

Prof. RNDr. Jiří Fajkus, CSc.

Masaryk University
Head of the Mendel Centre, CEITEC MU
Head of the Lab. of Functional Genomics and Proteomics,
NCBR, Faculty of Science

Kamenice 5, 625 00, Brno, Czech Republic

Contact: fajkus@sci.muni.cz

Reviewer's assessment of the dissertation of Božena Klodová:
Studying the dynamics of gene expression and the role of NAC complex in male gametophyte development

Božena Klodová's dissertation presents an extensive study of gene expression regulation within male gametophyte development in plants, emphasizing transcriptional, translational, and post-translational levels. In addition to the other results, the thesis introduces "GOLEM," an online tool for visualizing and analyzing motif distribution in plant DNA, using RNA-seq data for gene expression studies across different tissues and developmental stages. This research enriches the understanding of gene expression dynamics in male gametophyte development and identifies significant trends and categorizes gene families based on their expression patterns.

The **introduction** provides a thorough and highly informative background on gene expression regulation, emphasizing its significance in plant adaptation and development. I liked its reading very much.

In the first section of the **Results**, an integrated analysis of gene expression dynamics during development of Arabidopsis pollen is presented. Changes in transcriptomes, including expression of non-coding RNAs, and proteomes are detected, together with alternative splicing events, pointing to their role in pollen development. These results have been published in Plant Reproduction and contribution of the Candidate as the first author of this study is well described.

The second section of results is presented as a manuscript entitled *GOLEM: distribution of Gene regulatOry eLEMents within the plant promoters*. This MS introduces of the innovative bioinformatic GOLEM tool for analyzing motif distribution in plant DNA and is a notable methodological advancement in current "postgenomic" era. Again, the Candidate's contribution, here as the second author of this study, is sufficiently described.

The next section of Results is a review entitled *A Decade of Pollen Phosphoproteomics*, authored by the candidate and her supervisor, and published in IJMS. This comprehensive review provides very nice and in detail overview of current knowledge in this young field of research, as well as on perspective future topics to be studied. I have only minor formal comment to this part of the dissertaion - for unknown reasons, pages 59-70 and 72 are left empty in the end of the reprint of the paper.

The results section then continues with the original paper *The beta subunit of nascent polypeptide associated complex plays a role in flowers and siliques development of Arabidopsis thaliana*, with a candidate and supervisor as the joint first authors with equal contributions. This part of results is particularly relevant to the main aim of the dissertation as it provides a functional characterisation of the role of two genes (*NAC β 1* and *NAC β 2*) using their *A. thaliana* double mutants and showing phenotype consequences of their loss of function in flower and silique development. The results are well supported by transcriptomic and proteomic analyses.

The next part of results, following up the previous study, has been processed into a manuscript *The functional conservation of beta subunits of the nascent polypeptide associated complex in liverworts and angiosperms*. It shows the functional conservation of NAC β subunit between *Arabidopsis thaliana* and the liverwort *Marchantia polymorpha*. A complementation study on *A. thaliana* mutants of the NAC β subunits by *M. polymorpha* NAC β subunit reveals a conserved function of NAC β subunits across these divergent plant lineages of vascular and non-vascular plants. The authors propose that NAC function is conserved across all plant species and is crucial for plant development, as demonstrated by lethality of knock-out mutation. The study also includes analysis of functional divergence of Arabidopsis NAC subunits with the phylogeny and interaction analysis. The results support the notion that NAC β function is conserved among land plants despite vast evolutionary distances, probably due to its essential role in plant survival.

Q1: The studies on the NAC complex focus primarily on its role in male gametophyte development. However, the latest MS indicates broader implications of this protein family in overall plant development, stress responses, and adaptation mechanisms seem underexplored. What follow-up experiments would the candidate suggest to investigate these roles?

The last section of results is represented by a manuscript entitled *β subunits of the nascent polypeptide associated complex play a role in protein folding and protein sorting to chloroplasts in A. thaliana*. Here the authors analyze interactions of NAC β . The results provide a link between NAC and cytoplasmic chaperone system. NAC association with ribosomes and some other components of translational machinery was also demonstrated. In addition to results, this study shows an excellent complexity of methodology approaches.

Concluding discussion provides overview of interesting topics in the field, such as the role of ncRNAs in regulation of gene expression and development, new methodology approaches, including single cell transcriptomics, and useful database tools. Last but not least, both the demonstrated and potential functions of the nascent polypeptide-associated complex are discussed.

My only general comment to the overall positive evaluation of the results of the thesis is that their impact could be enhanced with more detailed and clearer graphical representations of the data to aid in interpretation and understanding.

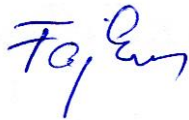
Overall, Božena Klodová's thesis contributes significantly to the field of plant biology, particularly in understanding the complex mechanisms governing gene expression in male gametophyte development. The introduction of tools like GOLEM and the detailed study of the NAC protein family provides valuable resources and insights for future research in plant genomics and reproduction.

Results have been published in four papers in WoS journals, and processed into two manuscripts.

Q2: is there any progress in finishing the preparation of the manuscripts and/or their publication?

In conclusion, the dissertation of Božena Klodová is of very high quality, meeting all the standards of Ph.D. thesis. Therefore, I recommend to award her with the Ph.D. title after a successful defense.

In Brno, Feb 12th



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