COST Action FA0807 Integrated Management of Phytoplasma Epidemics in Different CropSystems

Short-term Scientific Mission (STSM) Report

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STSM Topic: New technologies applied to fruit trees phytoplasma detection and their molecular characterization

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Purpose of the visit:

Since some phytoplasmas have been reported affecting fruit trees in Chile, the aim of this STSM was to study the molecular variability among them using different molecular techniques and approaches in order to understand the epidemiology of the associated diseases.

Description of the work:

The work started with a monitoring of different plant species showing symptoms possibly related to phytoplasma infection. A number of samples of peach, nectarine, apricot, plum were collected in different orchards, mainly located in the Metropolitan Region of Chile. More samples collected during January/February of 2011 and 2012 from symptomatic kiwi and fig plants that were available in the lab as freeze dried material were also employed for the testing to verify phytoplasma presence.
One gram of midribs and phloem scrapes from the newly collected and the freeze dried samples were subjected to DNA extraction using a phenol/chloroform protocol (Lee *et al*., 1991). Aliquots of 1 to 2 g were maintained at -80°C or as freeze dried samples in order to assess different extraction methods if needed. Detection and identification of phytoplasma presence in the samples was confirmed to be difficult because of the presence of many Taq polymerase inhibitors. This is a known problem in Chilean plant materials especially from previous work carried out on grapevine samples: the problem is likely due to the climatic conditions and to the ozone hole that induced different oxidative responses in to the plants especially into those that are not healthy.

Aliquots of 20 ng/µl of total extracted nucleic acid were then used in the nested-PCR analyses performed in parallel on 2 different genes, 16SrDNA and tuf, in order to avoid the presence of false negative.
After PCR analyses carried out on 16Sr gene, using universal primers P1/P7 followed by nested PCR with both, R16F2n/R2 and $16R_{758f}$/$16R_{1232r}$ (M1/M2) all the samples tested resulted as negative, while the majority of the samples were positive when tuf amplification strategies were used (Contaldo *et al*., 2011). Direct sequencing of positive amplicons to verify and confirm the presence of phytoplasma is in progress.
Therefore in order to improve the detection methods we tried other DNA extraction protocols (i.e. CTAB, kits) and different primer set combinations amplifying the 16S rDNA gene. The tests to verify the usefulness of these systems are now carried out in parallel in the two laboratories (Santiago and Bologna) and the comparison of the results will allow to verify the phytoplasma presence in Chilean fruit trees and also to determine the best procedure for phytoplasma detection in those 'difficult' environmental conditions.
Besides this work with conventional molecular technique, we exploited as well the deep amplicon sequencing technique therefore primers already employed for deep sequencing (Nicolaisen *et al*., 2011) were tested on positive Chilean control samples from periwinkle and resulted in amplification of the expected 480 bp fragments. However all the verification of cost for applying deep sequencing technique in Chile do not allow to continue the experiments under the present budget conditions.

During the STSM I gave a seminar titled "Phytoplasma impact on agricultural relevant crop" to explain the impact of these diseases in the chain of fruit production. The seminar was presented at the Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Sanidad Vegetal,Santiago, and it was attended by a number of students, researcher and specialists. Interesting discussion followed the seminar about phytoplasma detection methods and their control in the field.

Conclusion and future collaboration

An informal collaboration was agreed on the full identification and molecular characterization of phytoplasmas detected in all collected and shared samples. The data will be very useful to implement management of phytoplasma – associated diseases in COST countries as well as in Chile fruit tree orchards.

References

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