COST STSM Scientific Report - Seçkin Eroğlu

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STSM Title: Genotyping methods applied to stolbur phytoplasma isolates from Turkey.

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Genotyping methods applied to stolbur phytoplasma isolates from Turkey

Background: Phytoplasmas are non cultured microorganisms. In order to prevent the damage they cause, early detection techniques are of vital importance due to their long latency period. Different phytoplasmas may use different plants as reservoirs, and can be transferred by different bugs. Developing effective prevention strategies against phytoplasma epidemics would only be possible with the identification of the specific phytoplasma strains, vector bugs and plant reservoirs for the corresponding strains.

Phytoplasma strains can be characterized by comparison of the nucleotide sequences of ribosomal and non-ribosomal marker genes. Recently, new marker genes and specific primers to amplify these regions by PCR were developed. Phytoplasma strains in Europe were characterized intensively by those genotyping techniques; however there is a lack of information on the diversity and distribution of phytoplasmas in eastern Europe and the Mediterranean region.

Purpose of the visit: The aim of this scientific mission was to explore genetic diversity of phytoplasmas in East Europe-Mediterranean countries. This included (i) to amplify the non-ribosomal marker genes by nested PCR and (ii) to sequence the amplicons and analyse the results to explore genetic diversity.

Description of the work carried out during the visit:

In order to gain experience on phytoplasma genotyping from the beginning to the end, all steps were discussed separately and practiced.

i) *Quality control of isolated phytoplasma DNA stock:* Stored DNA can be degraded by time, especially when it is dissolved in water or subjected to continuous freeze-thaw cycles. DNA degradation results in false negatives in attempts to amplify phytoplasma DNAs by PCR. Therefore; all the stocks of phytoplasma DNAs used in the project so far were analysed to ensure DNA is not degraded. The DNA concentrations were measured by a nanospectrophotometer and results were recorded.

ii) *Isolation of phytoplasma DNA from new plant samples:* DNA isolation with CTAB protocol was practiced by using fresh leaf midribs from newly collected ornamental plants as a plant material. Positive (infected periwinkle from the

greenhouse [DAB and P-7]) and negative controls [non-infected periwinkle]) were included.

iii) *Amplification of marker genes by* direct and/or *nested PCR:* Following primer pairs were used in nested PCR reactions to amplify marker genes from the samples (Table 1). During my stay, samples which were collected from Turkey, Lebanon, Israel and Bulgaria were analyzed.

Primer Pairs used in Nested PCR	**Target Gene**	**Expected band size**
poSECF1/poSECR1 and PosecN2/PosecR3	*secY*	1052 bp and 887 bp
StolH10F1/StolH10R1 and TYPH10F/TYPH10R	*vmp*1	1189 to 1438 bp
Stamp-F/Stamp-R0 and StampF1/StampR1	*stamp*	637 bp and 578 bp

Table 1: Primer pairs used in PCRs and the expected band size

iv) Electrophoresis of amplicons: Capillary electrophoresis was used (Qiaxcell). Band sizes and intensities were recorded. Amplicons which have the expected size and considerable intensity were chosen to be prepared for sequencing.