

Identification of different phytoplasmas infecting grapevine in Turkey

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Abstract

Grapevines with severe redness and inward curling of leaves were collected from the main viticulture production areas, Aegean, Central Anatolia and Western Anatolian regions of Turkey. Collected samples were subjected to nucleic acid extraction followed by nested PCR/RFLP analyses that allow to identify different phytoplasmas in symptomatic grapevines. The majority of samples were infected with phytoplasmas belonging to 16SrXII group and referable to the 'bois noir' phytoplasmas, while in some samples 16SrIX or 16SrI-B phytoplasmas were identified. The 16SrIX phytoplasmas are firstly reported in grapevine.

Key words: Grapevine, PCR/RFLP analyses, phytoplasma identification, 'bois noir'.

Introduction

Turkey is one of the nations native to grapevine in the Middle East, where table and vine grape varieties have been grown in Thrace, Central Anatolia, Mediterranean, Aegean and Eastern Anatolia regions of Turkey. The country is the 4th and 6th in the world, respectively, for grapevine cultivation and production of table and wine grape. Grapevine is affected worldwide by several phytoplasma diseases named grapevine yellows. Phytoplasmas belonging to different ribosomal groups were identified such as aster yellows (16SrI group), elm yellows (16SrV group) and stolbur (16SrXII group), together with 16SrII, 16SrIII, 16SrVII and 16SrX groups in different countries (Varga *et al.*, 2000, Boudon-Padieu, 2005, Gajardo *et al.*, 2009, Duduk *et al.*, 2004, Milkus *et al.*, 2005). Two of these phytoplasmas are associated with specific diseases such as 'flavescence dorée' (16SrV-C and V-D subgroups) and 'bois noir' (subgroup 16SrXII-A). Grapevines with severe redness and inward curling of leaves were observed in the main viticulture production areas of Turkey therefore surveys were carried out to verify phytoplasmas presence and identity.

Materials and methods

The main viticulture production areas, Aegean, Central Anatolia and Western Anatolian parts of Turkey, were surveyed in the summer of 2009 and 167 leaf samples were collected from symptomatic plants. Severe redness and inward curling of leaves were the major symptoms of the collected plants. Nucleic acid was extracted from midribs according to a chloroform/phenol protocol (Prince *et al.*, 1993). The phytoplasma strains stolbur (STOL, ribosomal subgroup 16SrXII-A), aster yellows (PRIVA, ribosomal subgroup 16SrI-B) and Naxos (ribosomal subgroup 16SrIX-C) maintained in collection in periwinkle were employed as reference strains in restriction fragment length polymorphism (RFLP) analy-

ses. Direct PCR with ribosomal P1/P7 universal primer pair, followed by nested PCR with R16F2n/R2 (Gundersen and Lee, 1996), and R16(I)F1/R1 and R16(V)F1/R1 (Lee *et al.*, 1994) primer pairs were carried out. R16(I)F1/R1 products were subjected to RFLP analysis with *TruI* and *HhaI*. One uncloned R16F2/R2 amplicon was purified using Qiagen PCR Purification Kit (Qiagen GmbH, Hilden, Germany) and sequenced in both directions with R16F2 and R16R2 primers, using the BIG DYE sequencing terminator kit (PE Biosystems, Warrington, UK) for molecular characterization. The obtained sequence was aligned by using Clustal W and BioEdit (Hall, 1999) softwares and deposited in GenBank.

Results

R16F2n/R2 amplicons of the expected size were obtained for half of the samples tested. The majority of positive samples exhibited *TruI* RFLP pattern indistinguishable from those of 16SrXII ribosomal group. These phytoplasmas are also referred to as stolbur phytoplasmas, and reported to be associated in grapevine to 'bois noir' disease. Phytoplasmas belonging to aster yellows group (16SrI-B) were also identified by RFLP analyses on amplicons obtained with primer R16(I)F1/R1 in a few cases with *TruI* (figure 1) and *HhaI* (data not shown). In some of the symptomatic samples 16SrIX phytoplasmas were identified (data not shown). One of the latter samples showing 16SrIX phytoplasma infection was employed for sequencing, and the obtained sequence of 1,063 bp was deposited in the GeneBank under ID HQ714331. This sequence showed a 99% identity with that of pigeon pea witches' broom phytoplasma and other phytoplasma members of group 16SrIX, including the strains related to 'Candidatus Phytoplasma phoenicium' present in the Genbank. Comparison of the obtained sequence with those available in GenBank for 16SrIX group phytoplasmas allow to verify that the sequence show five mismatch with other sequences.

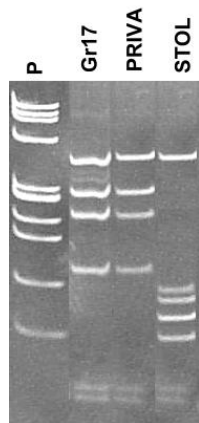


Figure 1. RFLP profiles of R16(I)F1/R1 amplicons after *TruI* digest. Acronyms: Gr., grapevine sample; PRIVA, primula yellows from Germany (16SrI-B); STOL, stolbur from pepper from Serbia (16SrXII-A); P, marker Φ X174 *Hae*III digested.

Discussion

'Bois noir' disease is widespread and occurs from Spain to Ukraine and from Germany and Northern France to Lebanon and Israel (Maixner, 2011). The disease was also recently reported in Turkey (Canik *et al.*, 2011). Stolbur group-related grapevine phytoplasmas have been also recently been reported from Iran (Karimi *et al.*, 2009) and China (Duduk *et al.*, 2010). Aster yellows phytoplasmas were reported in grapevine in several countries after the first finding in Italy (Alma *et al.*, 1996). The 16SrIX group phytoplasmas are severely infecting plants in different regions, especially in those bordering Turkey (Choueiri *et al.*, 2001; Abou-Jawdah *et al.*, 2002) so their first identification indicates the susceptibility of the species to this pathogen and the urgent need to further verify its presence in grapevine to prevent any further epidemic. Work on finer classification of the identified phytoplasmas, as well as their further detection in grapevine and in other host species and in potential insect vectors, is in progress.

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