COST Action FA1103 – Endophytes in Biotechnology and Agriculture

Scientific report for Short Term Scientific Mission (STSM)

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Title: **Response of plant roots and mycorrhizal fungi to soil hypoxia**

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Host institution: University of Ljubljana (UL). Biotechnical Faculty (BF).

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Host person: Dr Irena Maček

Participating researchers: Nataša Šibanc, Ph.D. researcher at Biotechnical Faculty, University of Ljubljana; Dr Peter Železnik, Ph.D. researcher at Slovenian Forestry Institute, Ljubljana.

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1. Purpose of the STSM

The main purpose of this STSM was to learn the methods to assess the diversity and ecology of fungal endophytes and plant root development under hypoxic conditions in soil (lack of O_2). These conditions occur in natural CO_2 springs or mofettes, with severe long-term changes in soil gas composition (high CO_2 concentration and hypoxia) due to geological ambient temperature CO_2 exhalations. This natural environment is present in north-eastern part of Slovenia.

As our research object we used a C_4 weed barnyard grass (*Echinochloa crus-galli (L.) Beauv*.) that has been reported before to grow well and to be colonized by endophytic fungi in these environments.

The methodology for the evaluation of root fungal colonization as a response to long-term stress, on *E. crus-galli* root system was applied. The evaluation of the root development of the barnyard grass in those conditions (seeded and grown on an agricultural soil exposed to geological CO_2) was assessed as well.

2. During the period of the STSM, the following tasks were carried out:

- **<u>Field sampling and measurements:</u>**

The experiment was conducted in the area of natural CO_2 springs (mofettes) in Slovenia, and was designed to follow the growth and root colonization with fungal endophytes in plant roots of *E. crus-galli* in three different CO_2 soil exposures: control (<1 % CO_2), medium CO_2 and high CO_2 exposed plot with peak CO_2 concentrations 7.7 ± 2.0 % CO_2 and 34.5 ± 6.7 % CO_2, respectively for medium and high CO_2 exposed plot, respectively. For each location, holes in the ground were dug and were filled with homogenized soil from a control site (before the start of the STSM).

Soil CO_2 concentration were measured as well during sampling. Five measurements per plot (4 plots per CO_2 soil exposure) were done with Gas analyzer GA 2000. In total, 120 soil CO_2 concentration were collected to improve the previous data on the gas regime in this area.

Within the field sampling extraction of the ingrowth soil cores that were installed two months before the sampling (before the start of the STSM) was done in order to obtain the root system.

Soil samples were taken, dried and stored for latter chemical analyses.

Chlorophyll content of *E. crus-galli* was measured in every plot with *SPAD* 502 Plus *Chlorophyll* Meter. 10 repetitions per subplot were done (4 subplots are in each CO_2 soil exposure).

Figure 1: extraction of the ingrowth soil cores in the field in the Stavešinci mofette area.

- **<u>Assessment of root parameters (biomass, root length, average diameter and specific root length)</u>**:

Samples were soaked in water and sieved through sieves of different sizes, in order to clean the substrate from the roots.

Figures 2 and 3: cleaning process of ingrowth soil cores.

Afterwards, the roots were divided into alive roots, dead root and debris under binocular.

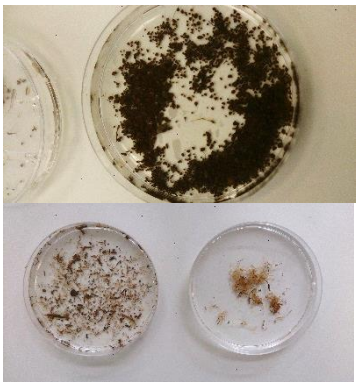
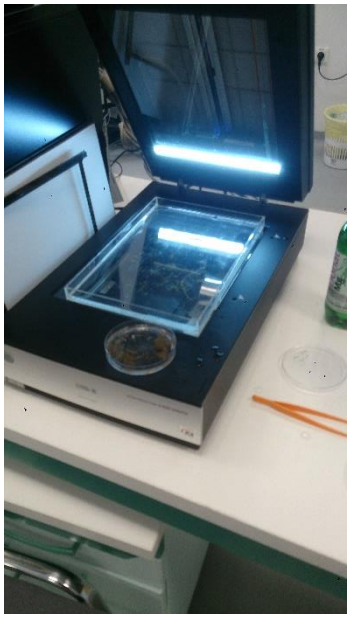


Figure 4 and 5: Sorting roots under binocular.

Roots were scanned in water on an optical scanner and root parameters were measured with WinRHIZO® (Régent Instruments Inc., CA) software. Subsequently, the roots were dried in an oven and weighed.



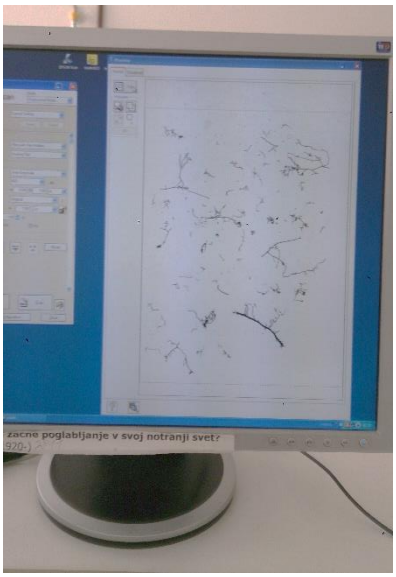


Figure 6 and 7: scanning roots for WinRHIZO® analysis.

- **<u>Estimation of root colonization with arbuscular mycorrhizal (AM) fungi:</u>**

AM fungal root colonization was assessed after clearing with 10% KOH and trypan blue staining by using an Olympus Provis AX70 microscope according to Trouvelot et al. (1986). Half of the dried root sample was stored for molecular characterization of AM fungal communities.

Two slides of 10 fragments were prepared per sample in order to evaluate the AM fungal presence in each fragment. Presence of arbuscules was estimated as well.

The following parameters were calculated using Mycocalc software (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download):

- F (frequency of mycorrhizal colonization in the root system),
- M (intensity of mycorrhizal colonization in the root system),
- m (intensity of mycorrhizal colonization in the root fragments),
- a (arbuscle abundance in mycorrhizal parts of roots fragments) and
- A (arbuscle abundance in the root system).

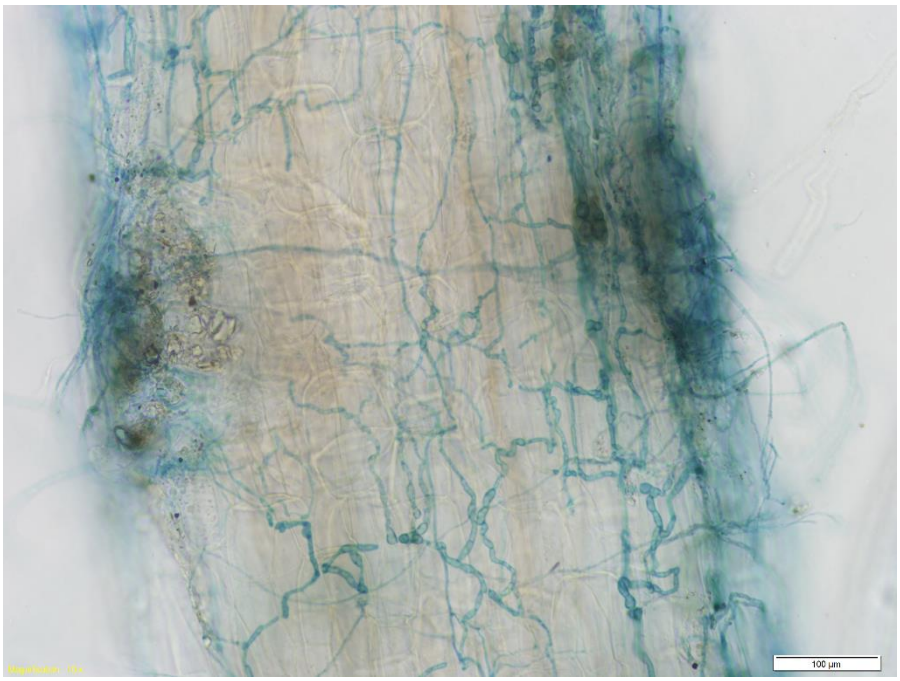


Figure 8: AM fungal root colonization.

- **Soil analysis:**

I was making soil analyses that includes the determination of soil pH (Reference method: ISO 1039D), carbonate content (Reference method: ISO 1039D) and available phosphorous (Calcium acetate lactate method) that involved preparation of the samples for soil analysis and application of the mentioned protocols on the sampled soil.

Figure 9: determination of carbonate content.

- **Introduction to new methods in molecular characterization of AM fungal communities:**

  During the visit I was introduced to molecular techniques by working alongside researchers in Dr Maček's lab. In addition I have attended a two day seminar on 'Molecular Advances in Microbial Ecology', covering the topics of next generation

sequencing and ecoinformatics at the University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Koper, Slovenia.

3. Description of the main results obtained

- No statistical difference in AM fungal colonization (m% and M% parameters) among different CO2 exposed plots was founded.
- Presence of AM fungi (hyphae) was observed on every root fragment analyzed (F = 100 %).
- At high and medium CO_2 exposed plots, root biomass (measured as root length) was lower compared to control plots.
- The concentration of available phosphorus in the samples was low: values for available phosphorus were <11 mg P/Kg in all the sites.
- Basic pH: pH values were in the range between 5.7 to 6.1, with higher values in the control samples.

4. Confirmation by the host institution of the successful execution of the STSM

I would kindly like to thank STSM Panel, for award me with this STSM funding.

Please find attached the Confirmation letter by the host institution.