**New insights on Grapevine yellows disease in North-Eastern Italy**

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**INTRODUCTION**

Grapevine yellows (GYs) is one of the most damaging phytoplasma-associated diseases that causes severe yield losses in every geographic area where grapevines are cultivated. The main yellows diseases in grapevine in Europe are “flavescence dorée” (FD, 16SrV-C/D ribosomal subgroups) (Martini et al. 1999) and “bois noir” (BN, '*Candidatus* Phytoplasma solani', 16SrXII-A ribosomal subgroup), transmitted by *Scaphoideus titanus* Ball and *Hyalestes obsoletus* Signoret, respectively. Recently the mosaic leafhopper *Orientus ishidae* (Matsumura) (Cicadellidae; Deltocephalinae) was found to be positive to 16SrV-C and -D phytoplasmas in Slovenia, Italy and Switzerland (Mehle et al., 2010; Gaffuri et al., 2011; Trivellone et al,. 2015); it was also shown as capable to transmit 16SrV phytoplasmas from broadbean to grapevine (Lessio et al., 2016). To verify the reasons of the continuous GY spreading in the Veneto region (North-Eastern Italy), “Prosecco areas”, the identification and molecular characterization of phytoplasmas in symptomatic and asymptomatic grapevine and insects captured in selected vineyards during a three year-survey was carried out.

**MATERIALS AND METHODS**

Total nucleic acids were extracted from 1 g of fresh plant tissue (leaf midribs) from 137 symptomatic and 24 asymptomatic grapevines belonging to four grapevine cultivars (Chardonnay, Glera, Pinot Gris and Perera) collected in 17 different vineyard, using a phenol/chloroform protocol. Following a CTAB- based DNA extraction procedure 29 batches of *S. titanus* (50 individuals), 26 of *H. obsoletus* (32 individuals), 69 of *O. ishidae* (89 individuals) and 2 of *H. hamatus* (4 individuals) were processed for molecular analyses to verify phytoplasma presence. Phytoplasma detection was carried out by nested-PCR using P1/P7 (Deng and Hiruki, 1991; Schneider et al. 1995) followed by 16R758f/23SR1804 (Gibb et al., 1995; Padovan et al., 1995) and/or U5/U3 (Lorenz et al., 1995) primer pairs. Additional characterization was performed on *rp* gene with group specific primers (Lee et al., 2004; Martini et al., 2007). Direct sequencing of selected *16Sr* and *rp* gene amplicons was performed and assembled sequences were deposited in GenBank.

**Table 1**: Phytoplasmas detected in plants and insect samples during surveys in Treviso province vineyards. Insect numbers are referred to batches of 1 to 2 individuals.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Samples | Samples positive/collected | 16Sr group/subgroup | | | | | | | | | | | | | |
| V-C | V-D | XII-A | VII-A | VI | X-B | I-B | V-C + V-D | V-C + XII-A | | | V-C + VII-A | | V-A |
|
| *Grapevines* | 103/161 | 49 | 14 | 11 | 9 | 4 | 5 | 6 | 1 | | 1 | 2 | | 1 | |
| *S. titanus* | 14/29 | 2 |  | 4 | 3 | 1 | 2 | 2 |  | |  |  | |  | |
| *O. ishidae* | 22/69 | 4 |  | 7 | 5 | 1 |  | 3 |  | | 2 |  | |  | |
| *H. obsoletus* | 10/27 |  |  | 6 |  |  |  | 4 |  | |  |  | |  | |
| *H. hamatus* | 0/2 |  |  |  |  |  |  |  |  | |  |  | |  | |

**Results and discussion**

The three years monitoring highlighted a significant percentage of phytoplasma positive plants in both, symptomatic (about 75%) and asymptomatic (about 40%) grapevine plants, with a prevalence of FD strains. During 2015 the presence of phytoplasma strains belonging to 16SrVI (4 samples) and 16SrVII ribosomal group (9 samples), both in single and in mixed infection was also detected in the 18% of the tested samples, mainly in asymptomatic plants. Moreover, phytoplasmas belonging to ribosomal groups 16SrI-B, 16SrV-A and 16SrX-B were occasionally detected in 12 samples (Table 1). Identification of phytoplasmas from insects showed the presence of 16SrXII-A, 16SrVII and 16SrVI in specimens of *S. titanus* and *O. ishidae*, while 16SrXII-A and 16SrI-B phytoplasma strains were identified in *O. ishidae* and *H. obsoletus*, and 16SrX-B in *S. titanus*. (Table 1). The results of this study confirm that GYs diseases in one of the most important viticultural areas in Italy are associated with the presence of different phytoplasmas and diverse insects vectors. The number of *O. ishidae* captured in the selected vineyards is significantly higher than previously reported in North-West Italy and Switzerland, where the insect was quite uncommon and was collected under low density situations (Casati et al., 2017). Moreover the three insect species positive to phytoplasmas were carrying indeed different ribosomal groups reported as associated to GY diseases in Chile and Iran respectively (16SrVII; Gajardo et al., 2009; Zamharir et al., 2017) and occasionally in Syria (16SrVI; Contaldo et al., 2011). The 16SrVII-A and 16SrVI phytoplasmas were never detected before in Europe in grapevine, *S. titanus* and *O. ishidae* and their epidemiologic relevance is under further monitoring.

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