**Scientific report for Short Term Scientific Mission (STMS) in the frame of the COST Action FA1103: Endophytes in Biotechnology and Agriculture**

# Species and strain specific detection and visualization of root endophytic fungi.

## Applicant

Wael Yakti, Leibniz institute for vegetable and ornamental crops, Grossbeeren (DE)

## Host

Dr. Gábor Kovács, Eötvös Loránd University, Institute of Biology, Department of Plant Anatomy, Budapest (HU).

## Time of Visit

6th of April 2015 – 23th of April 2015

**COST STSM Reference Number**: COST-STSM-FA1103-24755

- **Purpose of the STSM**

The main aim of my visit was to learn the microscopy techniques used and developed by the host group, particularly rRNA fluorescent in situ hybridization (FISH) (Vági et al, 2014). Additionally, to get an insight on other projects dealing with the same dark septate endophytes I Investigate in my PhD, carried out by the host group.

- **Description of the work carried out during the STSM**

On the first week of my stay I started, with the help of the host group, applying the FISH protocol on root samples from one of my experiments which I brought with me to Budapest, in addition to positive samples provided by the host group. The specific FISH probes were applied to detect the two species (*Periconia macrospinosa*, and *Cadophora* sp.) and to visualize them. Furthermore, WGA labeling was also used and compared with the FISH method.

After preparation, the samples were observed under Epifluorescence microscopes on both the first and the second week and I acquired the skills needed to use fluorescence microscopy to visualize and localize the fungi for my work at the home institution.

On the second and the third week I continued the visualization of more of my samples, and started to optimize a protocol combining both methods (FISH and WGA) and applying them on one specimen. Additionally, I joined the group in the optimization of newly designed qRT-PCR which will help us in the molecular detection and quantification of the dark septate endophytes in the root.

Furthermore, the host group arranged a trip to the great Hungarian plain, the region from which the two dark septate endophyte species I am working on were isolated, and had a closer insight into conditions of that region.

- **Description of the main results obtained**

Since the Dark septate endophytes are, in many cases, very hard to visualize using conventional staining methods, FISH and WGA allowed me to see the fungi and their structures in the roots (Figures above)

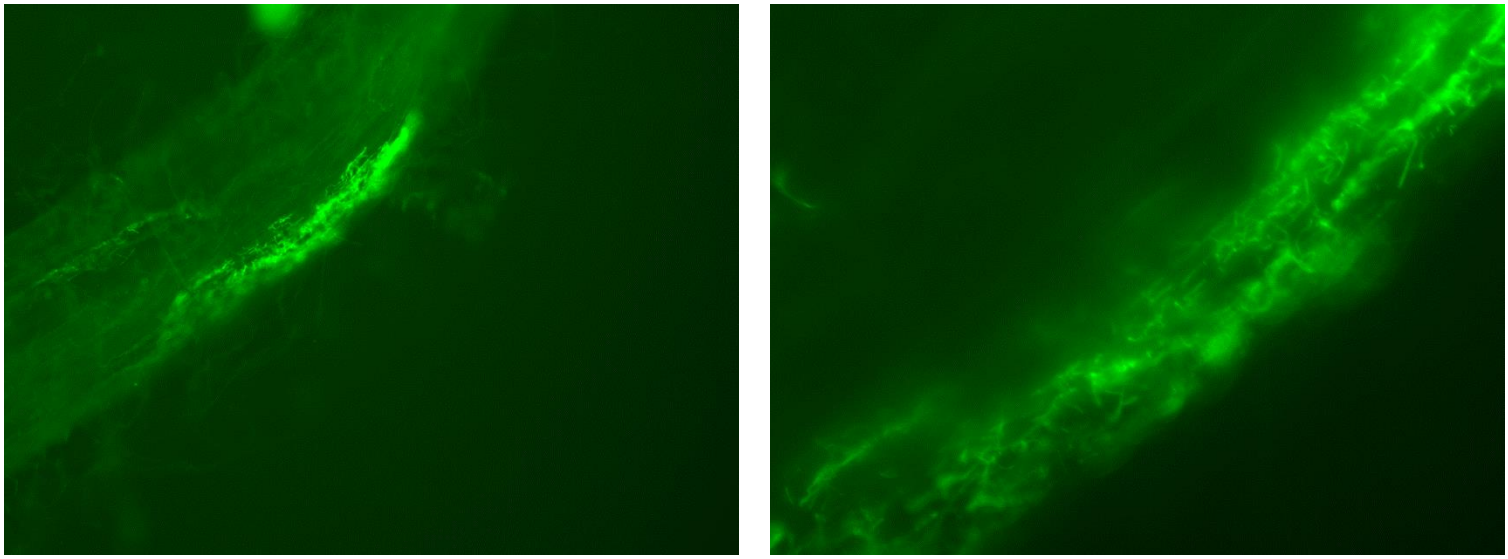

Figure A: tomato root colonized by *Cadophora* sp., the sample was labeled using the *Cadophora sp*. Specific FISH probe.

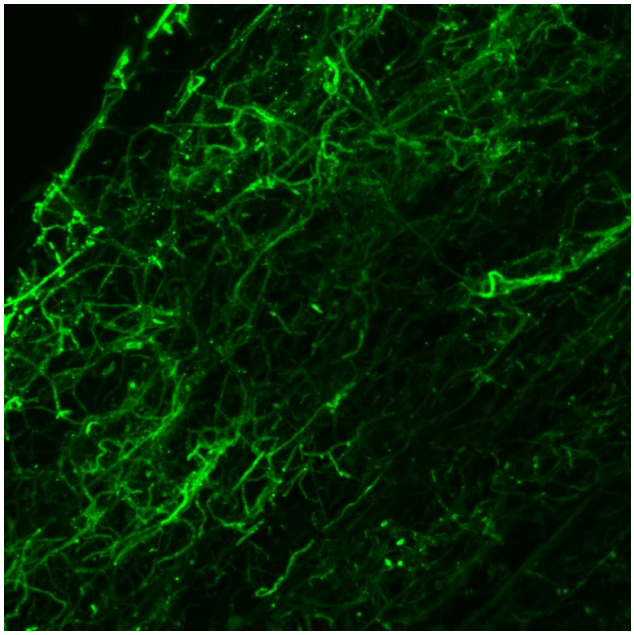

Figure B: tomato roots colonized by *Cadophora* sp., Image taken using confocal laser microscopy

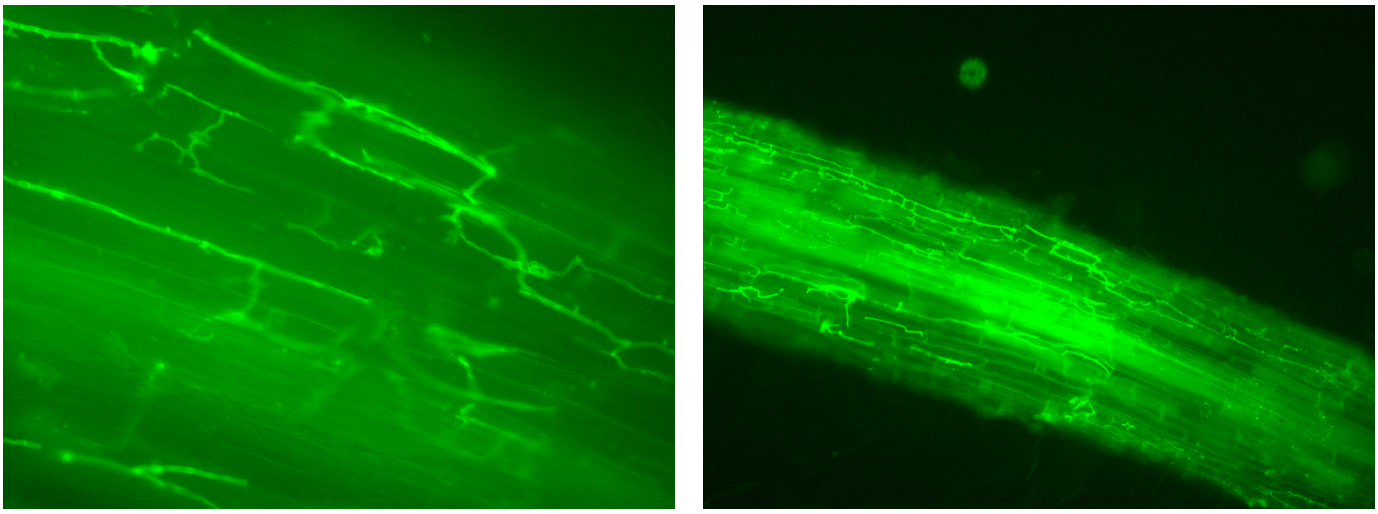

Figure C: tomato root colonized by *Periconia Macrospinosa.* Images taken using confocal laser microscopy.

- **Future collaboration with the host institution.**

My stay in Budapest enabled me to have a better contact with the host group. Many discussions and meetings with the group members took place during the period of my stay, and the work of both my home and host groups on the DSE species was coordinated to have a better Future collaboration.

**Reference**

Vági, Pál and Knapp, Dániel and Kósa, Annamária and Seress, Diána and Horváth, N. Áron and Kovács, M. Gábor (2014)*Simultaneous specific in planta visualization of root-colonizing fungi using fluorescence in situ hybridization (FISH).*MYCORRHIZA, 24 (4). pp. 259-266. ISSN 0940-6360