Grapevine “Bois noir”: What is New Under the Sun?

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***Abstract*—The diseases associated with phytoplasmas in grapevine are collectively called yellows and occur in the majority of grapevine growing regions over the world. Among these diseases the “bois noir” is the most widespread and in most of the cases is it endemic in vineyards. Molecular variability was reported in the ‘*Candidatus* Phytoplasma solani’ (“stolbur”, 16SrXII-A) infecting grapevines, and in some cases a link to epidemiological aspect of the genomic features detected by MLT was hypotized. Statistical modeling to allow the prediction of the disease in specific grapevine growing areas is also developed. Studies about grapevine- “bois noir” phytoplasma interaction were focusing on some of the main genes regulated in the plant response. A study linking wine production and quality from healthy and “bois noir” infected grapevines showed the negative influence of the disease on wine production but only when the weather conditions were favorable to the production. Perspectives for improved management of the “bois noir” disease were also explored.**

***Key words*-- characterization, epidemiology, symptoms, *Vitis vinifera*, management.**

I. INTRODUCTION

Phytoplasmas in grapevine are associated with “grapevine yellows” (GY) which occur in the majority of grapevine growing regions worldwide. All GY diseases show similar symptoms in *Vitis vinifera* independent from the phytoplasma present, however the most widespread disease is “bois noir” (BN) associated with the presence of “stolbur” (16SrXII-A), ‘*Candidatus* Phytoplasma solani’. It was first reported in 1961 in vineyards of north-eastern France when, on the basis of its lack of transmissibility by *Scaphoideus titanus*, it was distinguished from “flavescence dorée” (Caudwell et al. 1971). A few years later, similar symptoms had been observed in vineyards of the Mosel and the Rhein Valley in Germany. Experimental evidence proved that this disease, originally named “Vergilbungskrankheit” (VK), is transmitted by the planthopper *Hyalesthes obsoletus* Signoret (Sforza et al. 1998). Following the phytoplasma molecular identification this disease was detected from 1995 in 30 countries worldwide (Table 1).

II. MOLECULAR IDENTIFICATION

BN is widely spread and it is in some cases responsible for serious grapevine losses. The ‘*Ca*. P. solani’ strains associated with BN in Europe were assigned to the subgroup 16SrXII-A (Bertaccini et al. 1995), but recently more diversity was found and subgroups 16SrXII-F, -G, -J and -K were also differentiated and associated to BN disease (Quaglino et al. 2009, 2017a). It is widespread in almost all European grapevine growing countries (Table 1) and the most recent reports are from Moldova (Bondarciuc et al. 2018) and Azerbaijan (Absheron peninsula). In the latter case leaf reddening, drying of grapes and leaf rolling symptoms were observed in the main grape growing areas with a disease incidence and severity ranging from 3 to 16% (Balakishiyeva et al. 2016).

Table 1. First identification of “bois noir” in grapevine in the various countries worldwide.

Continent	Country	First literature report
America	***Canada***	***Rott et al. 2007***
	Chile	***Gajardo et al. 2009***
Europe	Austria	Riedle Bauer et al. 2006
	Bosnia & Erzegovina	Delic et al. 2006
	Bulgaria	Sakalieva et al. 2007
	Croatia	Saric et al. 1997
	Czech Republic	Starý et al. 2013
	France	Caudwell et al. 1961
	Germany	Maixner et al. 1995
	Georgia	Quaglino et al. 2014
	Greece	Davis et al. 1997
	Hungary	Kolber et al. 1997
	Italy	Bertaccini et al. 1995
	Moldova	Bondarciuc et al. 2018
	Montenegro	Kosovac et al. 2016
	North Macedonia	Mitrev et al. 2007
	Portugal	de Sousa et al. 2013
	Rumania	Chireceanu et al. 2013
	Serbia	Duduk et al. 2004
	Slovenia	Saric et al. 1997
	Spain	Battle et al. 2000
	Switzerland	Gugerli et al. 2002
	Ukraine	Milkus et al. 2005
Africa	***South Africa***	***Botti and Bertaccini 2006***

(continued)

Table 1.-continued

	Azerbaijan	Balakishiyeva et al. 2016
Asia	China	Duduk et al. 2010
	Iran	Mirchenari et al. 2015
	Israel	Davis et al. 1997
	Jordan	Salem et al. 2013
	Lebanon	Choueiri et al. 2002
	Turkey	Ertunc et al 2015

The sequence analysis of *tuf* gene revealed three BN tuf types in grapevines and alternative plant hosts, according with ecological diverse pathosystems; however the biological complexity of BN disease has stimulated research on additional molecular markers focused mainly to the verification of genetic diversity presence that could be related to diverse strain pathogenicity. MLST on variable genes, such as *secY*, *vmp1* and *stamp*, evidenced a large variability among BN strains within the reported tuf-types. The most recent analyses report that the cluster vmp1/stamp-4 included BN strains (tuf-type a) associated with nettle-related biological cycle, while the other four clusters included BN strains (tuftype b) associated with bindweed-related biological cycle (Quaglino et al. 2016). Based on phylogenetic analysis of concatenated nucleotide sequences of *vmp1* and *stamp* genes of 76 '*Ca*. P. solani' strains, 49 sequence variants were grouped into five vmp1/stamp clusters (Angelini et al. 2018).

It is interesting that '*Ca*. P. solani' was detected in the 30% of Chardonnay recovered grapevine plants where the phytoplasma populations in symptomatic and recovered plants were distinguishable on *vmp1* gene (Quaglino et al. 2017b).

Genetic diversity among BN strains and their prevalence and possible association with grapevine symptom severity were investigated also in a cv Sangiovese clone organic vineyard in the Chianti Classico area (Tuscany). Field surveys over 2 years revealed a range of symptom severity in grapevine and an increase of disease incidence. Only tuf-type b was detected, suggesting the prevalence of the bindweed related ecology. Nucleotide sequence analyses of vmp1 and stamp genes identified 12 vmp1 and 16 stamp sequence variants, showing an overall positive selection for such genes. The prevalent genotype was Vm43/St10, reported for the first time and closely related to strains identified only in the French Eastern Pyrenées. BN strains identified in the examined vineyard and mostly grouped in separate bindweed-related phylogenetic clusters showed statistically significant differences in their distribution in grapevines exhibiting distinct symptom severity. These results suggest the possible occurrence of a range of virulence within BN strain populations in the Chianti Classico area (Pierro et al. 2018).

III. MOLECULAR EPIDEMIOLOGY

Several weeds, such as *Chenopodium album* and *Malva sylvestris*, host the '*Ca*. P. solani' in or around infected vineyards and can therefore play a role in BN diffusion (Marchi et al. 2015; Mori et al. 2015).

In the Euro-Mediterranean regions *Reptalus panzeri* and *R. quinquecostatus* have been reported as vectors of BN in Serbian and France vineyards, respectively (Cvrković et al. 2014; Chuche et al. 2016). The direct epidemiological role of *V. agnus-castus* in the *H. obsoletus*-mediated BN transmission to grapevine was recently demonstrated in Montenegro (Kosovac et al. 2016), and the ability of *R. panzeri* to transmit '*Ca*. P. solani' from corn with reddening disease to grapevine was proved in Serbia (Cvrković et al. 2014).

In Italy *Euscelis incisus* and *Dicranotropis hamata* resulted able to transmit '*Ca*. P. solani' to grapevine (Mori et al. 2018), the same phytoplasma was also detected in vineyard-collected *Euscelidius lineolatus*, *Mocydia crocea*, *Neoaliturs fenestratus* and *Psammotettix alienus* (Minuz et al. 2017). During a survey in South Moravia, Czech Republic *H. obsoletus* was confirmed as the main BN vector with 43.8% of phytoplasma positive individuals. However, a significant role of *Anaceratagallia ribauti* (22.6% of phytoplasma positive specimens) was also observed based on its occurrence and incidence of infected individuals. Eleven insect species were identified as new carriers of '*Ca*. P. solani' or suggested as potential BN vectors: *Macrosteles quadripunctulatus* (64 individuals of which 4.2% positive), *Neoaliturus fenestratus* (13 individuals of which 61.5% positive), *Mocuellus collinus* (51 individuals of which 36.1% positive), *Lygus rugulipennis* (69 individuals of which 46.2% positive). The phytoplasma was also detected for the first in *Doratura homophyla* (65 individuals of which 16.1% positive), *Empoasca pteridis* (165 individuals of which 35.0% positive), *Psammotettix confinis* (139 individuals of which 19.4% positive), and *Aphalara avicularis* (449 individuals of which 11.6% positive). New potential vectors of '*Ca*. P. solani' were also *Ophiola decumana*, *Streptanus aemulans*, *Dicranotropis hamata*, *Javesella pellucida*, *Adelphocoris lineolatus*, *Liocoris tripustulatus*, and *Trioza urticae* (Safarova et al. 2018).

A proof-of-concept statistical model using experimental data, different statistical tools and data mining approaches for '*Ca*. P. solani' infection of cv. Chardonnay grapevine plants. Individual plants from a single vineyard were monitored over a period of six years. Phytoplasma presence and expression of 21 selected grapevine genes and environmental conditions were recorded and related to disease severity. Under the described conditions BN is a function of the expression of grapevine gene VvDMR6, summer rainfall and abundance of '*Ca*. P. solani'. The greatest importance in this model was attributed to the pathogen presence independently of its titer (Rotter et al. 2018a, 2018b).

IV. PLANT-PHYTOPLASMA INTERACTION

A study aimed to understand whether salicylate- and jasmonate-defense pathways might have a role in the recovery from the BN disease was carried out using leaves from healthy, BN-infected and recovered plants. In symptomatic

diseased plants (late summer), unlike symptomless plants (late spring), salicylate biosynthesis was increased and salicylate responsive genes were activated. In contrast, jasmonate biosynthesis and signalling genes were up-regulated both in recovered and diseased plants at all sampling dates. Activation of the salicylate signalling pathway that is associated with the BN presence seems to antagonize the jasmonate defence response. BN presence however fail to activate or suppressing both the expression of some jasmonate responsive genes that act downstream of the jasmonate biosynthetic pathway, as well as the first events of the jasmonate signalling pathway. On the other hand, the activation of the entire jasmonate signalling pathway in recovered plants suggests the possible importance of jasmonate-regulated defences in preventing BN infections and disease (Paolacci et al. 2017). This has also been suggested following studies that showed that infection with '*Ca*. P. solani' induces salicylic-acid-dependent systemic acquired resistance in grapevine, which delays phytoplasma multiplication. This was supported by significant upregulation of the PR-1, PR-2 and PR-5 genes in leaf samples of the infected plants. It was also observed a significantly increased transcription of the salicylic acid carboxyl methyltransferase, the gene that encodes the enzyme S adenosyl-L-methionine which is responsible for biosynthesis of methyl salicylate, and a 26-fold increase in salicylic acid glucopyranoside and a significant increase in free and total salicylate (Dermastia et al. 2017, Paolacci et al. 2017). On the other hand, the abundance of PR-1, PR-2 and PR-5 gene transcripts in grapevine plants recovered from BN disease did not differ from their levels in uninfected plants. These combined data suggest that although plants react to phytoplasmas through salicylic-acid-mediated signalling, the activation of these responses does not confer resistance against the disease. In addition, it has been shown that during the interactions between grapevines and '*Ca*. P. solani', activation of the salicylic acid signalling pathway antagonizes the jasmonic acid defence responses. However, in plants recovered from "bois noir" disease the jasmonic acid signalling pathway was activated, which was suggested to prevent the subsequent development of the disease (Paolacci et al. 2017).

The impact of BN on yield, berry composition and wine characteristics of *Vitis vinifera* L. cv. 'Chardonnay' was comprehensively characterized in a 3-year field experiment in Hungary. Additionally tuf- type b1 genotype was identified to be involved in the BN pathosystem in the experimental vineyard where severe yield losses were registered with the average decrease in number of bunches and total yield per plant was calculated to be 56.7% and 68.4%, respectively. Analyses of wines produced from BN infected plants revealed decreased alcohol, epicatechin and iron contents; and increased organic acids, titratable acidity, catechin and calcium contents. Sensory evaluation of these wines confirmed higher acidity, bitterness, and usually pinkish discoloration. Negative impact on berry composition and wine quality were pronounced in the vintage with favourable weather conditions for grapevine production, whereas the negative effects of BN infection were less marked, or even masked, in the vintages with unfavourable weather (wet and cool) (Ember et al. 2018).

V. DISEASE MANAGEMENT

Volatiles emission from BN recovered grapevine after treatment with acibezolar-S-methyl (BTH) and two glutathione oligosaccarin based products applications was evaluated in an Italian cv. Chardonnay vineyard. These volatiles were repellents to *H. obsoletus* adults while one of them strongly attracted cixiids showing interesting potential in practical application for organic farming BN management (Riolo et al. 2017).

The complexity of BN disease epidemiology renders it difficult to design efficient control strategies. The management of *H. obsoletus* host plants in the vineyards and surrounding areas is therefore considered crucial for BN control (Mori et al. 2012). Thus, preventive measures such as checking the sanitary status of propagation material, and treating diseased mother plants through thermotherapy must be applied to limit long distance dissemination and in-field spread of the disease. Other strategies for reducing BN spread or incidence are based on (i) preventive removal of the grapevines suckers on which *H. obsoletus* could feed after grass mowing; (ii) trunk cutting above the grafting point on the symptomatic grapevines; (iii) treatments by resistance inducers (Mori et al. 2015).

"BN" management could also be improved by developing new molecular detection tools that can have wide application on large number of samples. The production of specific antisera and the use of grapevine cv. that are less susceptible to the disease could have a positive impact in the fields. Both approaches are exploited in the frame of the recently launched program H2020 TROPICSAFE for grapevine grown in the subtropical areas where epidemics of BN and of other phytoplasma-associated diseases are recently reported (Zambon et al. 2018; Fiore et al. 2018). Isolation of phytoplasmas from infected grapevine (Figure 1) is in progress toward having phytoplasma colonies to be used as a focused tool for antisera production to the phytoplasmas and to be used as challenge to verify the resistance of grapevine genotypes to phytoplasma-associated diseases.

Figure 1. Left, plate with a few phytoplasma colonies in agar medium (Contaldo et al. 2016) and right, "bois noir" infected grapevine plant showing typical yellows symptoms due to '*Ca*. P. solani' presence.

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