Detection and Identification of Phytoplasmas in Two Vineyards Located in a Restricted Geographic Area in Italy

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***Abstract*—A survey to verify the presence of grapevine yellows was carried out in a restricted area of the Marche region (central east Italy) in a small geographic area. In two locations six grapevine varieties were examined and phytoplasmas belonging to four ribosomal groups were detected by PCR/RFLP analyses. The surveyed varieties were Passerina, Pecorino, Merlot, Sangiovese, Fedit and Montepulciano. The different phytoplasmas detected were present in a scattered manner in all the varieties, but only Passerina, Pecorino and Sangiovese where shown to be infected by "bois noir" (16SrXII-A) phytoplasmas. Considering the landscape shape and the age of the vineyards the source of BN infection and of the other phytoplasmas is very likely in the surrounding environment or vineyards.**

***Key words*—Epidemiology, grapevine yellows, identification, molecular detection, phytoplasmas**

I. INTRODUCTION

Grapevine yellows (GY) are widespread in all the grapevine growing areas worldwide, however while "bois noir" (BN) and "flavescence dorée" (FD) diseases are studied and have known and distinct distribution areas, the presence of other phytoplasmas is associated with GY in several viticultural areas worldwide (Dermastia et al. 2017). Recently the occurrence of diverse phytoplasmas was reported in the Prosecco area (Italy), where traditionally only FD and BN associated phytoplasmas were reported (Zambon et al. 2018). In 2017, sampling to verify phytoplasma association with typical yellows symptoms such as downward curled leaves in some varieties accompanied with partial yellowing or reddening of leaf lamina (Fig. 1), and reduced production were observed in vineyards located in a restricted area of Marche region (central eastern Italy) in Ascoli Piceno province (Fig. 2). A survey was therefore carried out in order to verify phytoplasma presence and identity.

II. MATERIAL AND METHODS

Total nucleic acids were extracted from 1 g of fresh plant tissue (leaf midribs) from 29 symptomatic grapevines collected in two vineyards and belonging to the six varieties Passerina, Pecorino, Merlot, Sangiovese, Fedit and Montepulciano (Table 1). All the vineyards were more than 20 years old, only the variety Fedit was about 6 year old. Samples were ground in liquid nitrogen using a phenol/ chloroform protocol (Prince et al. 1993). PCR was performed on 20 ng/µl DNA template

Figure 1. Typical GY symptoms in sample MJ-1 of Sangiovese variety.

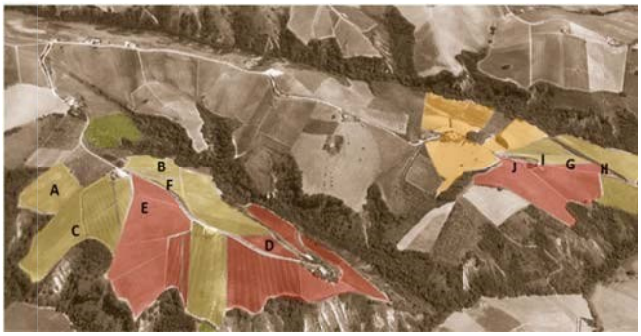

Figure 2. Google map of the surveyed vineyards located in Ascoli Piceno province (Marche, Italy) the different plots are marked with different letters (see Table 1 for grapevine varieties).

using phytoplasma universal primer pair P1/P7 (Deng and Hiruki, 1991; Schneider et al. 1995), followed by nested-PCR on 1: 29 dilution of the obtained amplicons (1 µl) with primers R16(I)F1/R1 and R16(X)F1/r1 (Lee et al. 1994, 1995) or U5/U3 (Lorenz et al. 1995) on P1/P7 dilution 1: 29.

Samples lacking template DNA were run as negative controls, and DNAs extracted from periwinkle maintained in micropropagation (Bertaccini 2014) were used as positive controls. Amplification and detection of phytoplasma presence was performed as reported by Zambon et al. (2018). Identification of detected phytoplasmas was done by RFLP analyses with informative restriction enzymes. The digested

DNA fragments were separated by electrophoresis in a 6.7% polyacrylamide gel, stained with ethidium bromide, and visualized and photographed under UV transillumination.

Table 1 Samples collected in the two vineyards and phytoplasma identified.

Sample/ variety	16Sr DNA[a]	Group/subgroup
Locality San Basso		
M-A1 Passerina	+	16SrIII
M-A2 Passerina	+	16SrXII-A
M-C1 Pecorino	+	16SrXII-A
M-C2 Pecorino	+	16SrXII-A
M-D1 Merlot	+	16SrIX
M-D2 Merlot	+	16SrIII
M-D4 Merlot	-	
M-D5 Merlot	+	16SrX-B
M-D7 Merlot	+	16SrIII
M-E2 Montepulciano	+	16SrIII
M-E3 Montepulciano	-	
M-F1 Passerina	-	
M-F10 Passerina	-	
M-F2 Passerina	-	
M-F3 Passerina	-	
M-F4 Passerina	+	16SrIII
M-F5 Passerina	+	16SrIII
M-F7 Passerina	-	
M-F8 Passerina	+	16SrIII
Locality Tarà		
M-G1 Sangiovese	-	
M-H1 Fedit	-	
M-H3 Fedit	+	16SrX-B
M-I2 Passerina	+	16SrX-B
M-I3 Passerina	-	
M-I4 Passerina	-	
M-I6 Passerina	-	
M-I7 Passerina	+	16SrVII-A
M-J1 Sangiovese	+	16SrXII-A
M-J2 Sangiovese	+	16SrXII-A

a. +, amplification in nested PCR, - no amplification in nested PCR

III. Results

The majority of the collected samples were positive, but 38% randomly distributed samples in all cultivars examined were negative in respect to phytoplasma presence (Table 1).

The RFLP analyses allowed to classify the detected phytoplasmas to the ribosoml group/subgroup level and the presence of BN phytoplasmas (16SrXII-A) was detected only in Sangiovese, Pecorino and Passerina varieties in both localities surveyed. Among the other phytoplasmas detected the group 16SrIII was the most prevalent and was identified in Passerina, Montepulciano and Merlot of the San Basso locality only. Phytoplasmas of the 16SrX-B subgroup were detected in both localities in Passerina, Fedit and Merlot varieties. One plant of Merlot in the San Basso locality was found infected with 16SrIX phytoplasmas and one Passerina grapevine in the locality Tarà was infected with 16SrVII phytoplasmas.

IV. Discussion

The presence of diverse phytoplasmas in a restricted geographic area in grapevine showing yellows symptoms is indicating the possible presence of diverse phytoplasma sources in or around these vineyards. Considering the age of the majority of the vineyards it can be speculated that the environment play a key role in the presence of diverse phytoplasmas making it difficult to manage the disease spreading. While the BN phytoplasmas are quite common in vineyards worldwide for their open cycle spreading from insect vectors from weeds and also their survival to thermotherapy, for the other phytoplasmas little is known about epidemiology. They were detected in some other areas of Italy and worldwide in grapevine, but never together in a single restricted geographic area. In particular group 16SrIX was reported in Turkey and in Iran (Ertunc et al. 2015; Zamharir et al. 2017), 16SrIII in USA (Davis et al. 2015) and in Chile (Gajardo et al. 2009), and 16SrX-B in Italy, Hungary and Serbia (Bertaccini et al. 1996; Varga et al. 2000; Duduk et al. 2004). Phytoplasmas of the 16SrVII were reported mainly in Chile, but recently also in Iran and in Italy (north and south) (Gajardo et al. 2009; Zamharir et al. 2017; Zambon et al. 2018; Fiore et al. 2018). The results of the survey indicates the need for more accurate surveys, not only with focus on BN and FD in order to verify the presence of other phytoplasmas and their potential epidemic dissemination.

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