Phytopathogenic Mollicutes
Vol. 9 (1), June 2019, 39-40

doi: 10.5958/2249-4677.2019.00020.3

Epidemiology

New insights on "bois noir" epidemiology in the Chianti Classico area, Tuscany

Roberto Pierro[1], Alberto Materazzi[1], Andrea Luvisi[2], Fabio Quaglino[3], Augusto Loni[1], Andrea Lucchi[1] and Alessandra Panattoni[1]

[1]Department of Agriculture, Food and Environment (DAFE), University of Pisa, Italy
[2]Department of Biological and Environmental Sciences and Technologies - University of Salento, Lecce, Italy
[3]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DiSAA), University of Milan, Italy

Abstract

"Bois noir" (BN) is the most widespread disease of the grapevine yellows complex in several countries worldwide. BN is associated with the presence of '*Candidatus* Phytoplasma solani', transmitted from herbaceous plants to grapevine by polyphagous insect vectors. In the present study, a preliminary investigation on the epidemiology of BN in the Chianti Classico area was carried out in an organic Sangiovese vineyard. '*Ca.* P. solani' strains identified in symptomatic grapevines and insects were typed through the analysis of the *stamp* gene nucleotide sequences. Obtained results revealed the presence of 4 *stamp* sequence variants in grapevines and the exclusive presence of the sequence variant St10 in *Reptalus quinquecostatus*, the sole insect species found infected by this phytoplasma in the studied vineyard.

Keywords: grapevine yellows, *Reptalus quinquecostatus*, *Vitis vinifera*, nucleotide sequence analysis

Introduction

Grapevine yellows (GY) diseases associated with phytoplasmas constitute a major threat to the worldwide viticulture. "Bois noir" (BN) is one of the most important GY in the European and Mediterranean regions and is associated with '*Candidatus* Phytoplasma solani' (Quaglino *et al.*, 2013). Its main insect vector is the planthopper *Hyalesthes obsoletus* Signoret (Maixner, 1994), even though BN incidence is not always correlated to high densities of this insect. Interestingly, recent studies evidenced the capability of other insects to transmit '*Ca.* P. solani' to grapevine (Cvrkovic *et al.*, 2014). Up to now, *Convolvulus arvensis* and *Urtica dioica* have been reported as being the main host plants of '*Ca.* P. solani'. Moreover, other wild and cultivated plants within or near vineyards were found infected by the phytoplasma suggesting their role in BN epidemiology (Mori *et al.*, 2015). The use of molecular markers for '*Ca.* P. solani' strain typing increased the knowledge of BN epidemiology in vineyard agro-ecosystems (Kosovac *et al.*, 2016, 2019). The present study aimed to describe the genetic diversity of '*Ca.* P. solani' strains identified in grapevines and insects. Obtained results allowed to gain new insights into the role of putative insect vectors in BN epidemiology.

Materials and Methods

The study was conducted in an organic Sangiovese vineyard located in Greve in Chianti (Chianti Classico area, Florence province). In September 2018, about 10 leaves were collected from each of 48 symptomatic *Vitis vinifera* cultivar Sangiovese plants. In July and August, yellow sticky traps, placed inside and at the vineyard borders, were used to collect Auchenorrhyncha species, considered potential phytoplasma vectors. Plant DNA was extracted following the protocol described by Li *et al.* (2008). Insect DNA was extracted from captured specimens, maintained in 70% ethanol, as described by Marzachì *et al.* (1998). Detection of phytoplasmas belonging to groups 16SrI and 16SrV, and subgroup 16SrXII-A ('*Ca.* P. solani') was carried out by quantitative PCR according to the protocol by Angelini *et al.* (2007). '*Ca.* P. solani' strains, detected in grapevines and insects, were typed by PCR-based amplification of the *stamp* gene and nucleotide sequence analysis, as described by Pierro *et al.* (2018a).

Results

A total of 347 Auchenorrhyncha specimens were collected, with a prevalence (186 out of 347 specimens) of *Reptalus quinquecostatus* (Table 1). '*Ca.* P. solani' was detected in 45 out of 48 symptomatic grapevines, and in 76 out of 186 specimens of *R. quinquecostatus*, the sole species found phytoplasma infected. Phytoplasma groups 16SrI and 16SrV were not detected neither in grapevines nor in insects. Nested-PCR reactions allowed the amplification of the *stamp* gene in 43

Corresponding author e-mail: Roberto Pierro (rob.pierro@outlook.it)

out of 45 '*Ca.* P. solani'-infected grapevines and in 67 out of 76 '*Ca.* P. solani'-infected *R. quinquecostatus* specimens.

Based on sequence identity, four *stamp* sequence variants were identified within the '*Ca.* P. solani' strains infecting grapevines: 3 sequence variants shared 100% sequence identity with St10 (46.6%), St18 (30%) and St5 (20%) sequence variants, while 1 (St59) was identified for the first time in this study and differed from St10 for 2 single nucleotide polymorphisms (nucleotide position 335 and 500) (Figure 1). Sequence analysis revealed that the *stamp* sequence variant St10 was present in all the 67 '*Ca.* P. solani'-infected *R. quinquecostatus* specimens (Figure 1).

Table 1. Insects identified in the surveyed vineyard.

Family	Genus	Species	Collected
Aphrophoridae	*Phylaenus*	*spumarius*	72
Aphrophoridae	*Neophylaenus*	sp.	4
Cicadellidae	*Psammotettix*	sp.	21
Cicadellidae	*Zygina*	*rhamni*	57
Cicadellidae	*Macrosteles*	sp.	1
Cixidae	*Reptalus*	*quinquecostatus*	186
Cixidae	*Cixius*	sp.	1
Dictiopharidae	*Dictyophara*	*europaea*	5

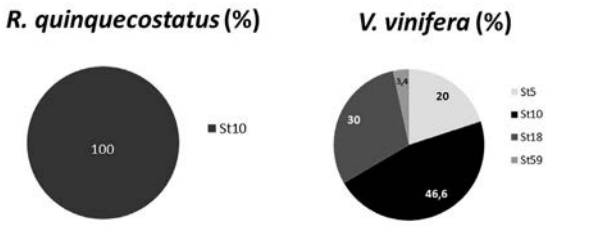

Figure 1. Prevalence of *stamp* sequence variants (%) of '*Ca.* P. solani' strains identified in *V. vinifera* and *R. quinquecostatus* in the studied vineyard.

Discussion

The survey on Auchenorrhyncha surprisingly showed the absence of *H. obsoletus* and the massive occurrence of highly '*Ca.* P. solani'-infected (41%) *R. quinquecostatus* in the examined vineyard. In accordance with previous results obtained in Tuscany (Pierro *et al.*, 2018b), '*Ca.* P. solani' strains infecting grapevines were typed as *stamp* sequence variants St5, St10, and St18, with the prevalence of St10. Interestingly, nucleotide sequence analysis showed the unique presence of the *stamp* sequence variant St10 in *R. quinquecostatus*. Such results could reinforce previous evidences suggesting an important epidemiological role of *R. quinquecostatus* in the transmission of '*Ca.* P. solani' to grapevine (Trivellone *et al.*, 2006; Chuche *et al.*, 2016), at least for phytoplasma strains harboring the St10 *stamp* sequence variant.

Considering the exclusive presence of '*Ca.* P. solani' strains harboring the *stamp* sequence variants St5, St18, and St59 in grapevines, it is reasonable to hypothesize the existence of additional epidemiological pathways for these '*Ca.* P. solani' strains, including other insect vector(s) and/or plant reservoir(s). Further studies based on '*Ca.* P. solani' transmission trials to grapevine are necessary to clarify the role of *R. quinquecostatus* in "bois noir" epidemiology.

References

Angelini E, Bianchi GL, Filippin L, Morassutti C and Borgo M 2007. A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay. *Journal of Microbiological Methods*, 68: 613-622.

Chuche J, Danet J-L, Salar P, Foissac X and Thiery D 2016. Transmission of '*Candidatus* Phytoplasma solani' by *Reptalus quinquecostatus* (Hemiptera: Cixiidae). *Annals of Applied Biology*, 169: 214-223.

Cvrkovic T, Jovic J, Mitrovic M, Krstic Q and Tosevski I 2014. Experimental and molecular evidence of *Reptalus panzeri* as a natural vector of "bois noir". *Plant Pathology*, 63: 42-53.

Kosovac A, Jakovljevic M, Krstic O, Cvrkovic T, Mitrovic M, Tosevski I and Jovic J 2019. Role of plant-specialized *Hyalesthes obsoletus* associated with *Convolvulus arvensis* and *Crepis foetida* in the transmission of '*Candidatus* Phytoplasma solani'-inflicted "bois noir" disease of grapevine in Serbia. *European Journal of Plant Pathology*, 153: 183-195.

Kosovac A, Radonjic S, Hrnèic S, Krstic O, Tosevski I and Jovic J 2016. Molecular tracing of the transmission routes of "bois noir" in Mediterranean vineyards of Montenegro and experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and associated *Hyalestes obsoletus* (Cixiidae). *Plant Pathology*, 65: 285-298.

Li R, Mock R, Huang Q, Abad J, Hartung J and Kinard G 2008. A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. *Journal of Virological Methods*, 154: 48-55.

Maixner M 1994. Transmission of German grapevine yellows ("Vergilbungskrankheit") by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, 33: 103-104.

Marzachì C, Veratti F and Bosco D 1998. Direct PCR detection of phytoplasmas in experimentally infected insects. *Annals of Applied Biology*, 133: 4554.

Mori N, Quaglino F, Tessari F, Pozzebon A, Bulgari D, Casati P and Bianco PA 2015. Investigation on "bois noir" epidemiology in north-eastern Italian vineyards through a multidisciplinary approach. *Annals of Applied Biology*, 166: 75-89.

Pierro R, Passera A, Panattoni A, Casati P, Luvisi A, Rizzo D, Bianco PA, Quaglino F and Materazzi A 2018a. Molecular typing of "bois noir" phytoplasma strains in the Chianti Classico area (Tuscany, Central Italy) and their association with symptom severity in *Vitis vinifera* L. cv. Sangiovese. *Phytopathology*, 108: 362-373.

Pierro R, Passera A, Panattoni A, Rizzo D, Stefani L, Bartolini L, Casati P, Luvisi A, Quaglino F and Materazzi A 2018b. Prevalence of a '*Candidatus* Phytoplasma solani' strain, so far associated only with other hosts, in "bois noir"-affected grapevines within Tuscan vineyards. *Annals of Applied Biology*, 173: 202-212.

Quaglino F, Zhao Y, Casati P, Bulgari D, Bianco PA, Wei W and Davis RE 2013. '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur and "bois noir" related diseases of plants. *International Journal of Systematic and Evolutionary Microbiology*, 63: 2879-2894.

Trivellone V, Pinzauti F and Bagnoli B 2006. *Reptalus quinquecostatus* (Dufour) (Auchenorrincha: Cixiidae) as a possible vector of "stolbur"- phytoplasma in a vineyard in Tuscany. *Redia*, LXXXVIII: 103-108.