Short Term Scientific Mission (STSM) report

Scientific visit to the AgResearch laboratories with the objectives of learning new endophyte-related techniques and exchange of research experiences (techniques, protocols, outcomes and related)

Applicant: Daria Rybakova, Graz University of Technology, Austria
Host: AgResearch Ltd, Lincoln, New Zealand
Dates: 07/10/2015-16/10/2015

The two main objectives of Daria Rybakova´s short scientific visit (STSM) with the AgResearch team in their Lincoln facility was to learn new techniques from the hosting organization, and foster collaboration in the field of endophytic research for plant production.

Both objectives were successfully carried out. The participant gave a lecture on the current work of her working group in the area of endophytic research. The lecture opened a broad field for discussion about the ongoing projects in endophyte-related research areas of both organizations. The talk was followed by a forum of questions and answers that was widely attended. Following the lecture, individual appointments with AgResearch employees working on plant endophytes were scheduled. The focus of the appointments was on the exchange of experience, methods and protocols as well as discussing possible collaborations between both organizations. For example, the participant consulted Dr. Mark Hurst from the Soil Biology group (AgResearch) on the recent techniques in the Confocal Laser Scanning Microscopy (CLSM) that is well established in her group. They discussed the possibility of using the fluorescence *in situ* hybridization (FISH) technique vs. labeling the bacteria of interest with fluorescent proteins in order to localize the target bacteria in the plant tissue and determine the ideal plant growth conditions for this experiment. The working protocols for FISH and CLSM were exchanged and future consultations per email and phone were agreed upon. The discussion with Dr. Steve Wakelin (Soil Biology group, AgResearch) was focused on the exchange of knowledge and protocols on the MiSeq rRNA amplicon sequencing from roots and soil. The meeting with Jayanthi Swaminathan (Soil Biology group, AgResearch) resulted in the discussion of a collaboration in the area of formulation of endophytes with biological control properties for the applicant’s project. Additionally, several meetings were

organized with the BioProtection centre (a partner organization of AgResearch). A possible collaboration in area of research of endophytic microorganisms of Brassicaceae was discussed with the group of Prof. John Hampton (BioProtection Centre, Lincoln). Another interesting collaborative project may result from the new connection with the group of Prof. Hayley Ridgway (Lincoln university) that works on endophytes of medical plants, in particular of Manuka (*Leptospermum scoparium*), native New Zealand tree. The discussion was focused on the potential for the TU Graz to carry out bioinformatics research and confocal microscopy of Manuka endophytes in a possible joint project between both organizations.

The second part of the STSM was dedicated to learning new endophyte-related techniques. The participant assisted the employees of the host institutions with their ongoing tasks while learning new techniques and sharing experiences on related topics. The main focus was on the learning of techniques such as seed formulations with mycorrhiza inoculum and identification of the rhizosphere and endosphere microbes responsible for pest suppressive and plant growth responses. The participant worked on the mycorrhiza project with the Soil Biology group, (AgResearch Lincoln). Her task was to determine the Most Probable Number (MPN) of propagule densities which is described below in detail.

Background:

Arbuscular mycorrhiza are symbiotic relationships between fungi and plant roots. They are known to protect host plants from the harmful effects of drought and to improve the nutrient uptake and growth of plants under water stress conditions (Fig. 1).

Fig. 1: Effect of mycorrhiza treatment on plants growth. Left: plants that were supplied with arbuscular mycorrhiza; right: plants grown without arbuscular mycorrhiza.

One of the mechanisms of the mycorrhizal symbiosis on host plant water balance is the increased root biomass and the subsequently plant size. In particular the mobilization and uptake of phosphorus is often related to an increase in plant size. One of the successful areas of research that AgResearch is doing in collaboration with GrassLands (New Zealand) is the propagation of arbuscular mycorrhizal fungi.

Description of the work carried out:

In the preliminary project the *Plantago lanceolata* seedlings were grown in a series of 10-fold dilutions of mycorrhizal formulation in soil. Each seedling was grown in a separate cell of a seedling tray (Fig. 2).

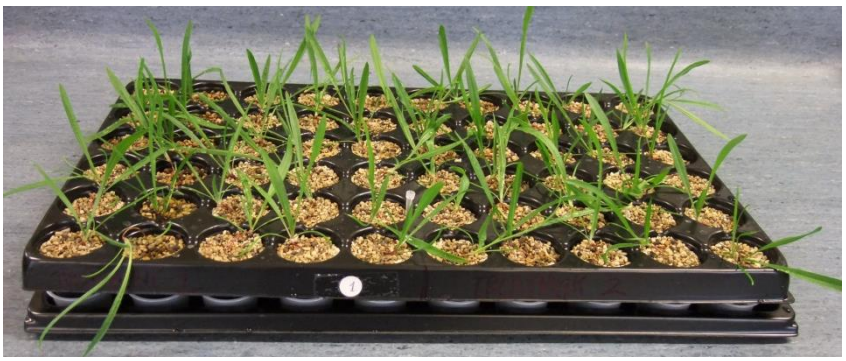

Fig. 2: Assay set up for the MPN determination. The seedlings were inoculated with different dilutions of formulated product and grown in cells.

The presence of infective mycorrhizal propagules was detected by clearing and staining the roots and examining them for mycorrhizal colonization The number of viable mycorrhizal propagules present in each sample was estimated by determining the presence or the absence of colonization. The roots of five replicates were checked for absence or presence of mycorrhizal microscopically (Fig. 3). MPN was calculated from the numbers of roots in successive dilutions with and without mycorrhiza using equations based on probability theory. The results of the test were provided to AgResearch and cannot be published due to a confidentiality agreement.

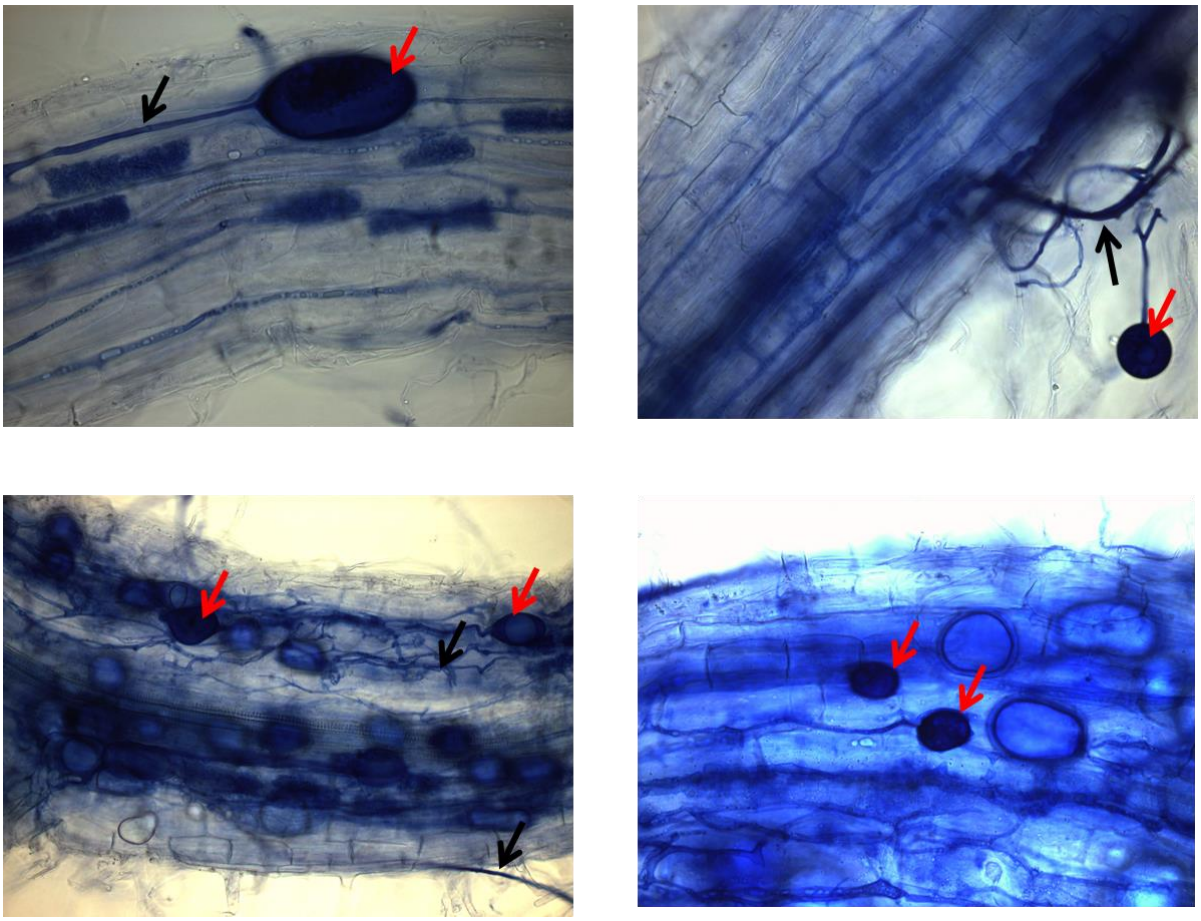

Fig. 3. Examples of microscopical images of plant roots containing arbuscular mycorrhiza. The arbuscular mycorrhizal hyphae (black arrows) are stained dark blue; the dark blue mycorrhizal spores are highlighted with red arrows. The plant tissue is stained light blue.