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Molecular characterization of stolbur isolates collected in grapevines, weeds and insects in central and southern Italy

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Abstract

One of the main grapevine yellows (GY) associated to phytoplasma presence is "bois noir" (BN). The causal agent of the disease is spread in a wide range of host plants including wild and cultivated herbaceous hosts. In order to evaluate possible correlations among BN infections and the presence of alternative host plants and insect vectors into the vineyards, a molecular investigation was carried out on different phytoplasma hosts from vineyards placed in different viticultural areas of central and southern Italy. A different geographical distribution of BN isolates in grapevine samples and in weeds and insect samples was observed.

Key words: Grapevine yellows, phytoplasma, molecular characterization, VK-I, VK-II.

Introduction

"Bois noir" (BN) is an important grapevine disease associated with phytoplasmas belonging to ribosomal subgroup 16SrXII-A, commonly spread in a wide range of host plants including wild and cultivated herbaceous hosts. It is naturally transmitted by the Cixiidae planthopper *Hyalesthes obsoletus* Signoret, a polyfagous vector that complete its cycle on herbaceous plants. Its complete epidemiological cycle, as observed in German vineyards, was reported and it was underlined the importance of weeds, as *Urtica dioica* and *Convolvolus arvensis,* where the vector overcomes winter season as immature stages (Maixner *et al*., 2006) These plants, thus, could play a key role in the epidemiology of the disease since they influence the population density of the polyphagous vectors and act as source of inocolum.

Similar information has been reported in Italy where*, H. obsoletus* has been demonstrated to be able to transmit the phytoplasma to grapevine (Alma *et al.*, 2002) and the presence of 16SrXII-A phytoplasmas in *U. dioica* and *C. arvensis*, (Credi *et al.,* 2006; Pasquini *et al.,* 2006) has been ascertained. Anyway, it is important to underline that many other weeds and Auchenorrhyncha species, commonly present into the Italian vineyards, resulted positive for the presence of the phytoplasma by molecular analysis (Trivellone *et al.*, 2005; Pasquini *et al*., 2006), even if their role in the epidemiological cycle of this disease is still under evaluation. The analysis of *tuf* gene sequence showed that it is possible to distinguish two types of BN phytoplasmas, named VK-I (tuf-type I) and VK-II (tuf-type II), with an high specificity for alternative host plant species (Langer and Maixner, 2004).

To improve knowledges on the epidemiological cycle of this importan disease, the characterization of phytoplasma population present in grapevine plants, weeds and vectors collected in the same orchard has been performed throught molecular analysis of t*uf* gene of 16SrXII-A phytoplasmas.

Materials and methods

Samples of symptomatic vines, belonging to different varieties, were collected in September from six monitored vineyards, located in Central (Tuscany and Latium) and southern (Calabria) regions.

Leaf samples of the most common perennial and annual weeds, present in each orchard were collected during the summer. Within each vineyard, adult insects, belonging to different Auchenorryncha species, were caught by sweep-net and yellow sticky-traps from May to October. Insects were caught either from vine canopy or interrow and edge vegetation.

Direct PCR, using universal ribosomal primers P1/P7 (Schneider *et al.*, 1995; Deng and Hiruki, 1991), followed by a nested amplification with the group-specific primer pairs R16(I)F1/R1 (Lee *et al.,* 1994), was used to identify samples infected by 16SrXII-A phytoplasmas. Amplification reactions and cycling conditions were programmed as previously described (Pasquini *et al.*, 2001).

The molecular characterization of the phytoplasma was performed analyzing the non-ribosomal sequence of the *tuf* gene, encoding for the elongation factor Tu (EF-TU). The primer pair fTufAY/rTufAY (Schneider *et al*., 1997) was used in direct PCR, followed by a nested amplification performed with the primer pair TufAYf2/TufAYr2, specifically designed on the phytoplasma *tuf* gene sequences retrieved in gene banks, after multiple alignment. Amplified products were analyzed by RFLP with *Hpa*II endonuclease, to identify the presence of different patterns.

Results

PCR analysis performed both on ribosomal and non ribosomal phytoplasma genes allowed to amplify DNA fragment of expected size. The primer pair TufAYf2/r2 amplified a fragment of *tuf* gene (898 bp) idoneous for

the characterization of 16SrXII-A isolates. The RFLP pattern obtained using *Hpa*II enzyme allowed to distinguish tuf-type I and tuf-type II isolates as previously described by Langer and Maixner (2004). The molecular characterization of grapevines, wild species and insect samples, collected in different vineyards, showed that both isolates are present in Central and Southern italian vineyards, even if with a different geographical distribution. Both types were found in grapevine plants grown in the vineyard of Tuscany regions, whereas only tuf-type I was detected in plants of Latium plantation and tuf-type II in plants cultivated in Calabria. It is important to underline that only in Tuscany was found a mixed population of the two phytoplasmas types.

An identical phytoplasma type distribution was observed also in the Auchenorrincha population, no matter the diffent species analyzed.

Results were not so clearly in agreement in the case of weeds. In fact, in spite of the type of stolbur phytoplasmas present in vineyards of the three monitored regions, *C. arvensis* was always found infected by tuf-type II and *U. dioica* by tuf-type I.

Discussion

A geographical distribution of the two different BN types detected in the surveyed areas was pointed out. An high frequency of infection by tuf-type I was observed in the investigated areas of Latium region, where an increasing diffusion of BN disease has been reported in the last few years (Pasquini *et al*., 2007). Both types, often contemporaneously present into the same vineyard, were found grapevine growing areas of Tuscany region. On the contrary, tuf-type II resulted predominant in the inspected vineyards of Calabria, where an endemic presence of BN has been observed (Albanese *et al*., 2006).

Preliminary results on insect vector confirmed that several species, other than *H. obsoletus* are able to acquire Stolbur phytoplasmas. Particularly, *R. quinquecostatus* (Trivellone *et al.,* 2005) and *R. panzeri* seem to have a potential role in BN cycle, since their presence is often abundant in the grapevine canopy and interrow vegetation. *U. dioica* has been confirmed to be an important host plant of *H. obsoletus* and the host-type-specificity is clear, being tuf-type I isolated in infected grapes, in individuals of *H. obsoletus* and in infected nettle plants. The role of *C. arvensis*, is still not clear because it resulted always infected by tuf-type II, independently from the isolate detected in grape and in insects.

Other wild species should be considered alternative host plants for the epidemiology of BN: *A. retroflexus* and *S. nigro,* very common in central and southern Italian vineyards, were found always infected by the same tuf-type identified in grape plants suggesting that they could act as reservoir of inoculum. *C. arvensis* and *C. arvense*, both present in southern Italy could also play an epidemiological role.

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