Bulletin of Insectology ***64*** *(Supplement): S217-S218, 2011*
ISSN 1721-8861

Preliminary identification of phytoplasmas associated with grapevine yellows in Syria

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Abstract

Symptoms of grapevine yellows diseases have been observed in some grapevine growing areas near to the cost in Syria. Symptoms are similar to those associated with phytoplasmas, know as yellows diseases. In order to verify the presence of phytoplasmas using molecular techniques, leaf samples were taken from symptomatic grapevine plants that were exhibiting leaf rolling and red coloration during September 2010. Total DNA was extracted from leaf midribs of these samples and tested by nested-PCR followed by RFLP assays. Two phytoplasmas were identified in mixed infection: one related to stolbur (16SrXII) and the other tentatively related to clover proliferation group (16SrVI). To our knowledge, this is the first detection and identification of phytoplasmas infecting grapevine in Syria. Further research about final classification of the phytoplasma identified in mixed infection and about their spreading and insect vector(s) in vineyards are in progress.

Key words: grapevine yellows, stolbur phytoplasmas, 'bois noir' disease, PCR/RFLP.

Introduction

Grapevine production is of economic importance in many countries of the world including Syria, where it is a source of income and livelihood for a large number of Syrian farmers (Baghasa, 2006). In the recent years, Syria has kept its rank of 28th grape producing country in the world, accounting for 0.4% of total world production (FAOSTAT, 2004).

The grapevine is cultivated throughout Syria, although the cultivated area of grape in Syria has decreased significantly since 1997 especially the in rained area due to climate changes such as, frost, temperature rise and plant pests (Baghasa, 2006). Grapevine yellows (GY) are widespread diseases caused by different types of phytoplasma including 'bois noir' (BN). BN is a GY diseases associated with a stolbur phytoplasma which belongs to 16SrXII ribosomal group (Lee *et al*., 1998) and reported in Asia Minor, and in the Mediterranean and European countries (Maixner, 2006).

Since the symptoms are very similar among the different grapevine yellows diseases, the molecular investigation is needed in order to determine which phytoplasmas are associated to these diseases.

Materials and methods

In September 2010, the grapevine leaves used in this study were taken from two fields near Latakia that locates on the Mediterranean in north-west of Syria, these leaves were sun-dried and leaf veins were isolated from samples and ground in liquid nitrogen to a fine powder in order to extract the total DNA according to a chloroform/phenol procedure (Prince *et al*., 1993).

Phytoplasmas were screened by nested PCR using P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) primer pair, followed by the primer pair R16mF1/R16mR2 (Gundersen and Lee, 1996). Final nested PCR was carried out with R16F2/R2 (Lee *et al*., 1995). Template without nucleic acid was used as negative control. RFLP analyses was performed on the amplicons from first and second nested PCR reaction with *Tru*I, *Hha*I, and *Alu*I restriction endonucleases (Fermentas, Vilnius, Lithuania). After electrophoresis the restriction patterns obtained from these samples were compared with those of reference phytoplasma strains STOL (from pepper from Serbia, 16SrXII-A). Further nested PCR was carried out on P1/P7 amplicons using B5/P7 (Padovan *et al*., 1995) followed by M1/V1731 (Martini *et al*., 1999) using as reference strain ULW (elm yellows from France, 16SrV-A).

Results

Nested PCR results with generic phytoplasma primers B5/P7 and R16F2/R2 provided positive results from all grapevines tested. RFLP analyses on R16F2/R2 amplicons with *Tru*I allow to identify phytoplasmas as stolbur or 16SrXII-A phytoplasmas (figure 1). A second RFLP profile indicated the possible presence of 16SrV, VI or VII phytoplasmas. Further analyses were carried out to identify the possible second phytoplasma detected in mixed infection. RFLP analyses with *Hha*I excluded the presence of 16SrVII group phytoplasmas (data not shown); while *Alu*I suggested the presence of groups 16SrV or 16SrVI phytoplasmas. A nested PCR with 16SrV group specific primers provided negative results (figure 2) indicating the possible presence of 16SrVI phytoplasmas.

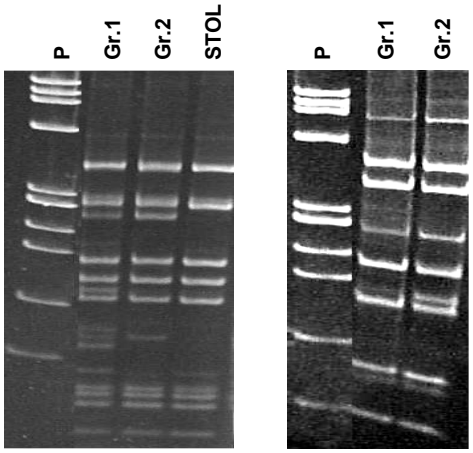

Figure 1. RFLP profiles of R16F2/R2 amplicons after *Tru*I (left) and *Alu*I (right) digest. Acronyms: Gr., grapevine samples; STOL, stolbur from pepper from Serbia (16SrXII-A); P, marker ΦX174 *Hae*III digested.

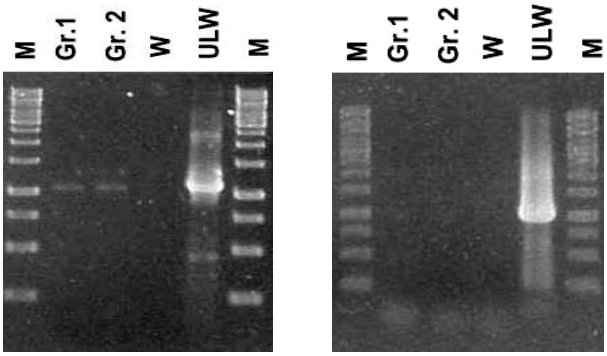

Figure 2. Nested PCR amplification with primers B5/P7 (left) and M1/V1731 (right) on grapevine samples. The lack on amplification with M1/V1731 primers indicates absence of 16SrV phytoplasmas. Acronyms: Gr., grapevine samples; ULW, elm yellows from EU (16SrV-A); M, marker 1kb DNA ladder.

Discussion

Stolbur phytoplasma is widespread in Europe in several different crops. BN disease associated with stolbur phytoplasmas has expanded geographically and increased in occurrence within the recent years (Maixner, 2006). There is no investigation up to now on the phytoplasma infections of grapevine in Syria, although phytoplasma infections have been reported in neighbouring countries to Syria (Boudon–Padieu, 2003, 2005). The finding of BN-associated phytoplasmas is in agreement with literature since it was reported in almost all grapevine growing areas worldwide.

The presence of mixed infection with 16SrVI group phytoplasmas is to be confirmed. This phytoplasma group was reported in other crops such as sesame in region near to Syria (Sertkaya *et al*., 2007). In the future, surveys for the GY phytoplasmas will be carried out in a number of grapevine samples from different areas such as Damascus Rural, Homs, Hama, and Aleppo. In addition, further investigations in vineyards will verify BN incidence , as well as, both insect vector and alternative hosts, considering the wide host range for BN phytoplasmas.

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