REPORT of the SHORT TERM SCIENTIFIC MISSION, COST FA1208-ID n°250116-071242

Title: Identification of nuclear plant targets of *Meloidogyne incognita* effectors

Start date: 14/03/2016 End date: 28/03/2016

Applicant: Nhat My TRUONG, UMR ISA, Sophia Antipolis France

Host: Dr. Maëlle Jaouannet

Dr Jorunn Bos's Lab, the James Hutton Institute (JHI), Dundee, Scotland, UK.

Background: The root-knot nematodes (*Meloidogyne* spp.) are extremely polyphagous and are on the top most important pests affecting tomato production worldwide. They are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks and induce the differentiation of root cells into specialized multinucleate feeding cells, named giant cells. Nematode effectors synthesized in the oesophageal glands and injected into the plant tissue through the syringe-like stylet play a central role in giant cells ontogenesis. In the Plant-Nematode Interactions group at Institut Sophia Agrobiotech (ISA), in Sophia-Antipolis, we aim at identifying plant cellular functions directly targeted and manipulated by *Meloidogyne incognita*. We have searched for tomato targets of *M. incognita* secreted proteins that are directed into the host cells (named effectors or EFF) using a yeast-two hybrid (Y2H) approach.

Objectives/ Purpose of the visit: As Y2H screens may generate a significant number of false positives, my aim was to learn how to perform co-immunoprecipitation (Co-IP) following transient expression in *Nicotiana benthamiana* in order to validate whether the identified nematode effector-tomato prey interactions occur in plant cells. I hoped to validate two nematode effector-tomato prey interactions that I identified in the course of my PhD. Later on, I would be able to bring back this expertise in our laboratory in Sophia-Antipolis.

Description of the work: I had already isolated *Agrobacterium tumefaciens* carrying GFP-effector or RFP-prey fusions constructs to be expressed *in planta* (two *M. incognita* nuclear effectors EFF1 and EFF18 and their respective tomato preys). In Dundee, I agro-infiltrated *N. benthamiana* leaves with these two pairs of nematode effectors and tomato preys in order to express both proteins *in planta*. Proteins were extracted from tobacco leaves, and extracts used for Co-IP according to the lab protocol and under the supervision of Dr. Maëlle Jaouannet. Both input and co-immunoprecipitated proteins

were loaded on a SDS-PAGE before western-blot analysis using GFP and RFP specific antibodies.

Main results:

The signals of GFP and RFP were first detected by confocal microscopy, showing that the GFP-effector and RFP-prey fusions were well produced in *N. benthamiana* and localized in the correct cellular compartment, i.e. the nucleus. Proteins were extracted from tobacco leaves at 3 days after infiltration, and Co-IP performed. After several assays and optimizations, we were able to show that each nematode effectors (EFF1 and EFF18) co-immunoprecipitated with their respective targets. Consequently, the physical interaction between the 2 nematode effectors and their targets was validated.

Beyond the experimental results obtained during this short-term mission, this training has provided me the opportunity to practice and get familiar with the co-immunoprecipitation method. The use of Co-IP to study protein-protein interactions has been discussed several times in my laboratory but, due to the lack of appropriate expertise, we have been unable to use it up to now. This training opportunity in Dundee will not only allow me to implement this technique for my own research project but also to transfer knowledge to other members of my group at the Institute Sophia Agrobiotech.

Future collaboration with the host institution (if applicable):

During my mission at the James Hutton Institute, I had been invited to give a talk about my research project during a weekly seminar of the institute. In order to expand my professional network and for future collaboration, I had the opportunity to discuss with several senior researcher of the institute, especially Prof. John Jones and Dr. Craig Simpson. I also had the opportunity to participate to a Synthetic Biology Workshop that was held at the institute during my stay. I really hope that these meetings will lead to further collaborations between our groups.

Projected publications/articles related to or resulting from the STSM:

The result from the STSM will be a major contribution for a publication that will be prepared at the end of my thesis.