

COST FA 0807

WG1 School

Methodologies to Improve Phytoplasma DNA Extraction from Plants and Insects

3-7 September, 2012

Ankara University

Turkey

The aim of the course was to teach phytoplasma extraction methods from different host plants and insects.

Monday - September 3, 2012

Prof. Assunta Bertaccini, Prof. Piero A. Bianco, Dr. Nicoletta Contaldo, prof. Filiz Ertunc, mrs. Didem Canik prepared final course schedule and materials needed. Fresh pear, peach, grapevine and plum samples were collected to be used in the training course. Profs. Ertunç and Bertaccini made a welcome speech and gave brief information about the course. Each participant introduced himself to the audience and gave brief report on what they are working on and also their purposes for joining such a training school. A list was prepared including the names of the participants and the different infected host samples which they would work with during the course. Plant samples were distributed to the participants according to that list. Participants prepared their plant material for next day's work: they cut off the main veins which includes phloem tissue by using electron microscopy forceps, weighted as 1 g, put into the sealed plastic bags and put them all to the refrigerator.

Tuesday - September 4, 2012

Samanta Paltrinieri, Didem Canik, Dr. Rosemarie Tedeschi and Dr. Luca Ferretti trained the DNA extraction. Trainees took their samples from the refrigerator and grinded them in liquid nitrogen with sterile mortar and pestle. Plant total nucleic acid (DNA) was extracted according to modified protocol of Lee *et al.*, 1991 and Prince *et al.*, 1993. The protocol was applied up to the stage of overnight staying in the refrigerator, then all of the samples were maintained at 4°C overnight. After the end of that days schedule, a tour to Hamamönü (old central town of Ankara) was carried out.



Wednesday - September 5, 2012

Samples were taken from 4°C and plant total nucleic acid extraction protocol was completed. Obtained DNA's concentrations were determined by nanodrop reading. Trainees started DNA extraction from insect tissues (supplied by Dr. Rosemarie Tedeschi) according to the insect DNA extraction protocol of Marzachi *et al.*, 1994. *Hyalesthes obsoletus* and *Cacopsylla melanoneura* were used and insect total nucleic acid extraction protocol was completed on the same day. Concentrations of obtained insect vector DNAs were measured by nanodrop reading. DNAs were prepared for direct PCR and direct PCR was started using the primers P1/P7.

In the evening, social dinner was held in a traditional Turkish meat restaurant.



Thursday - September 6, 2012

In the morning, participants diluted direct PCR products for nested PCR at the dilution of 1/30. PCR master mix was prepared and nested PCR was started with the primer pair R16F2n/R16R2.

Participants left the department for field trip to Kalecik province. Kalecik is a famous province of Ankara with a special wine grape variety and also the wine called "Kalecik Karası". Experimental vineyard of Ankara University was visited in Kalecik which is a small town and 70 km far from the centrum of Ankara. Participants observed and collected grapevine yellows phytoplasma (mainly 'bois noir') infected plants and tasted different grapevine varieties. Turkish traditional style of lunch (Pide) was also tasted. After lunch, a wine factory (Bak Şarapçılık) was visited and different kinds of wines they produced were tasted. Winery was visited and information about the vineyards belonging to the company and their wine production were provided.



Friday - September 7, 2012

An agarose gel was prepared and amplified PCR products were loaded to the gel, the gel was run at the conditions 100 V, 45 min. Gel was stained by EtBr for 10 min. and visualized under image analysing system of Syngene. Obtained results and course outcome were discussed between trainers and trainees.

Participants left the department of Plant Protection, Faculty of Agriculture, University of Ankara in the afternoon for the departure to their countries.

