Reviewer´s opinion of diploma thesis

Reviewer of the thesis: Dr. Katarzyna Retzer

Date: 14.08.2020

Author of the thesis: Bc. Karel Raabe

Name of the thesis: Characterization of subunit A of the Eukaryotic translation initiation factor 3 in *Arabidopsis thaliana*

Objectives of the thesis

In silico analysis of plant eIF3A protein sequence and its comparison with other eukaryotic eIF3A orthologues

Phenotypical characterization of Arabidopsis thaliana T-DNA insertion lines in the AteIF3A gene locus At4g11420

Expression analysis of AteIF3A gene in Arabidopsis thaliana stable transgenic lines expressing GFP-GUS fusion protein driven by the native eIF3A promoter

Subcellular localization of AteIF3A protein in Arabidopsis thaliana stable transgenic lines expressing the AteIF3A protein fused to GFP, driven by native, constitutive sporophytic or male gametophyte specific promoters

Stable transformation and regeneration of Nicotiana tabacum plants expressing fluorescent markers or heterologous Arabidopsis AteIF3A subunit fused to GFP

Structure of the thesis

Size of the thesis (number of pages): **110**

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 very well written, in a clear but highly scientific language, including up-to-date literature citations and is well structured. The figures are in high quality and are representing the high amount of work done to specify the structure-function relationship of AteIF3A. Especially I want to point out he impressive work done for the *in silico* analysis of the individual domains of *AteIF3A*, this data set will in future allow to solve open question about the importance of the protein for translational regulation, which at the end allows the proper adaptation of cell/plant growth.

Logical structure and language quality of the thesis

The thesis is well structured, it is easy understandable which methods were chosen why, how the work was executed, and how the new established plant material was tested. The methods part is so well written, it can be used as a methods handbook for further students, it is visible that the candidate put a lot of effort to write the thesis. The Figures and Results are well and clear described, and the Discussion is on point and clearly shows that additional experiments/lines are in the pipeline and in which direction the project will be continued.

Literature overview:

Does it correspond to the topic and is it logically structured? **Yes, excellent executed**

Is it written comprehensibly? **Yes, very much**

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. **Yes**

Do described methods correspond to results presented? **Yes**

Are methods comprehensibly described? **Yes, excellent executed**

Experimental part:

Are the aims of particular experiments explained? **Yes, very well explained**

Is the documentation of the results adequate? **Yes, very much**

Is the number of conducted experiments sufficient? **Yes, extraordinary amount of work was done**

Discussion:

Is it really a discussion, is it not just a repetition of previously mentioned results? **Yes**, the discussion is written well; **No, the discussion is giving a good outlook on how the project will be continued.**

Are the results related to the literature? **Most of the gained data are novel insight for plant eIF3A, but they correlate with the described homologues in mammals/yeast. Yes, the results are related to literature.**

Are there any hypotheses or suggestions for further research?

Yes, a lot of additional work is obviously ready and described in the discussion how the project will be continued with the prepared plant material.

Conclusions (Summary):

Are the main findings supported by the data? **Yes**

Are they formulated appropriately? **Yes, excellent described and well written**

Achievement of aims and overall assessment:

Karel demonstrates an extraordinary understanding of the topic, the aims were either fully reached or he prepared additional plant material to continue to solve the asked questions. Overall the amount of work corresponds more to a PhD thesis. **Karel was able to collect a high amount of *in silico* data to study the structure-function relation of AteIF3A, which are completely novel insights for the plant homologue.** Further he achieved a detailed map of distribution of the protein in Arabidopsis and tobacco. He prepared a lot of material to understand the physiological role of the target protein.

He presented the results in a clear and understandable way, which supports the applicability of his research work in further studies.

The title and aims of the thesis are clearly formulated and sufficient addressed in the written thesis. The abstract is well structured and informative. The thesis is written in a logical and orderly manner, supported by well composed figures, which is reflecting the high amount of work invested into obtaining the data for the master thesis. The discussion summarizes and describes the results detailed and incorporated current knowledge from the literature. **His work will allow to understand the regulatory mechanisms behind translational initiation *in planta* better and therefore Karel has successfully fulfilled the objectives of his master thesis.**

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

- What are the advantages/disadvantages of single copy gene vs. gene family characterization in plants?
    - What could be the benefits of eIF3 subcomplexes composed of different eIF3 subunit paralogs, particularly with the plant-specific eIF4 isocomplex?
    - Is it possible to easily target more members of one gene family with available reverse genetic methods?
- Is eIF3 complex, in general, essential for plant viability or more for specific stages of plant development?
- What is the structural background of the eIF3 complex on which most of the Cryo-EM analyses are based on?
- Why is the *AteIF3A* expression stronger in roots than in shoots?
    - What are the advantages of performing the GUS staining aseptically?
- What adjustments would you suggest to prevent degradation/clipping of the extracted AteIF3A fusion proteins?
    - How would you further verify that the protein product detected with western blotting is truly the AteIF3A fusion protein?
- What is the difference between protein sequence identity and similarity?
- How would you verify that particular segment of the AteIF3A protein possess similar function that was described for the same conserved segment in other Eukaryotes?
- What could be the evolutionary reason for plant specific regions inside the PCI domain?
- How would you proceed with the AteIF3A protein localization analysis, particularly with the signal foci in roots and the filamentous localization in pollen tubes?
- Considering your hypothesis that eIF3A is essential in plants, how would you explain why T-DNA insertion lines with confirmed insertion in the *AteIF3A* do not lead to any observable phenotype?

Reviewer´s final classification proposal:
☒ **excellent (výborně)** ☐ very good (velmi dobře) ☐ good (dobře) ☐ unsatisfactory (nevyhověl/a)

Signature of the reviewer