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

Yuri Zambon, Diego Marchetti, Alessandro Canel, Assunta Bertaccini and Nicoletta Contaldo\*

Alma Mater Studiorum *- University of Bologna, Viale G. Fanin, 42, 40127 Bologna, Italy.*

\*Corresponding author: nicoletta.contaldo2@unibo.it

**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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**REFERENCES**

Casati, P., Jermini, M., Quaglino, F., Corbani, G., Schaerer, S., Passera, A., Bianco, P. A. 2017. New insights on “flavescence dorèe” phytoplasma ecology in the vineyard agro-ecosystem in southern Switzerland. Annals of Applied Biology, 171(1): 37-51.

Contaldo, N., Soufi, Z., Bertaccini, A. 2011. Preliminary identification of phytoplasmas associated with grapevine yellows in Syria. Bull. Insectol. 64(suppl.):S217-S218.

Deng, S.J., Hiruki, C. 1991. Amplification of 16S ribosomal-RNA genes from culturable and nonculturablemollicutes. Journal of Microbiological Methods, 14: 53–61.

Gaffuri, F., Sacchi, S., Cavagna, B. 2011.First detection of the mosaic leafhopper, *Orientus ishidae*, in northern Italy infected by the “flavescence dorée” phytoplasma. New Disease Reporter, 24: 22.

Gajardo, A., Fiore, N., Prodan, S., Paltrinieri, S., Botti, S., Pino, A.M., Zamorano, A., Montealegre, J., Bertaccini, A. 2009. Phytoplasmas associated with grapevine yellows disease in Chile. Plant Disease, 93(8): 789-796.

Gibb, K.S., Padovan, A.C., Mogen, B.D. 1995. Studies on sweet potato little-leaf phytoplasma detected in sweet potato and other plant species growing in Northern Australia. Phytopathology, 85: 169-174.

Lee, I-M., Martini, M., Marcone, C., Zhu, S.F. 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of ‘*Candidatus* Phytoplasma ulmi’ for the phytoplasma associated with elm yellows. International Journal of Systematic and Evolutionary Microbiology, 54: 337–347.

Lessio, F., Picciau, L., Gonella, E., Mandrioli, M., Tota, F., Alma, A. 2016. The mosaic leafhopper *Orientus ishidae*:host plants, spatial distribution, infectivity, and transmission of 16SrV phytoplasma to vines. Bulletin of Insectology, 69(2): 277-289.

Lorenz, K.H., Schneider, B., Ahrens, U., Seemüller, E. 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. Phytopathology, 85: 771-776.

Martini, M., Murari, E., Mori, N., Bertaccini, A. 1999. Identification and epidemic distribution of two “flavescencedorée”-related phytoplasmas in Veneto (Italy). Plant Disease, 83: 925-930.

Martini, M., Lee, I-M., Bottner, K.D., Zhao, Y., Botti, S., Bertaccini, A., Harrison, N.A., Carraro, L., Marcone, C., Khan, A.J., Osler, R. 2007. Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. International Journal of Systematic and Evolutionary Microbiology, 57: 2037-2051.

Mehle, N., Seljak, G., Rupar, M., Ravnikar, M., and Dermastia, M. 2010. The first detection of a phytoplasma from the 16SrV (Elm yellows) group in the mosaic leafhopper *Orientus ishidae*. New Dis. Rep. 22: 11.

Padovan, A.C., Gibb, K.S., Bertaccini, A., Vibio, M., Bonfiglioli, R.E., Magarey, P.A., Sears, B.B. 1995. Molecular detection of the Australian grapevine yellows phytoplasma and comparison with a grapevine yellows phytoplasma from Emilia-Romagna in Italy. Australian J. Grape and Wine Research, 1: 25-31.

Schneider, B., Seemüller, E., Smart, C.D., Kirkpatrick, B.C. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. 369-380. In S. Razin and J.G. Tully (ed.), Molecular and diagnostic procedures in mycoplasmology, vol.1. Academic Press, San Diego, CA.

Trivellone, V., Filippin, L., Jermini, M., Angelini, E. 2015. Molecular characterization of phytoplasma strains in leafhoppers inhabiting the vineyard agroecosystem in Southern Switzerland. Phytopathogenic Mollicutes, 5(1-Supplement), S45-S46.

Zamharir, M. G., Paltrinieri, S., Hajivand, S., Taheri, M., Bertaccini, A. 2017. Molecular identification of diverse ‘*Candidatus* Phytoplasma’ species associated with grapevine decline in Iran. Journal of Phytopathology, 165: 407–413.