey of wild plant and potential planthopper vectors led to the detection the same genotype of '*Ca.* P. solani' in the field bindweed *Convolvulus arvensis* in the two vineyards as well as in several sites in the Bekaa valley and in *Hyalesthes obsoletus* populations collected on this plant species. These data suggest the local propagation of '*Ca.* P. solani' through a classical epidemiological cycle involving bindweed reservoir hosts and *H. obsoletus* planthopper vectors. Surprisingly, a '*Ca.* P. omanense' -related strain was also detected in an old plantation of Syrah as well as in *C. arvensis* and two cixiid planthopper species.

Keywords: grapevine yellows, insect vector, *Hyalesthes obsoletus*, *Convolvulus arvensis*

Introduction

'*Candidatus* Phytoplasma solani', known also as "stolbur" phytoplasma, is a phloem-limited wall-less bacterium that belongs to the phytoplasma group 16SrXII-A. All over Europe, '*Ca.* P. solani' infects a wide range of annual and perennial crops (EFSA, 2014), but it is also occurring in the eastern part of the Mediterranean basin where it associated with the "bois noir" (BN) disease of grapevine (Daire *et al*., 1997; Davis *et al*., 1997; Orenstein *et al*., 2001; Choueiri *et al*., 2002; Ertunc *et al*., 2015). In Europe, the main '*Ca.* P. solani' insect vectors are planthoppers of the family Cixiidae that transmit the phytoplasma from wild plants acting as reservoir, to crops. *Hyalesthes obsoletus* is considered the most important vector of "stolbur" phytoplasmas (Fos *et al*., 1992; Maixner, 1994), while *Reptalus panzeri* acts as BN vector in Balkans (Cvrkovic *et al*., 2014). Deciphering epidemic cycles is essential for controlling BN as it rely on the removal of wild plant reservoirs for '*Ca.* P. solani'. In the Near East, '*Ca.* P. solani' reservoir plants are not yet identified although *H. obsoletus* is suspected to transmit it to grapevine (Sharon *et al*., 2015). Surveys for phytoplasmas have been conducted in Lebanon in grapevine, wild plants present in the vineyards as well as in planthoppers collected on bindweeds. '*Ca.* P. solani' strains detected in grapevines have been genetically characterized and compared to those detected in infected weeds and potential insect vectors.

Materials and Methods

Plant collection and total nucleic acid extraction

Grapevine samples displaying leaf discoloration and rolling, shriveling of grapes and incomplete lignification of canes, were collected in Bekaa valley from 2008 to 2015. Symptomless grapevine plant materials were also sampled as negative control. Yellowing and stunted weeds including *Convolvulus arvensis* (bindweed), *Lactuca serriola*, *Carthamus* sp. were collected in 2014 and 2015. Total nucleic acids were extracted from 1 g of fresh leaf vein using the CTAB method described by Maixner *et al*. (1995).

Insect collection, identification and total nucleic acids extraction

Planthoppers were captured on *C. arvensis* using sweep nets in June 2014 in Ammiq and Kanafar, Bekaa valley. They were identified according to taxonomy handbooks for planthoppers (Holzinger *et al*., 2003), and specimen of *H. obsoletus* and *Reptalus sp.* were finally kept in 70% ethanol at –20°C before nucleic acids extraction using the same CTAB method used for plant samples.

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Phytoplasma detection and genotyping

Total nucleic acid extracts were tested by nested PCR using the 16Sr phytoplasma-specific primers R16mF2/R16mR1 followed by R16F2n/R16R2 (Gundersen and Lee, 1996). *Tuf* and *stamp* genes were amplified as previously reported (Balakishiyeva *et al*., 2018; Fabre *et al*., 2011). PCR products were analysed by electrophoresis in 1% agarose gel, stained with ethidium bromide and visualized under UV light. All PCR products were directly sequenced on both strands by Beckman Coulter Genomics Company (Takeley, UK) on MegaBACE capillary sequencing instruments. Sequences were assembled, quality edited and consensus sequences were used for phylogenetic analyses as previously described (Balakishiyeva *et al*., 2018).

Results and Discussion

Almost all phytoplasmas detected in BN grapevine samples were '*Ca*. P. solani'-related strains genotypes tuf-b1 and stamp ST14 corresponding to the Lebanese strain P7 maintained in periwinkle. A '*Ca*. P. omanense'-related strain (Al-Saadi *et al*., 2008) was detected in one old plot of Syrah (Foissac *et al*., 2019). Survey from 2008 to 2014 of two plots of Chardonnay indicated that BN symptomatic grapevine plants were aggregating and progressing in some parts of the plots where *C. arvensis* bindweeds were found abundant. Many of them were stunted with yellowish and purplish small leaves. Symptomatic *C. arvensis* were in majority positive for '*Ca*. P. solani' and carrying the same tuf-b1-ST14 genotype. A few of them were positive for a '*Ca*. P. omanense'-related strain. Both phytoplasmas were also detected in *H. obsoletus* collected on *C. arvensis* and '*Ca*. P. omanense' was also detected in a *Reptalus* sp. collected on bindweed. Diseased *L. serriola* were positive for group 16SrIX phytoplasmas as reported in Lebanon (Verdin *et al*., 2003) and diseased *Carthamus* sp. were positive for a 4 SNP variant of the '*Ca*. P. trifolii'–related strain previously reported in tomato and pepper in Lebanon (Choueiri *et al*., 2007). These results suggest that the wild plant reservoir for '*Ca*. P. solani' in Lebanon is the bindweed as reported in Western Europe (Langer and Maixner, 2004). Removal of diseased '*Ca*. P. solani' infected *C. arvensis* in the vineyards and their vicinity should help to reduce the BN incidence in the Bekaa valley.

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