Dr. Tomasz Nodzyński

Developmental and Cell Biology of Plants

CEITEC Masaryk University

Kamenice 753/5

To whom it may concern.

This review concerns the Doctoral Thesis entitled " *Characterization of factors participating in regulation of intracellular dynamics of auxin carriers* " written by Ing Jozef Lacek and supervised by prof. RNDr. Eva Zažímalová, CSc. where the advisor of the thesis was Dr. nat. techn. Katarzyna Retzer RNDr.

The work is written in correct English with just very minor grammatical and textual short comings. The manuscript is readable, the style is not great sometimes but I had no problems following the text. This is no surprise since a lot of the thesis has been already published in impacted journals.

Chapters: 3.1.1 (**1st author review** - Polar AuxinTransport - 2017); 3.1.2 (**co-1st author review** – dark side of the root - 2021); 3.2.1. (co-author on a research paper - cysteines in minor loops of PIN2 - 2017); 3.2.2 (**co-1st author research paper** – 2021 Dissecting Hierarchies between Light, Sugar and Auxin Action Underpinning Root and Root Hair Growth; 3.2.3 (**co-1st author on a research paper** - Throttling Growth Speed: Evaluation of aux1-7 Root Growth Profile by Combining D-Root system and Root Penetration Assay); 3.2.4. (co-author Arp2/3 Complex Is Required for Auxin-Driven Cell Expansion Through Regulation of Auxin Transporter Homeostasis); 3.2.5. (2022 co-author on a research paper - An Arabidopsis mutant deficient in phosphatidylinositol-4-phosphate kinases ß1 and ß2 displays altered auxin-related responses in roots). This makes 7 published contributions in total, certainly a very solid result.

The structure and parts of the thesis are appropriate. I find that also adequate references where selected that are relevant for this work, however I give a suggestion for adding some references. The thesis is a composite of published works that the candidate authored or co-authored, and I congratulate the candidate on the solid publication output and work that he has done. That means that the work was reviewed during the publication process. Therefore, my attention was primary focused on the non-published parts of the thesis, that is the abstract, introduction, and discussion.

The abstract seems a bit disjointed maybe due to the need to include so many publications.

The discussion is written correctly but is sometimes so generalised that the sentences seem even vague, for example at the page 119 of discussion start. As the questions that fill follow try to illustrate, that even with a short text there is space for making some insights and deliberations. Later the discussion gets better and more substantial.

Although I am far from the opinion that the amount of text relates directly to the quality of the thesis. However, the short introduction could include some parts of the relatively recently published PIN auxin carrier findings.

On page 4 in the introduction section 2.2.2 concerning the auxin efflux does not mention any of the recent PIN structures, that even in the time of AlphaFold protein structure predictions, are a major achievement in the field. I understand that the author tried to stay focused on the PIN2 very relevant for directional root growth but still two other long PINs structures were solved and before them one short PIN that deserve to be mentioned, I find.

1.Here my fist question arises, in spring of 2022 PIN8 structure was published but the title of the publication seemed to generalise the structural findings for all plant PINs. Are there differences between the ER, so called short and the long PINs. Because there are cell biology and functional studies going back a decade earlier (for example 2010, 2014) that investigate such differences and could the candidate discuss those in the view of the more recent structural findings.

a) Does the candidate think that PIN8 auxin transport mechanism can be used as a prototype, example for all the Arabidopsis PINs?

b) Also, can the PIN8 used as a prototype for all the Arabidopsis PINs if we omit the loop.

c) Is it a given that the other Arabidopsis ER PIN, the number 5 is also almost same as PIN8.

2.On page 5 at the end of the 2.2.2.1. chapter the author writes.: ” Although several post-translational modifications of PIN2 are known, they still cannot be assigned to particular developmental or growth adaptation processes ”.

a) But is that true? Approximately two years back there was a phosphorylation site identified that was quite specifically tied to the root system adaptation – can the thesis author discuss this result. Would it meet the specificity criteria of the PhD candidate?

b) What is more, latter on just in next chapter beginning (page 5 again) it is written “The complex relationships between PIN2 protein modifications and their impact on root physiology have been particularly dissected (47)”. Is this not a contradiction to point 2a)? Or please discuss it if not…

3.In the chapter of the published review - Lessons Learned from the Studies of Roots Shaded from Direct Root Illumination – there are few illustrations that illustrate key but rather uncomplicated matters, like how a dark root plate looks. But it is a pity that the more complex influences of root illumination are not depicted on some scheme that would provide the synthesis of the other parts of this review, depicting the consequences of growing the roots in light the arising ROS stress, impact on hormones and their transporters – that would be some reconciliation also with the auxin part of the thesis. Please make the scheme for the defence presentation. This could also help advertise the benefits of dark root system.

Textual corrections:

In the thesis abstract and multiple times in the introduction and discussion: in the abstract line 4, “..tropistic growth..” – did not find the word tropistic used often in English writing.

Page 122 – “…This requires response of several regulation mechanisms. ” maybe word – regulatory mechanisms - would be better stylistically

Minor comments

1.Add chapter 3.2.4. Arp2/3 Complex Is Required for Auxin-Driven Cell Expansion Through Regulation of Auxin Transporter Homeostasis, the way the published pdf was pasted leaves a poor quality of the text, it is hard to read at times, for example page 89 of the thesis.

2.Page 5: one can find the sentence: “Intracellular distribution of proteins relies on flawless establishment of the endomembrane system, which is tightly connected to properly and flexible arrangements of the cytoskeleton.” What the author means by this – it hard to understand this sentence – there is phrases like establishment of the endomembrane system.

3.The word “manifold” is used to indicated plurality, multitude – maybe other expressions could be used at times as a replacement to enhance the style of the text.

Yet overall, I very much enjoyed reading the thesis and I certainly find the scientific work is sufficient and I recommend the thesis for defence.

With regards!

Tomasz Nodzyński

02.03.2023