Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. Markéta Šámalová, Ph.D.
Habilitation thesis	Development of molecular tools for experimental biology of plants and fungi
Reviewer	Enrique Rojo de la Viesca, Ph. D.
Reviewer's home unit, institution	Centro Nacional de Biotecnología-CSIC

Review of the habilitation thesis “Development of molecular tools for experimental biology of plants and fungi” submitted by Markéta Šámalová, PhD

The title of the habilitation thesis very well summarizes what has been a unifying and successful theme throughout the Applicant’s research career, namely to develop sophisticated tools for biological research in plants and fungi. It is well documented that, often, key scientific advances are only made after the development of new technologies and tools that allow to probe deeper into the workings of nature. In this regard, the applicant has made very important contributions in developing useful tools for the research community and has used them herself to make a number of significant findings in different fields of research and experimental systems.

After a brief commentary on her scientific itinerary, in the main body of the thesis the applicant addresses the main topics investigated during her career and the contributions made in each of them. The thesis is thus divided in 5 chapters, which include as introduction a concise and current review of the topic at hand.

The first chapter is focused on the development and use of pOp6/LhGR, an universal system for chemical (dexamethasone) induced expression in plants. This work was initiated by the applicant during her PhD in the lab of Prof. Moore and has continued over the years, having published four articles (all of them as first or shared first authorship), the latest one in 2021. This work has been very influential (over 220 citations for the two initial papers presenting the technology) , and the tools developed have been used by many laboratories both for basic and applied research. It is safe to say that pOp6/LhGR remains the best inducible system for use in plants, in terms of efficiency, sensitivity, dynamic range and minimal secondary effects. As one example of the utility for the community of plant biologists, a set of LhGR activator lines has been recently developed in Arabidopsis to attain pOp6-dependent cell-type specific inducible gene expression (Schurholz et al., 2018). Moreover, the applicant has recently extended the use of the pOp6/LhGR system to rice and plans to combine it with CRISPR-cas editing, which opens a very promising venue to use this technology for precision crop breeding.

The second chapter explains the generation of ratiometric fluorescent microscopy assays to study endomembrane trafficking and organization, which was published by the applicant as

first author of a research paper in the journal Traffic in 2006. These assays are fundamental for robust and reproducible measurements of protein localization and transport, and were used by the Moore's lab for several forward and reverse genetic screens for trafficking mutants, including an article characterizing RabD1 and RabD2 mutants to which the applicant contributed (Pinheiro et al., 2009). They have also been used many labs studying endomembrane trafficking in plants.

The third chapter is devoted to work done on the development of fluorescent-sensors and assays to measure redox state and nitric oxide (NO) in the fungus *Magnaporthe oryzae.* These and other tools were used to determine the role of NO and ROS signalling in the growth and infectivity of *Magnaporthe oryzae*, results that were published in two highly cited papers in New Phytologist (over 50 citations each), with the applicant as first author of both. Moreover, the applicant contributed to a review on emerging fungal pathogens.

Finally, the fourth and fifth chapter are centred on the study of fungal and plant cell wall proteins, respectively. The candidate has done very relevant work to uncover the role of GPI-anchored protein in pathogenicity of *Magnaporthe oryzae,* a pathogen of rice causing important losses worldwide, and *Aspergillus fumigatus*, an opportunistic pathogen causing deadly infections in immunocompromised human patients. Lastly, the candidate presents a new and ongoing line of research on the role of expansins in cell wall biomechanics. Again, as has been a constant throughout her career, the applicant has implemented the use of Brillouin spectroscopy in a label-free assay for measuring mechanical properties of the plant cell wall. She has combined this with advanced confocal and atomic force microscopy techniques for a profound characterization of four expansins in Arabidopsis, including their localization at the cell wall, their hormonal control and their role in root elongation (Samalova et al. 2020). Most importantly, in a clear example of the advances made possible with technological improvements in our measuring capacity, they have made the unexpected observation that overexpression of EXP1 actually increases cell wall stiffness, which constitutes a paradigm shift.

It is clear from this thesis that Dr. Markéta Šámalová has made very important contributions to science, in a dual way, by developing very useful tools and assays for research and by using those tools and assays to advance our knowledge of plant and fungal development.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

The overexpression of LhGR might cause off target gene activation or secondary effects. In an experiment using this technology, what is the best way (best control) to assure that the observed phenotypes are due to induced expression of the target gene and not to side effects?

Have you thought of a possible proof of concept project for the use of inducible gene editing in crop breeding? What gene, pathway or process would you target with this technology?

Considering your findings, GPI-anchored proteins might constitute a therapeutical target against fungal diseases in plants and animals. Do you have plans to pursue this possibility? How would you tackle it?

An enzymatic or hydrolytic activity has yet to be shown for expansins. Do you have any thoughts on how to identify the actual molecular activity of expansins that is responsible for them acting as catalyst of cell extension?

Conclusion

The habilitation thesis entitled “Development of molecular tools for experimental biology of plants and fungi” by Markéta Šámalová **fulfils** requirements expected of a habilitation thesis in the field of Plant Physiology.

Date:17th of March, 2023 Signature: