Institute of Biophysics, Czech Academy of Sciences
Královopolská 135
612 00 Brno
Tel. +420 541 517 500
Email: ibp@ibp.cz

Opponent Review of Doctoral Dissertation

The dissertation titled "Studying the dynamics of gene expression and the role of NAC complex in male gametophyte development" by Božena Klodová provides a comprehensive examination covering the highly attractive and actual topic of regulatory mechanisms driving gene expression in plants. The specific attention is devoted to the development of male gametophyte, a long-term interest of the author's "home" laboratory. The dissertation is divided into an abstract, introduction, results, general discussion, conclusion, and bibliography - as such can be considered complete, without missing any relevant chapter. Overall, the dissertation is very well structured, ideas presented clearly and concisely with a high level of proficiency in English.

The introduction effectively outlines the significance of precise gene expression regulation within the context of complex biological processes and plant survival strategies. The literature review provides a comprehensive outlook on the topic and demonstrates the author's solid and thorough understanding of the topic. In this part, the gene expression regulation in plants is described, from the level of transcription initiation, through co- and post-transcriptional regulation to the (post)translational regulatory mechanisms. The final part of this chapter is devoted to the specific regulatory networks controlling male gametophyte development. While this part offers truly deep insight into the regulation of gene expression, in my opinion, it should benefit from adding some of the developmental/epigenetic mechanisms such as DNA methylation or modifications of nucleosomal histones, both of which are merely mentioned in the literature review.

During the course of her Ph.D., the author gathered an impressive number of results, which are presented either as manuscripts (close to submitting) or published papers. These comprise three articles focusing on the characterization of NAC complex, the "omic" study (combination of the transcriptomic and proteomic approaches) which allowed the identification of regulatory dynamics of gene expression during male gametophyte development, one review and a paper describing the bioinformatic tool. Although the number and quality of articles/manuscripts are undoubtedly sufficient for the successful finish of Ph.D. programme, it is not clear whether and to what extent the author fulfilled the dissertation aims/objectives, as such aims are not very well defined in the text. It is regrettable that the dissertation lacks a separate chapter, that would clearly state the major aims/objectives.

Chapter general discussion is thoroughly considered and provides a synthesis of obtained results together with their comparison with existing data and methodology. Moreover, the author discusses and compares the relevant computational biology tools, that are currently available online. I really appreciate the way how the author presents artificial intelligence and its use to develop the pipeline for differential expression analysis of RNA-seq data. Finally, the discussion is closed by mentioning future scientific directions and experiments that should be performed in order to better understand the role of NAC

complex in biotic and abiotic stress tolerance and also the involvement of NAC in floral meristem development and maintenance.

Question on author:

In your "omic" paper, you identified numerous lncRNAs. If you couple lncRNAs data with the transcriptomic/proteomic data, do you see any effect of lncRNAs on gene expression in male gametophyte?

Despite ubiquitous expression of NACβ, the strongest phenotypic defects in *nacβ1nacβ2* mutants were observed in reproductive organs. Do you have any idea why?

How do you explain very low (or even zero) expression of NACβ in *Marchantia polymorpha* sperm cells?

What is the role of NAC domain for forming NAC dimers (you mentioned potential dimerization sites in NAC domains)? Do you have any data on this or do you know any from literature?

Summary:
The thesis brings new insights to the problematic of gene expression in male gametophyte as well as uncovers the role of NAC complex proteins in plants. It answers research questions and by its content, it brings an innovative knowledge related to the research topic. The author was able to show, that she is capable of carrying out methodological as well as theoretical scientific work. In my opinion, the presented dissertation by Božena Klodová fulfills the demands on dissertations of a Ph.D. study programme. Therefore, I recommend the Ph.D. dissertation "Studying the dynamics of gene expression and the role of NAC complex in male gametophyte development" to be defended.

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Vojtěch Hudzieczek
Institute of Biophysics ASCR,
Kralovopolska 135
61200 Brno
Czech Republic
tel.: +420-541-517-203
e-mail: hudzieczek@ibp.cz