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Review Report to the PhD Thesis by Mgr. Michal Daněk:

**Characterization of *Arabidopsis thaliana* FLOTILLINs and HYPERSENSITIVE
INDUCED RESPONSE Proteins - Dynamics, Interactions and Functions**

Mgr. Michal Daněk has submitted excellent PhD Thesis. His Thesis is composed of three parts: the introduction part which is represented by the review paper 1, the results part which is based on two research papers (2, 3) followed by one submitted research manuscript (4), and the final part composed of the general discussion finishing with conclusions. Especially, the *Frontiers in Plant Science* paper (3) and the *Plant Journal* manuscript are relevant for this Thesis with Michal Daněk being the first author on both these publications.

This Thesis characterizes *Arabidopsis* flotillins (FLOTs) and hypersensitive induced reaction proteins (HIRs) which belong to the stomatin /prohibitin/ flotillin/ HflK/C (SPFH) proteins. These are evolutionarily ancient proteins are very nicely covered in the review paper 1 (Daněk M et al. published in the *Critical Reviews in Plant Sciences*, 2016) which provides very informative introduction to the topic covered by this Thesis. Although Mgr. Michal Daněk is not the first author of the *Biologia Plantarum* paper (2), he published several other papers not included topically into his Thesis which makes it clear that he will be very productive in his future scientific career. Data presented in the *Biologia Plantarum* paper include transcriptomics analysis of FLOT1 and FLOT2 expressions under salt and cold stress, as well as after exposure to the flg22 peptide and infection with *Botrytis cinerea*. Besides higher expression levels of AfFLOTs under both biotic and abiotic stress situations, also generation of ROS and callose deposits were reported. For example, the AtFLOT2 expression increased up to sixty times after

flg22 exposure. Research paper published in the *Frontiers in Plant Science* paper (3) is reporting on identification and characterization of 19 proteins interacting with the AtFLOT2. Most of these proteins, including AHA1, SYP71, ABCG36, AVP1, HIR2, NHL3, are relevant of signaling and vesicle trafficking connected to lipid rafts. The final part of the Results section is the *Plant Journal* manuscript in which role of the cell wall integrity for nano- and micro-domain distributions of the AtFLOTs and AtHIRs are probed, analyzed and reported. There are five very important conclusions resulting from this analysis:

- 1/ Alterations of cell wall (CW) synthesis and structure resulted in changes in lateral mobility of AtFLOT2 and AtHIR1.
- 2/ Partial enzymatic disturbances to the CW synthesis and integrity increased dynamics and microdomain mobility of these two proteins.
3. Surprisingly, pharmacological treatments suggest no roles for the actin and microtubular cytoskeleton in the AtFLOTs and AtHIRs microdomain organization.
4. PM-associated AtFLOTs contain significantly higher immobile fraction than AtHIRs.
5. Tonoplast AtHIR3 has higher motilities than AtHIRs associated with the plasma membrane.

The Thesis of Mgr. Daněk finishes with the Discussion which is, with respect of the extensive Introduction (around 30 pages) and excellent results sections including breakthrough cutting-edge data, rather short - counting only 9 pages. Nevertheless, it is followed by informative Conclusions section and Michal Daněk has managed to discuss all the important points in relevant manner. In conclusion, this PhD Thesis certainly fulfills all the relevant criteria for PhD Thesis and I am happy to recommend acceptance of this excellent PhD Thesis.

I have five questions and comments:

- 1/ FLOT1, FLOT3, HIR2 and HIR3 are enriched at the end-poles (under cross-walls) – Figure S2 of Danek et al. In Press (*Plant J.*). On the other hand, FLOT2 and PIP2 are not enriched at the same domains. Can you comment on this issue?
- 2/ FLOT2-GFP was reported to be localized to the end-poles (under cross-walls) of plasmolyzed root epidermal cells (Figure 1B in Junková et al. – the *Frontiers in Plant Science* paper 3). Why are plasmolyzed root cells (but not for cotyledons - Figure 1A) shown? Is FLOT2-GFP localized to the end-poles of root cells also in the control non-plasmolyzed cells?

3/ Although the PM-localizations of AtFLOTs and AtHIRs are restricted by corrals co-aligning with the cortical microtubules, pharmacological treatments suggest no roles for the microtubular cytoskeleton in the AtFLOT and AtHIR microdomain organization.

What is your explanation?

4/ AtFLOT and AtHIR lacks trans-membrane domains – how could be their immobilities at the plasma membrane connected to the cell wall integrity?

5/ What could be the role AtHIR3 at the tonoplast?

Súhlasím.

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