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Molecular identification of "bois noir" phytoplasmas in grapevine in Bulgaria

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Abstract

Field inspection in 4 vineyards located near Plovdiv (Bulgaria) allows observation of yellows symptom presence. PCR/RFLP analyses on 16S ribosomal gene identified phytoplasmas belonging to 16SrXII-A ribosomal subgroup in both symptomatic grapevines and bindweed growing in infected vineyard. Grapevine mother plant and young plants were also infected suggesting that, even if this is the first report of this disease in Bulgaria, the environment is epidemically affected.

Key words: Phytoplasmas, grapevine, "bois noir", molecular identification.

Introduction

Yellows diseases of grapevine were detected and phytoplasma associated were identified in the majority of grapevine grown countries worldwide. Several molecularly distinct phytoplasmas were identified, however "flavescence dorée" (FD) and "bois noir" (BN) are the main phytoplasma associated diseases detected in European vineyards (Boudon-Padieu, 2005). Since symptoms on plants are indistinguishable from each others molecular analyses to identify phytoplasmas involved, when typical symptoms are present, must be carried out.

Figure 1. Cane of a Merlot 5 year old grapevine plant showing irregular shape associated to "bois noir" phytoplasma infection.

During August 2006 a survey to evaluate phytoplasma presence was carried out in the region near Plovdiv that is one of the major regions of vineyards and wine production in Bulgaria. Different vineyards were surveyed for phytoplasma symptoms detection: a limited number of plants showing downward leaf curling, yellow or reddish leaf lamina discoloration, and some lack of cane lignification were observed. In some cases also reduced cane growth with irregular shape was also recorded (figure 1).

Materials and methods

A total of 22 grapevine samples were collected from different plants at the beginning August 2006 in 4 locations near Plovdiv as follows: location a) - 7 samples from symptomatic grapevine plants up to 30 year-old, mainly of cv Bouquet, located in the experimental fields of Plovdiv University (village Brestnik), one sample of bindweed (*Convolvolus arvensis* L.) showing yellows and stunting shape, collected under symptomatic grapevines; location b) - 2 samples from mother plants used for cutting production belonging to cv Chardonnay (village Tzalapitza); location c) - 4 samples from a private vineyard, cv Cabernet (village Tzalapitza); location d) - 9 samples from five year-old plants of cv Merlot (Bessa Valley).

Total nucleic acids were extracted from 1 g of fresh leaf midribs and phloem tissues following a chloroform/phenol procedure (Prince *et al*., 1993), dissolved in Tris-EDTA pH 8 buffer, and maintained at 4 °C. 20 ng/µl of nucleic acid were used for amplification that was carried out with primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al*., 1995) in direct reaction, followed in nested PCR by primer pair F1/B6 (Duduk *et al*., 2004) and in second nested PCR by R16F2/R2 primers (Lee *et al*., 1995). PCR and nested PCR reactions were carried out according with published protocol (Schaff *et al*., 1992). Identification of detected phytoplasmas was done using RFLP analyses on amplified ribosomal DNA fragments with *Tru*I, *Rsa*I, and *Taq*I

(Fermentas, MBI, Vilnius, Lithuania) restriction enzymes. Restriction profiles were compared with those of reference phytoplasma strains maintained in collection (Bertaccini *et al*., 2000).

Results

In all four locations phytoplasmas were identified in some of the symptomatic plants. Only 16SrXII-A ("bois noir") phytoplasma was identified after RFLP analyses with the enzymes described above in all PCR positive plant material. In particular one out of seven samples tested in location a) was positive to the "bois noir" presence, however in this location also the bindweed sample was positive to 16SrXII-A phytoplasmas.

Both samples collected from Chardonnay mother plants in location b) resulted positive to phytoplasma presence. From cv Cabernet and Merlot - locations c) and d) - respectively, samples from three and two plants resulted positive to "bois noir" phytoplasmas.

Discussion

This is the first molecular identification of phytoplasmas infecting grapevines in Bulgaria even if the BN disease was recently reported (EPPO, 2006). Even if only one grapevine growing area was surveyed the presence of "bois noir" phytoplasmas appear to be not of little distribution or erratic. All the localities show phytoplasma presence regardless to the cultivar and to the age of vineyards. Only 8 out of 22 symptomatic samples tested were positive to phytoplasma presence; however a PCR-inhibitory effect of pesticides employed in vineyards at the time of sampling can not be excluded, considering also the need of repeated nested PCR.

Two epidemiologically important aspects were observed: the presence of phytoplasmas in mother plants and in young vineyards. The same phytoplasmas were also detected in bindweed that is one of the major natural reservoirs in vineyards and play an important epidemiological role for insect vector maintenance in the environment (Weber and Maixner, 1998; Mori *et al*., 2007).

In many other European regions where "bois noir" was reported *Hyalesthes obsoletus* Signoret is the vector of the disease (Sforza *et al*., 1998; Alma *et al*., 2002), even if other insects, such as *Reptalus panzeri* in vineyards in Hungary as well as in Italy, were reported to be infected by 16SrXII-A phytoplasmas (Palermo *et al*., 2004; Botti *et al*., 2005).

No information about insect vectors in the surveyed vineyard was achieved, further research will be necessary to evaluate real risk for "bois noir" dissemination in Bulgarian vineyards.

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