

Reviewer's opinion of diploma thesis

Reviewer of the thesis: Clément Lafon Placette

Date: 08/09/2022

Author of the thesis: Oliver Pitoňak

Name of the thesis: Role of TCTP1 in plant reproduction

Objectives of the thesis

“• Phenotypic characterization of *tctp-1* T-DNA insertion line with focus on pollen tube growth and guidance

- Analysis of subcellular localization of TCTP1 in *Arabidopsis thaliana* mature pollen and pollen tubes
- Optimization of recombinant AtTCTP1 expression in *E. coli* and its purification
- Verification of AtTCTP1 dimerization *in vitro*
- Recombinant expression of selected potential TCTP1 interacting partners BRL2 and TTL3 and in vitro pull-down experiment”

I have a problem with these objectives, as to me, objectives of a thesis should be biological questions to answer, something like “understanding the role of TCTP1 in pollen tube guidance”. The objectives written here are rather a list of methodological tasks, the “how”, not the “why”. In other words, these objectives are not hypothesis-driven.

Structure of the thesis

Size of the thesis (number of pages): 94

Are the English and Czech abstracts and keywords given? yes

Formal level of the thesis (visual documentation, graphics, text, list of literature)

The thesis is well documented with figures and literature; the figures are clear and the fonts etc are easy to read.

Logical structure and language quality of the thesis

I have a general problem with the logical structure of the thesis, which to me, does not show the capacity of the student to critically think about the biological questions related to the topic (see my other comments). The English level is however pretty good.

Literature overview:

Oliver Pitoňak's literature overview is certainly thorough. However, the literature overview is a place for the student to show that he/she can critically think about the state of the art. This means: 1/ selecting the information which is important for the topic of the thesis and 2/ not stopping at doing a catalogue of the known facts, but going further by making links/proposing to unify interspersed evidence into a hypothesis proposed by the students, or by clearly identifying limitation/knowledge gap according to the students and in general, adding some personal reflection on the biological mechanisms (supported by scientific

arguments of course). I would rather see speculations rather than no thoughts from the student at all.

And I must say that Oliver Pitoňak's literature overview failed the two points. A large part of the overview is to me unnecessary for the topic, i.e. role of TCTP1 in pollen tube growth/guidance: the 3D structure of the protein, role in programmed cell death and other pathways which seems specific to animals... And in general, the literature review is very encyclopaedic, i.e. a catalogue of all the facts known about these proteins. So it seems there is no selection of information. And on the other hand, if TCTP1 is supposed to be a secreted protein from pollen helping tube guidance/growth, I would expect a much larger part dedicated to reflections about the mechanistic explanation on how this would happen. Indeed, it is not to me intuitive how the pollen could be guided to the ovule via a molecule pollen secretes (as explained in the literature overview, secretion by female tissues and reception by the pollen is a more intuitive scenario), so it would have been good to consider mechanistic scenarios on how this may happen. For this purpose, I would have expected the literature overview to finish with a part identifying the knowledge gaps, potential hypotheses on how TCTP1 may play a role in pollen tube growth/guidance, based on the previous literature, to finally formulate questions/hypotheses that will be answered/tested in the thesis (using the current "objectives" being the "how" to answer these questions). Again this is to me a crucial part, as this shows that the student is able to critically think about the topic.

Materials and Methods:

The thesis involves a very large range of methods, which is quite impressive. They are well described and look solid to me. It is however hard for me to evaluate if they are adequately chosen to answer the questions of the project, because the biological questions are lacking (see my comment above).

Experimental part:

The results part is quite solid technically and thorough: it is again a lot of experimental work; the data are clearly presented; the number of replicates and positive/negative controls for the experiments are sufficient in my opinion. As a downside, stemming from my criticism above, the point of some experiments are not clear to me. Because there are no clear biological questions/aims in the thesis, it is hard for me to see why studying the cellular localization of TCTP1, its dimerization, its interaction with some proteins would help in the frame of the topic of the thesis, i.e. the role of TCTP1 in pollen development...

Discussion:

Is it really a discussion, is it not just a repetition of previously mentioned results?

It is a discussion, however a mostly technical one. A large part is dedicated to discuss why some experiments were not conclusive, and propose additional experiments to perform. The conclusions are again often technical, such as "To my knowledge, this study is the first report of TCTP1 subcellular localization in pollen." It is great in itself, but if it is not to serve a biological question, it is not enough as conclusion/discussion of a scientific work.

Are the results related to the literature?

The candidate does include the literature in this discussion. However, the way the results relate to the literature is not convincing to me. It is mostly a superposition of the results of the thesis, followed by a mention of a few similar studies and results from the literature, and that's it. There is mostly no part from the student trying to unify his results and the literature, trying to ponder contradicting evidence and propose a (conceptual, not methodological) way

to resolve these contradictions.

Are there any hypotheses or suggestions for further research?

Here again, the suggestions for further research are mostly additional experiments to perform. There is no proposal of biological hypotheses.

Conclusions (Summary):

Here again, the conclusions are largely technical, i.e. based on the goals of the thesis, the student answer whether or not the goals (=methodological tasks) were completed or not, and if not what tasks to do next. No biological conclusions.

Achievement of aims and overall assessment:

The thesis is certainly thorough, solid and extensive, both for the literature review and experimental work, no doubts about it. I trust the experimental work and I imagine it is a great starting material for the lab. However, when it comes to critical thinking and interpretation skills that are expected at the Master level, to me the thesis lacks the signs that the student acquired these skills during his Master project.

Questions and comments of the reviewer (mandatory part of the report!):

Questions:

- You nicely show that the *tctp1* pollen tubes cannot reach the bottom of the siliques. From the results with GUS pollen, you conclude however about a problem of pollen guidance. But to me, Fig 9 & 10 largely suggest instead that the failure to reach ovules (your A category in Figure 11) is simply due to the pollen tube not able to grow fast/long enough to reach ovules before the WT pollen, nothing to do with pollen guidance. And Figure 9 also argues against a problem of pollen tube guidance. So what argument do you have to still favour a pollen tube guidance problem?
- The fact that seeds of *tctp1* are large and white + the fact that embryos can be rescued on with artificial medium makes me think of an endosperm problem actually, not an embryo one (and a failing endosperm would lead to embryo delay and death). Has anyone looked at that?
- Related to pollen tube guidance: it is hard for me to imagine that a protein secreted by the pollen would help its guidance. How would that work? I mean that to be guided, the pollen need external signals, not signals coming from itself. What mechanisms do you envision to explain that secreting TCTP1 would help the pollen to be guided?
- I largely criticized the lack of biological hypotheses/proposals in your thesis, sorry about this, and it is particularly unfair if you cannot respond to this criticism. So, from your results, what are the main biological conclusions you can make regarding how TCTP1 would be involved in pollen tube growth/guidance/embryo development?

Reviewer's final classification proposal:

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

Signature of the reviewer



Note: The usual length of a standard review is about 2-3 pages.

Instructions for the preparation and submission of the opinion:

- Use this form for evaluation of the thesis. The text in standard font serves as a guide.
- According to the University rules, the opinion must be made available 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 and lipavska@natur.cuni.cz. Furthermore, please, ensure the delivery of the signed original to the secretary's office of the Department of Experimental Plant Biology, Faculty of Science, Charles University (Ms. Elena Kozlova), Viničná 5, 128 44 Praha 2. The signed printed copy of the opinion must be delivered in advance, without it the defence cannot start!