SHORT TERM SCIENTIFIC MISSION

FINAL REPORT

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Improved Methods for Fruit Trees Phytoplasma Strains Characterization

This Short Term Scientific Mission (STSM) was held in DISTA, University of Bologna Phytoplasmology laboratory under the supervision of Prof. Dr. Assunta BERTACCINI between the dates 02.07.2012 /18.07.2010.

Background

The presence of fruit tree phytoplasmas in Turkey has been described since 1999. Since then, the presence of European stone fruit yellows, pear decline and apple proliferation phytoplasmas has been detected in the main fruit tree growing areas. Results from laboratory testing indicate the presence of these phytoplasmas in stone fruit orchards of Turkey. Molecular identification of 16SrX group (apple proliferation group) is performed by specific PCR on the 16S rRNA gene of fruit tree samples.

Purpose of visit

The main objectives of this work were to verify the presence of genetic variability in selected strains of fruit tree phytoplasmas detected in phytoplasma infected samples collected from Turkey, to improve knowledge in Turkey about detection methods for phytoplasma diseases in fruit trees and to clarify the presence of different groups among the identified strains.

Description of the Work During the Visit

Fresh plant tissues were collected during monitoring of the presence of fruit tree phytoplasmas in 2012 from different regions of Turkey. Total DNAs were extracted from leaf midribs and phloem tissues of collected samples according to Doyle and Doyle (1990). Totally 24 stone fruit samples (16 apple, 1 pear, 4 sweet cherry, 1 apricot, 2 Myrobalan) were extracted (Table 1).

In addition, thirty-eight DNA samples of stone fruit trees that were shown to be positive by PCR testing of the 16S rRNA gene were selected for the study. All DNAs were amplified using direct PCR with universal phytoplasma primers P1 (Deng and Hiruki, 1991) and P7 (Smart *et al*., 1996).

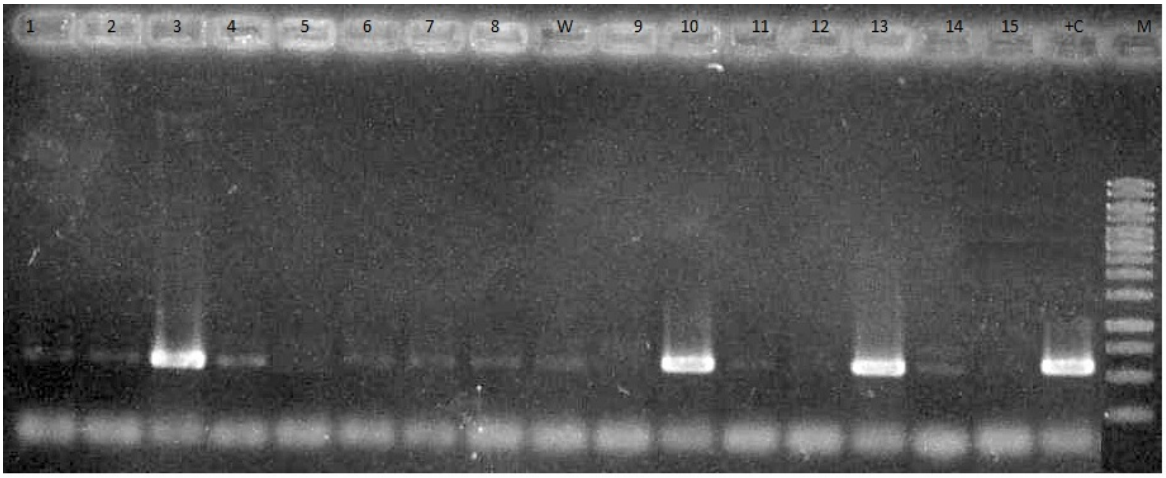
Amplicons produced were diluted (1:29) in sterile distilled water and 16RF2n/R16R2 primers were used for first nested PCR followed by RFLP analyses with *Tru1*I. According to the results group-specific primer pairs were then employed in further nested-PCR assays. All PCR reactions were performed with a mixture of 25 µl and for each PCR reaction one positive control and water as negative control were used to check the reliability of the reaction. PCR results were analysed on 1% agarose gels and visualized by staining with ethidium bromide under UV transillumination (Fig.1). Further restriction endonuclease (RE) enzymes used for RFLP (Restriction Fragment Length Polymorphism) analyses were *Rsa*I and *Ssp*I. The digested products were analyzed by electrophoresis using 6.7% polyacrylamide gels and visualized by staining with ethidium bromide under UV transillumination.

Table 1. Locations of collected plant samples and PCR results.

Number	Name /Variety	Location	PCR result
1	Apple/Starking	Malatya	-
2	Apple/Starking	Malatya	-
3	Apple/Starking	Malatya	+
4	Apple/Starking	Malatya	-
5	Apple/Starking	Malatya	-
6	Apple/Starking	Malatya	

7	Apple/Starking	Malatya	-
8	Apple/Starking	Malatya	-
9	Apple/Starking	Malatya	-
10	Apple/Starking	Malatya	+
11	Apple/Starking	Malatya	-
12	Apple/Starking	Malatya	-
13	Apple/Starking	Malatya	+
14	Apple/Starking	Malatya	-
15	Apple/Starking	Malatya	-
16	Pear/Deveci	Malatya	-
17	Apple/Starking	Malatya	-
18	Sweet cherry/Ziraat 900	Usak	-
19	Sweet cherry/Ziraat 900	Usak	+
20	Sweet cherry/Ziraat 900	Usak	+
21	Sweet cherry/Ziraat 900	Usak	+
22	Apricot	Hatay	-
23	Myrobolan 29C	Hatay	-
24	Myrobolan 29C	Hatay	+

Figure 1. PCR analyse results of tested samples. Lane 1-15: Apple samples, W: Water control, +C: Positive control, M: Marker



Phytoplasma DNAs were also subjected to molecular characterization using Tuf gene primers: TufA/B in direct PCR, and primers TufC/D in nested PCR reactions. RFLP analyses of all obtained amplicons were performed with *Tru1*I restriction enzyme (RE).

Profiles of the R16F2n/R16R2 PCR amplicons restricted with *Ssp*I and *Rsa*I show the presence of the 16SrX phytoplasmas. On the other hand some stone and pome fruit samples restricted with other enzymes showed different restriction profiles when compared with reference strains available in literature. The samples showing polymorphism different from the one of 16SrX group will be further studied by sequence analyses.

Selected samples were tested by PCR and RFLP analysis several times and have been sent for sequencing to clarify the identity of the phytoplasmas detected. Cleaning and aligning of these sequences are in progress.

These DNA and PCR product samples will be maintained at DISTA, University of Bologna for further studies on fruit trees phytoplasma diversity under framework of WG1 COST FA0807 action. Further analyses based on the sequences analyses of Turkey and other countries samples will give clearer insights into the fruit tree phytoplasma diversity, polymorphisms and they role in the disease epidemiology.