FELLOWSHIP SUMMARY REPORT

Prof. Dr. María Pérez-Fernández

Impact of the agricultural fungicide phosphite on the phosphoproteome of the *Arabidopsis thaliana*

Theme 3:

Host Institution: Queen's University, Kingston, Ontario, Canada
Host Supervisor: Prof. William Charles Plaxton
Dates: May 1st to September 4th

Consent is given to post this report on the Co-operative Research Program's Website

1. Relevance

To the Co-operation Research Program Objectives: The Co-operative Research Programme mainly aims to strengthen scientific knowledge and provide relevant scientific information and advice that will inform future policy decisions related to the sustainable use of natural resources, in the areas of food, agriculture amongst others. The present project demonstrates that Phi treatment causes a dramatic reduction in the growth and Pi content of Pi-deprived suspension cells of the model plant *Arabidopsis thaliana*. We have also demonstrated that Phi specifically interferes with the Pi-starvation response of *A. thaliana* and that it also alters the *in vitro* phosphorylation of several polypeptides during Pi stress.

As Phi is regularly sold in all world markets as a fertilizer to control fungal infection, it is indiscriminately used in crops, inducing severe P pollution and putative contamination of food products that will be use by either, humans or animals. Our findings place the right scenario to open political discussion on how to regulate the use of P, one of the fundamental plant-nutrient that is in severe depletion worldwide.

- **To the Theme:** This project addressed Theme III 'Transformational technologies and innovation'. Particularly the project falls into the Advanced breeding tools/Genetic and genomic technologies. Our research has concentrated on the identification of proteins whose phosphorylation status is significantly modified in Pi-starved vs. Pi-resupplied *Arabidopsis* cells and seedlings. We have also pay attention to the influence of Phi on this process and how Phi attenuates plant Pi starvation responses (positive or negative). The entire project requires the use of proteomic tools. Proteomics focuses on characterization of protein function so as to interpret the biological relevance of genome sequences. Protein function and cellular responses to external signals are often influenced by a pivotal post-translational protein modification known as reversible phosphorylation, which has been analysed in the model plant *Arabidopsis thaliana*.

- **To agricultural and food policy**: This project has proven, using the model plant *Arabidopsis thaliana*, how the phosphorylation status of plants is responsive to Pi nutrition in the presence of phosphite. Phosphite is a universally applied commodity used to prevent crop infection by fungal pathogens. It is widely used and with little control, contributing to P depletion, contamination due to excess P application that in turn induces decrease in plant growth and performance. Keeping in mind this information, the application of phosphate based fertilizers of fungal control agents should be revised and reformulated.

- **To society**: The project contributes to the agricultural and food policy in that it has proven the detrimental effect of phosphite (Phi). Phi is known to act as a fungicide that is general sold as a super-fertilizer in order to avoid strict regulations. This way, Phi is openly introduced in crops with little or no control. This chemical clearly reduces plant growth and can accumulate in the tissue of plants that are bound to feed both cattle and humans. Measurements on its regulation are needed and policy makers should take action to control the amount of Phi added to crops as fungicide and to make clear that it is a fungicide and not a fertilizer.

Studies that aim to reduce the use of chemicals in the food change are beneficial to the society because they contribute to enhance the quality of the food we intake, reducing illness and enhancing human and animal health.

2. Objectives of the fellowship

i. Overall aims of this proposal are to employ a proteomics approach to identify intracellular proteins of the model plant *Arabidopsis thaliana* whose phosphorylation status is responsive to Pi nutrition, and the influence of Phi on this process.
ii. We aimed at demonstrating that Phi treatment causes a reduction in the growth and Pi content of Pi-deprived suspension cells of Arabidopsis thaliana. At the same time we wanted to prove that Phi interferes with the Pi-starvation response and alters the in-vivo phosphorylation during Pi stress.
iii. At the personal level, the fellow sought to gain skills in proteomic techniques.

3. Major achievements (up to three)

From the scientific side of the project:

1. We have demonstrated that Phi treatment causes a deep reduction in the growth and Pi content of Pi-deprived suspension cells and seedlings (grown in magenta bosex) of *Arabidopsis thaliana*. Phi concentrations comparable to those required to control plant infection by pathogenic fungus have negative effects on the plant performance of cells and seedlings of A. thaliana grown under Pi deprivation. These results are consistent with previous ones on *Brassica napus* (Carswel et al., et al., 1996 and 1997)
2. We have also demonstrated that Phi specifically interferes with the Pi-starvation response of *A. thaliana*.
3. We have also concluded that the Lanthanun-chloride method (Pink et al., 2011) proposed to concentrate phosphorylated proteins does not work on cell cultures of *A. thaliana*, as it precipitates all proteins in the first step, without discrimination of their phosphorylation status.

From the training side of the project:

1. The fellow has learnt (i) how to culture cells of *Arabidopsis thaliana*; (ii) to extract entire proteome of both cell cultures and in vitro grown plants; (iii) to determine phosphatase activity and total protein concentration; (iv) to identify the phosphorilation status of proteins using three different methods (avidin alkaline conjugate, avidin-HRP and avidin-Fluor conjugates –pIMAGO reagent; (v) concentrate proteins using two different methods a) lanthanum chloride and b) methanol-chloroform precipitation method.
2. The fellow is now proficient at using Chemilumina and Typhon methods for the detection of total proteome and specific phosphorylation sites.
3. The fellow has also learnt how to work with specific antibodies for phosphorylation detection and has used the Pro-Q Phosphoprotein concentration method.

4. Follow-up

The project we conducted during the length of the stay is likely to be published in a scientific journal on either biochemistry or proteomics. The host institution is now working in the final stage of it, by identifying amino acids and proteins implied in the differential phosphorylation described. Once this part of the project is completed we will be in the position of writing up the manuscript for its submission. This project opens up a line of cooperation between the host institution and my own, via the joint application for funds to national and international funding bodies. All the new methodologies and protocols used in the project will be implemented in the

fellow's own laboratory, thus giving continuity to both the project and the cooperation. In addition and due to the fellow's interests, other future collaborations are envisaged with the host institution to explore proteomics in soils and their relevance in the system formed by soil-plants-microorganisms.

For the time being, there are no intellectual property issues arising from the project, at least in the short term.

5. Satisfaction

This has been one of the best fellowships I have ever had. I has been completely satisfying from all points of view. To start with, the personnel from OECD's office were always helpful and efficient in answering my queries and meeting committed dates. Simultaneously, my stay at Queen's was a really outstanding, learning experience that has let me to train myself in proteomics at a high level. My host researcher, his colleagues, and the staff at the Department of Biology and Biochemistry at Queen's University made my stay with them very easy and confortable, outreaching my expectations. I have to acknowledge the generosity of Prof. Plaxton who quickly accepted me in his laboratory without knowing me. It is remarkable the high quality of research they perform, as well as the scientific facilities (laboratories and their equipment, growth chambers, greenhouses, offices and space to work) they have and that made freely available to me.

As a consequence, the career opportunities of the fellow are likely to be enhanced after the stay at Queen's. In first place, the fellow has already incorporated some of the learnt techniques to her ongoing teaching and research in soil ecology and soil microbiology. Secondly, the fellow has established a new connection with the host laboratory, and with individual colleagues. Finally, the added value of the incorporated knowledge is very likely to improve the fellow's chances to obtain funding from competitive call for proposals as the first-line research learnt is, with no doubt a plus to both, her University and to the country.

No practical problems where encountered. The Fellowship Programme could be extended to other areas of knowledge.

6. Advertising the Co-operative Research Programme

I learnt about the Co-operative Research Program from the OECD's web page. Visibility of the program would be improved by advertising the different call from proposals of the various programs in the media. It would also be interesting to ask for dissemination through the Agriculture and Research departments of the Administration; they all have public web sites, publications and links to universities and other scientific organizations that would act as dissemination agents.

References

Carswell CM, Grant BR, Theodorou ME, Harris J, Niere JO, Plaxton WC (1996). The fungicide phosphonate disrupts phosphate-starvation response in *Brassica nigra* seedlings. *Plant Physiology* 110: 105-110.

Carswell CM, Grant BR, Plaxton WC (1997). Disruption of the phosphate-starvation resonse of oilseed rape suspension cells by the fungicide phosphonate. *Planta* 203: 67-74

Pink M, Verma N, Polato F, Bonn GK, Baba HA, Rettenmeier AW, Schmitz-Spanke S (2011). Precipitation by lanthanum ions: A straightforward approach to isolation phosphoproteins. *Proteomics* 11: 375-387.