Evaluation of bachelor thesis (reviewer´s form)

Author of the thesis: Sofie Dundrova

Title of the thesis: The Role of Cyclic Nucleotides in Plant Signaling

A. Evaluation of individual aspects of the thesis (mark X one of the options)

	1. The character of the thesis (BT) and its structure
A	A - proportionate, corresponds to the scope of BT and to the significance of individual parts
	B - unbalanced, the structure is not logical or the extent of individual parts does not correspond to their importance
	C - satisfactory, the extent of some parts is insufficient
	N - insufficient

	2. Scientific correctness
	A - excellent, no serious comments
B	B - very good, with minor imperfections (ambiguity of interpretation, errors in formulas or chemical nomenclature, incomplete description of methods or results)
	C - satisfactory, with numerous minor defects
	N - unsatisfactory, with serious mistakes

	3. Correctness of the literature resources survey
A	A - without objections, all literary resources properly cited, the total number of resources corresponds to the scope of the BT
	B - satisfactory, with occasional nuisances, especially in the reference placement, or with a lower total number of citations
	C - with more serious mistakes, such as "non-standard" references to textbooks, lectures, web pages, or the sporadic omission of a link to the downloaded data source
	N - unsatisfactory, very few references, or with possible features of plagiarism, references to the source data frequently neglected

	4. Language standard
	A - excellent, the work is well-written and comprehensible, without grammatical / spelling mistakes
B	B - very good, unique stylistic awkwardness, grammatical / spelling mistakes
	C - sufficient, more frequent stylistic awkwardness, frequent grammatical / spelling mistakes, rare sentences difficult to understand or ambiguous formulations
	N – unsatisfactory, frequent serious mistakes

	5. Formal and graphical level of the thesis
A	A – excellent, without spelling mistakes / text formatting errors
	B – very good, unique mistakes in reference format, misspellings, missing abbreviation, etc.
	C – satisfactory, with unique considerable mistake (such as text page skip) or multiple minor bugs
	N - unsatisfactory, frequent serious mistakes

Optional word comment (to points 1. - 5.):

Scientific correctness

Several slightly unfortunate statements such as "the discovery of the majority of the known plant CNGCs (Zelman, Dawe and Berkowitz, 2013), making them the only experimentally confirmed plant proteins regulated by cNs for two decades." Perhaps just a language error, but CNGCs are not confirmed to be regulated by cNs in plants, to my knowledge.

Language

I do not think spelling is important for science. Nonetheless, I recommend spelling "eukaryotic" with a "k" as the word is derived from the Greek word karyon (nucleus). This is very widely used in both textbooks and manuscripts and might distract the readers unnecessarily when spelled with "c".

B. The defense

Reviewer's questions for the student (mandatory part of the report!)
I have enjoyed reading this thesis. The candidate did an excellent job in presenting a reasonable snapshot of both the depth and breadth of cyclic nucleotide research in plants. This was in no way simple as the emerging field has a colorful history, controversial aspects, and sometimes conflicting reports. I am satisfied with the comprehensiveness of the report, although I will suggest below some more aspects that would have fitted well with the narrative. The aims of the thesis were all met. Regarding methods to study cNs, the candidate could have included a discussion of several other assays beyond listing LC-MS, immunoassays, and genetic sensors. (Question 9 below) The thesis advocates for a positive outlook on the past and future of cN research with a sound and logical presentation of all arguments. What I feel might improve the text is a critical assessment of the various inconsistencies or controversial aspects of the field. I would not necessarily expect this from a bachelor thesis but, given the candidate now has an excellent understanding of the topic, I feel it could be a rewarding exercise for the defense. I invite the candidate to choose any two questions from the list below for an exciting discussion during the defense: 1) I was surprised to see that the section about the controversial past of cNs omits a major controversy specifically pertaining to auxin. What was this controversy about and how did it affect cN research? (refer to Ichikawa, Takanari, et al., *Nature*, (1998): 390-390.) 2) The candidate discussed nucleotide cyclase activities of LRR-RLKs. Some people in the field argue that guanylate cyclase activity of their kinase domains is physically impossible. Why is that and what data suggest that? (refer to Bojar, Daniel, et al. *The Plant Journal* 78.1 (2014): 31-43.) 3) Could you compare and contrast the possible evolution of nucleotide cyclase activities in plants and animals? How many times would one assume it evolved in plants versus animals? How does the idea of moonlighting fit in that picture? 4) Many of the proposed roles for cNs in plants include very fast responses such as ion fluxes at the plasma membrane. Nonetheless, all currently known plant cyclases show very, very slow production of cNs. How does this consolidate? Do published works quote the kinetic parameters of the cyclases studied using well-accepted units (e.g. molarity for Km)? Do they quote the turnover number for the enzymes (number of molecules converted per second)? 5) Related to 4): The plant cyclases are not only slow but also the amount of cNs produced is very low. (refer to Ashton, Anthony R. *Proceedings of the National Academy of Sciences* 108.19 (2011): E96-E96.) In many cases, cNs are proposed to result in all-or-nothing responses like

Reviewer's questions for the student (mandatory part of the report!)

transcription. However, the observed increase of cN content in plants is often very small. What could explain this proportionality mismatch? (e.g. refer to recent Qi et al 2022 paper on TIR1 cyclase activity – how much does cAMP production increase after auxin treatment?)

6) How does one establish the physiological relevance of cyclic nucleotides for plants? What does it mean to say that something is "necessary and sufficient" in biology? How many reports show that cNs are necessary for something? How many reports also show that they are sufficient for the same function?
7) Related to 6). What experiments would you do to test whether cNMP binding to CNGC is necessary and sufficient for some CNGC function?
8) Have genetic screens ever returned strong mutations in the assumed AC/GC centres or PDE centers? Do you know any examples where a screen hit an AC/GC center, resulting in a plant phenotype?
9) Ultrasensitive mass spectrometry is notorious for being capable of detecting activities at the level of noise without biological relevance and confounding whole scientific fields. Maybe this has not yet arrived in plant biology but it strongly resonated in mammals in recent years. Almost every day, researchers detect new obscure modifications with dubious biological functions that are later disproven by careful metabolic labeling analyses. A sobering example is N6-adenosine methylation in mammalian cells. Discovery of this modification was seemingly ground-breaking and was made by ultra-sensitive mass spec (refer to Wu, Tao P., et al. *Nature* 532.7599 (2016): 329-333.). However, subsequent works showed that this was an error and that the detected m6A contamination came from bacterial enzymes used for DNA digestion or from host-cell nucleotide degradation pathways (Douvlataniotis, Karolos, et al. *Science advances* 6.12 (2020): eaay3335.). How would you establish the physiological relevance of cN production by plant proteins without the use of mass spec?

Opinion on the correction(s) of errors:

Errata/correction in the text **IS NOT** required for thesis acceptance.

C. Overall assessment

I recommend the thesis to be accepted for further processing: **YES**

Reviewer's final classification proposal: 1- excellent
(1 - excellent; 2 - very good; 3 - good; 4 - unsatisfactory/failed)

Date: 03/09/2024

Name and surname, signature of the reviewer (according to SIS):
Lukas Fiedler

Instructions for the preparation and submission of the review (delete after completing the review):

- The usual length of a standard opponent's review is about 2 pages
- Use this form for evaluation of the thesis.

- According to the University rules, the opinion must be made available to the student **at least three working days prior defence**.
- You can submit the fulfilled form by yourself to the SIS or send it in advance electronically to: hana.konradova@natur.cuni.cz. **Furthermore, please, ensure the delivery of the signed original printout to the secretary´s office of the Department of Experimental Plant Biology, Faculty of Science, Charles University, Viničná 5, 128 00 Praha 2.** The signed printed copy of the opinion must be delivered in advance, without it the defence cannot start!