



**Re.: Doctoral Thesis evaluation for Josef Lazek**

**Vienna, 5th March 2023**

I have been asked to review the Doctoral thesis “*Characterization of factors participating in regulation of intracellular dynamics of auxin carriers*” by Jozef Lacey, supervised by Prof. RNDr. Eva Zažímalová, CSc and advised by Dr. nat. techn. Katarzyna Retzer. The criteria applied will be the professional, linguistic and formal aspects of the thesis.

**Thesis**

The Doctoral thesis of Jozef Lacey starts with an introduction where an overview of the phytohormone auxin, and its transport is presented. The introduction further presents more detailed information about the auxin influx and efflux transporters. The following two sub abstracts describe the fundamental role of the auxin efflux transporter (PIN-FORMED (PIN) PIN2 for directional root growth control and the effect of post-translation modifications on its function. The introduction closes with an abstract about the interplay of environmental signals on the root growth and of the effect of growth conditions on the directional root growth. The next 118 pages covers published content with seven scientific papers, each introduced by a (short) summary and declaration of his contribution. The published content is followed by a discussion with the focus on intrinsic and exogenous signals orchestrating the root growth movements. Finally, the thesis closes with a conclusion and outlook, each one page.

In general, the Thesis was written clearly. However, the order of the formulated aims was different to the published content part: it would have been easier to follow the scientific story with the support of the published articles, not regarding if these articles are Reviews or Original Research articles. This may also explain that Jozef Lacey often jumps between the themes. Since the introduction is very short, some basic introduction topics are missing. For example, it would be very interesting, to explain the morphology of a root and its different zones (schema), since the effect of PINs on the root growth is tissue dependent.

Additionally, a short introduction into the used methods would have been helpful, since (i) the application of the a high-end horizontal microscopic setup, that allows the placement of samples

vertically, and (ii), the application of the D-root setup enabled a great impact on this research.

Additionally, I would have been appreciated to set the story of this thesis in the discussion in a broader context: for example, what is the connection auf auxin with other hormones? How is the auxin transport affected by abiotic stress?

#### Professional aspects

Jozef Lacek presents within his thesis seven published manuscripts between the years 2017 and 2022, all published in Q1 journals. From the seven publications, two are review articles (both first author) and five are original research paper (three shared first author). In all his research contributions, he has demonstrated a high expertise in live cell imaging and mass spectrometry techniques, and through the different investigations he also demonstrated a very good expertise in several disciplines which is a very important feature today for efficient research which requires a high level of multidisciplinary expertise.

#### Linguistic aspects

The English is correct, and the quality of writing is good.

#### Formal aspects

The Doctoral thesis of Jozef Lacek consist of an introduction of six pages, a published content of 118 pages, a discussion of four pages, and one page each of conclusion and outlook. As already mentioned above, the introduction, the conclusion and the outlook are kept very short. Additionally, the thesis could have been written more carefully since it shows several formal mistakes: e.g. page 72: mutant lines are not written in italic; page 120: "I summarize the impact of exogenous stimuli in Figure 1" which is Figure 2 in the thesis. Or: Jozef Lacek has contributed to both review manuscripts as first author, even though he indicated this differently (page 23). The summaries (in terms of their style and length) of the published articles are not consistent (e.g. page 10, the last paragraph is a repetition of the introduction). Additionally, the references are not formatted uniformly (e.g. page 131, reference 73: Year is missing), explanation of some abbreviations is missing and the abbreviations are wrongly used in the text (even though the abbreviation was used, the full name was written later in the thesis). Finally, auxin with its influx and efflux transporters is a broad scientific field and many research groups are working on this topic. However, Jozef Lazek used many references that are published before the year 2020 even though a large amount of data within this field has been generated in the last two years.

#### Prepared questions and suggestions for the thesis

- Jozef Lacek described in his thesis that post-translational modification of PIN2 determine its function. PIN2 is ubiquitinated and two cysteines of PIN2 determine the protein

abundance and subcellular distribution. It is briefly mentioned in the thesis, that PIN proteins can be phosphorylated, leading to its polarity regulation. However, I would be very interested in more detailed information about (i) which PIN proteins can be phosphorylated and (ii) what is the trigger for this phosphorylation. Is the phosphorylation stress induced?

- Jozef Lazek mentioned the effect of intrinsic and exogenous signals orchestrating together the root growth movements, including non-natural growth conditions of *Arabidopsis* on agar plates where roots are illuminated. He posted at page 119 following statement: “The more it is surprising that for many years, root were cultivated under light, thus producing results describing non-native conditions”. I would have loved to read more discussion about this topic: e.g. which experimental setup Jozef Lazek suggests for the future? And how to deal with this knowledge, that sugar, light and agar plates have been affecting the results?
- Within this thesis, the model plant was mainly *Arabidopsis thaliana*. Are there any results known about PIN proteins and the effect of posttranslational modifications, intrinsic and exogenous signals in other plant species? Many studies of Crops are undertaken directly in soil: would it be possible, to switch directly to a crop model plant to enhance natural growth conditions?

#### Final evaluation

The scientific output of Jozef Lazek is very solid and underpinned by his total output of 16 publications. The Doctoral Thesis could have been written in a more appropriate way with putting more effort into writing the introduction and specially to correlate his results with the current research on PIN proteins in the plant field in the discussion.

Overall, based on the very solid published content I got a very good impression of the scientific work of Josez Lazek and thus recommend the thesis for defensio.

Kind regards,

Verena Ibl