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 03.02.2010 / 26.02.2010.

Purpose of the Visit

Turkey is one of the main genetic origins of many grapevine and fruit tree varieties and rootstocks. Fruit and grapevine production are of major importance for Turkish agriculture and trade. Phytoplasma diseases and the vectors of these diseases are new research areas in Turkey. The purposes of this visit were to improve knowledge in Turkey about detection methods for phytoplasma diseases in fruit trees and grapevine and in their vectors, and to clarify the presence of molecular differences among the identified strains.

Description of the Work During the Visit

Fresh plant tissue, extracted DNA and PCR samples were the main materials of the work. Apple, pear, apricot and grapevine samples were the source of phytoplasmas. 35 (17 apples, 9 pears, 4 grapevines and 5 apricots) samples were analyzed. Plant species, suspected phytoplasmas, symptoms of the collected plants and provinces are shown on table 1.

Table 1: Plant species, suspected phytoplasmas, symptoms of the collected plants and provinces

Host	Suspected phytoplasma	Symptoms	Province
Apple	Apple proliferation	Enlarged stipules, Rosette formed growth of shoots, Autumn blossom	Isparta Yalova Ankara Antalya
Pear	Pear decline	Severe reddenning, Early defoliation after redening, Leaf rolling	Yalova Ankara
Apricot	European stone fruit yellows	Yellowing in summer, Leaf roll	Antalya
Grape	Bois noir Flavescence dorée	Severe redening, Leaf yellowing and rolling	Manisa Malatya

As the first step DNA was extracted from fresh plant tissue using a phenol/chloroform extraction method. Essentially 1g of fresh plant tissue was ground with liquid nitrogen in a grinding buffer, the sample was transferred to a 15 ml tube and centrifuged at 11,000 rpm for 30 min. The supernatant was discarded and the pellet was re-suspended in 4 ml extraction buffer. Eighty µl proteinase K and 400 µl 10% SDS were added and incubated at 55°C for 1 hour. The samples were centrifuged at 8,000 rpm for 10 min after incubation. The supernatant was mixed with 0.6 volume isopropanol and incubated at -20°C for 30 min. Samples were then cleaned by chloroform/isoamylalcohol/phenol and centrifuged at 8,000 rpm for 10 min. The supernatant was precipitated with 0.6 volume isopropanol. The pellet was washed with 70% ethanol, centrifuged and was diluted with TE buffer. The concentration of DNA was determined and all samples were amplified using direct PCR with universal phytoplasma primers P1/P7. Amplicons produced were diluted (1:29) in sterile distilled water and 16RF2n/R16R2 primers were used for first nested PCR. According to the results and to the different plant species, 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. For specific detection of PCR products, *Tru*I, *Rsa*I and *Ssp*I restriction endonuclease (RE) enzymes were used for RFLP (Restriction Fragment Length Polymorphism) analyses. 16RF2n/R16R2 and group specific primer pairs amplicons were restricted using these RE enzymes. Fragments were analysed on

5% polyacrylamide gels and visualized by staining with ethidium bromide under UV transillumination. Plant species, group specific primer pairs and RE enzymes employed are shown in table 2.

Table 2: Plant species, primer pairs employed for PCR reactions and RE enzymes

Plant species	**Primer pairs**	**Restriction Endonuclease enzymes**
Apple	P1/P7 R16F2/R2 (X)F1/(X)R1 (I)F1/(I)R1 mF1/mR1 rpAp15f/ rpAp15r	*Rsa*I *Ssp*I *Tru*I
Apricot	P1/P7 R16F2/R2 (X)F1/(X)R1 mF1/mR1	*Rsa*I *Ssp*I *Tru*I
Grapevine	(I)F1/(I)R1 (V)F1/(V)R1 mF1/mR1	*Rsa*I *Ssp*I *Tru*I
Pear	P1/P7 R16F2/R2 (X)F1/(X)R1 mF1/mR1 fO1/rO1 rpAp15f/ rpAp15r	*Rsa*I *Ssp*I *Tru*I

According to the restriction profiles of the amplicons, phytoplasmas associated with 16SrX group (apple proliferation group) subgroup–A (‘*Candidatus* Phytoplasma mali’) and 16SrX subgroup-C (‘*Candidatus* Phytoplasma pyri’) were detected.

Some from the apple, pear and apricot samples, and a grapevine sample showed different restriction profiles from the expected ones. Mixed infection-like profiles were observed on some of these samples. These 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. Identification of phytoplasmas detected in grapevine is also in progress.

Insects vector of potential vector of phytoplasma diseases were also analysed for phytoplasma detection. Male and female individuals of diverse species were extracted using a chloroform DNA extraction method.

In this working period, inoculation of the phytoplasmas from infected periwinkle to healthy ones by grafting was also performed.

Although pome and stone fruit trees phytoplasmas and their vectors have been searched for previously in Turkey, this is the first search of vineyards for grapevine phytoplasma diseases and their vectors in Turkey. Therefore this scientific visit was very important for the visitor country as well as for the researcher and it provided experience to the researcher.

The results of the work will indicate the direction for future work on both phytoplasma diseases and the insect vectors of these diseases in Turkey.