Reviewer´s opinion of diploma thesis

Reviewer of the thesis:
Dr. PARIS Nadine

Date:
19th June 2020

Author of the thesis:
Daša Wernerová

Name of the thesis:
Visualization of root apoplastic pH in plants

Objectives of the thesis
Evaluate performance of currently available pH sensitive dyes and genetically encoded sensors for their use to visualize apoplastic pH changes in growing roots upon gravistimulation and auxin treatments using *in vivo* live-cell imaging. Create a new genetically encoded pH sensitive sensor based on the above-mentioned findings.

Structure of the thesis
Size of the thesis (number of pages): 93. It is well written, very clear and nicely illustrated. The introduction of 18 pages is complete and gives all the information required to understand the status of the field at the beginning of her thesis and the objectives. 11 pages of methods clear and complete with even DNA sequences provided for the cellulose binding motifs.
Are the English and Czech abstracts and keywords given? Yes for english

Formal level of the thesis (visual documentation, graphics, text, list of literature)
High quality, well documented. To me nothing is missing. It is a shame that high quality versions of the result images were not provided especially for the results with the dye. The research field is vast but a nice selection of citations was made (88 in total).

Logical structure and language quality of the thesis
The document is well structured and logic. The results are well organized, presented and discussed with scientific rigor. From the non-english speaker that I am, the language is very good and clear except for some sentences in the discussion that would probably have required an extra checking.

Literature overview:
Does it correspond to the topic and is it logically structured? yes
Is it written comprehensibly? yes
Are the literature sources used relevant and up-to-date? yes
Are the literature sources used (including pictures) correctly quoted? yes

Materials and Methods:
The extend of methodologies used.
The methodologies were well described except for the SD chamber. A picture of the chamber with the seedling would have been helpful.
Do described methods correspond to results presented? yes
Are methods comprehensibly described? yes

Experimental part:

Are the aims of particular experiments explained? yes

Is the documentation of the results adequate? Yes, although, ideally, and additional electronic version of the imaging data would have been a plus to the images in a pdf format.

Is the number of conducted experiments sufficient? Ten times yes !

Discussion:

Is it really a discussion, is it not just a repetition of previously mentioned results? Yes, it is a fairly good discussion except it feels like it could have been slightly improved with one or two more re-reading.

Are the results related to the literature? Yes and there is a discussion provided for when results aren't quite the same as the published ones

Are there any hypotheses or suggestions for further research? Yes, plenty !

Conclusions (Summary):

Are the main findings supported by the data? Yes, absolutely

Are they formulated appropriately? Yes

Achievement of aims and overall assessment:

Dasa's thesis aimed to visualize pH changes in the root apoplasm using live imaging, especially in the context of gravitropic response in link with auxin fluxes. For this purpose, she explored already available genetically encoded sensors, dyes and finally she designed new genetically encoded sensors adapted to acidic pH and targeted to the cell wall.
Dasa achieved her goals and provided some work of excellent level in quantity as well as in quality. She made a very large and complete survey of various sensors available for pH measurement in plant. She selected and partially validated at least two nice systems to further explore the pH changes in arabidopsis root upon gravitropism, (1) a pHluorin anchored sensor will provide at very close proximity of the plasma membrane (2) a novel dye that is sensitive in pH down to pH 4.5 that detect pH changes further away from the surface.

Questions and comments of the reviewer (<u>mandatory part of the report!</u>):

Dasa's thesis covered an amazingly large catalogue of resources for measuring pH *in vivo* and she analyzed in much details their potential and limitation. Among the published genetically encoded sensors, she tested three and selected a plasma-membrane anchored pHluorin facing the apoplast. As the signal was patchy in the root tip, Dasa modified the promoter and/or the antibiotic selection. The three new lines she obtained showed a strong and uniform signal of plasma membrane anchored pHluorin. It is really unfortunate that Dasa could not further test these lines due to COVID19 shutdown as these plants should be very useful for future work. Concerning the dyes, Dasa tested 5 molecules including two that had never been used in plant to monitor apoplastic pH. Her data is extremely novel and very useful for people working in the field as, in contrast to published data, she provided a comparative analysis of these dyes with the same imaging system. She found that the pH sensitivity and the penetration of the dyes differ from each other, she observed some differences with published data and described an artefactual phenomenon at the vicinity of the root making pH measurement unreliable for some of dyes. Surprisingly, this seems to be associated with the presence of dextran in some of these dyes. Among the tested dyes, one is very promising as Dasa could detect an alkalin halo around the root transition zone and an

acidic halo just above. Most interestingly, she could detect some differences between the upper and the lower sides of a root submitted to gravitropism. It remains to be confirmed that these differences are indeed due to pH only since this dye has not been characterized in details and could also respond to other parameters that pH (such as Calcium, Redox....). Finally, Dasa developed entirely new genetically encoded pH sensors that she chose to target to the cell wall using cellulose-binding domains. The choice of targeting the cellulose part of the cell instead of leaving the sensor free to diffuse in the apoplast is based on Dasa's observation that the published apo-pHusion appears to diffuse out of the apoplast in the root tip. The fluorescent part of the sensor was designed in the objective to improve detection in acidic pH by associating two proteins of different but fairly low pKa. The cellulose binding domains, 5 in total, were selected from the literature or database but their ability to be sufficient for cellulose association *in vivo* was unpublished, to my knowledge. For all these reasons, this approach was at high risk and turned out to provide only partially localized sensor in the cell wall in tobacco epidermal cells for some fusion. Still, this is a very encouraging result and a good basis for future optimization for example by modifying the position of the CBD in the fusion proteins.

What are the advantages and limitation of the two best sensors you identified so far (the genetically encoded one and your favorite dye) ?

What can explain the presence of an alkaline and an acidic halo in the root ?

Based on you experience, what would you do next to improve the localization of the new genetically encoded sensors ?

Do you have any suggestion for other sensors ?

Reviewer´s final classification proposal:

X excellent (výborně) ☐ very good (velmi dobře) ☐ good (dobře) ☐ unsatisfactory (nevyhověl/a)

Signature of the reviewer