Current status of grapevine phytoplasma infections in Turkey

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Abstract
Grapevines with severe redness and inward curling of leaves were collected from the main Turkish viticulture production areas. Nucleic acid extraction followed by nested PCR/RFLP analyses and sequencing allowed phytoplasma identification in symptomatic grapevines. The majority of samples were infected with 'bois noir' phytoplasmas, while in some samples 16SrIX or 16SrI-B phytoplasmas were identified.

Key words: grapevine, PCR/RFLP analyses, sequencing, phytoplasma identification.

Introduction

Turkey is one of the nations native to grapevine in the middle east and table and vine grape varieties have been grown in the majority of Turkey regions. 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 and several phytoplasmas were preliminary identified (Canik *et al.*, 2011a; 2011b). Relevance and incidence of these phytoplasma are under study.

Materials and methods

The main viticulture production areas were surveyed. 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) analyses. 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. RFLP analysis were performed by *Tru*I to R16(I)F1/IR1 products. Further molecular characterization was carried out on one uncloned R16F2/R2 amplicon 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). The obtained sequence was aligned by using Clustal W and BioEdit (Hall, 1999) softwares and deposited in GenBank.

Results and Discussion

The majority of positive samples resulted as phytoplasmas affiliated to 16SrXII ribosomal group. These phytoplasmas are also referred to as stolbur phytoplasmas, and reported to be associated in grapevine to 'bois noir' disease. Using specific primers R16(I)F1/R1 in a few cases also phytoplasmas belonging to aster yellows group (16SrI-B) were identified. In some of the symptomatic samples 16SrIX phytoplasmas were identified: one of these was employed for sequencing. This sequence show 99% identity with phytoplasmas assigned to group 16SrIX.
'Bois noir' disease is widespread and stolbur group related grapevine phytoplasmas have 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 identification in grapevine for the first time indicates the susceptibility of the species to this pathogen and the urgent need to further verify its presence in grapevine to avoid its possible epidemic diffusion.

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