prof. UAM dr hab. Agnieszka Bagniewska-Zadworna
Department of General Botany
Institute of Experimental Biology, Faculty of Biology
Adam Mickiewicz University in Poznań

Review of the doctoral dissertation entitled:
'Post-Transcriptional regulation of mRNA mediated by decapping and degradation proteins in the cytoplasm and processing bodies of *Larix decidua*'
by ARASH MATINAHMADI

The formal basis for preparing the review is the letter from the Chair of the Scientific Council in Biological Sciences at the Nicolaus Copernicus University in Toruń, Prof. Dariusz Smoliński, together with the resolution of the Scientific Council of the Biological Sciences Discipline at NCU in Toruń dated 21st of November 2025, entrusting me with the duties of reviewer under Resolution No. 96.
I declare that no conflict of interest could affect the objectivity of my opinion.

Formal assessment

The doctoral dissertation submitted for evaluation was completed at the Department of Cellular and Molecular Biology of the Faculty of Biological and Veterinary Sciences at the Nicolaus Copernicus University in Toruń, under the main supervision of Prof. Dariusz Smoliński and co-supervised by Prof. Mehrdad Hashemi, from the Tehran Medical Sciences University, both are well-recognized specialists in cellular and molecular biology. The dissertation falls within the field of Natural Sciences, in the discipline of Biological Sciences. The reviewed thesis is an extensive monograph comprising 174 pages and follows the structure typical for experimental doctoral theses. It consists of seven main chapters, as well as a bibliography containing 232 references. The monograph is further supplemented with appendices, including a list of figures, supplementary data, and information on research funding. In addition, the entire work is accompanied by summaries in both Polish and English. The thesis, moreover, includes an overview of academic achievements reached by the candidate during his PhD study, focused on the list of two publications directly resulting from the dissertation, which are submitted and under review in scientific journals, as well as on other articles published during the doctoral study period (9 items). The summary clearly and comprehensively presents the key findings from the PhD study, allowing readers to easily understand the scope and significance of the work accomplished. The abstract also provides a clear introduction, framing the thesis's topic,

research gaps, and objectives, as well as methodology and implications, directly putting the results into perspective.
The proportions between the individual chapters of the whole dissertation are appropriate, as are the completeness of the content and the research tools used. It should be emphasized that both the text and graphics in the submitted dissertation meet the required standards. The scope aligns with the current field norms, and the work's components are organized clearly and logically. All research was conducted by the Author of the dissertation; therefore, the candidate's individual and distinct contribution to developing the concept, carrying out the experimental component, and interpreting the results is evident, which is consistent with the provisions of the Act on Academic Degrees and Academic Title.
The research was funded by grants from external sources, including the National Science Centre (two projects led by the supervisor and one additional project), as well as internal sources: Grants4NCU Students (from the 4th and 6th editions) and Mini Grants from the Academia Copernicana Interdisciplinary Doctoral School (in 2023 and 2024). A PhD student's involvement in so many research projects undoubtedly demonstrates their immense dedication, organization, and determination. Managing even smaller projects demonstrates strategic planning and effective resource management to achieve unique research goals within time and scope, requiring responsibility for the schedule and quality of results.

Substantive assessment of the dissertation

The doctoral thesis submitted for review addresses a very important and, in my opinion, previously unrecognized topic of the formation of P-bodies and their active mediation in 5′→3′ decapping-dependent decay during plant development. For the experiments, *Larix decidua* microsporocytes were used as experimental material. They constitute a unique and excellent plant model for studying meiosis with a prolonged diplotene stage (lasting several months), cyclical bursts of transcription, and a clear five-stage poly(A) RNA cycle. Microsporocytes of European larch are unusually large, with spacious cytoplasm and big nuclei, which facilitates high-resolution microscopy. All those advantages create a specific time window and plate that make it possible to link the fate of the mRNA with subsequent events programmed at a definite developmental stage. Thus, the main goals were formulated as: *i)* to determine whether P-bodies form and act as sites of decapping/decay during the five-stage poly(A) cycle and specifically whether decapping contributes to the late-stage poly(A) decline; *ii)* to quantify how P-body metrics (including size and distribution) and specific protein composition change across stages; *iii)* to identify sets of transcript associated with individual decapping components by RNA-immunoprecipitation sequencing (RIP-seq), thereby generating candidate substrates for functional tests. To achieve these goals, confocal microscopy was used to measure cytoplasmic poly(A) RNA and distributions of selected proteins: DCP5, DCP2, EDC4/VCS, LSM4, and XRN4, alongside P-body quantifications, and size classes assessments across 5 stages were combined. This approach to the topic and the presented research concept seems to be absolutely

correct. Taken together, this multi-level study demonstrates that only such multi-factorial analyses provide a comprehensive picture of the range of events occurring during plant development.
In the main part of the dissertation, the author presented an introduction with a literature review, the aim of the work, the materials used, the research methods applied, the results, and their discussion. The presented results are largely innovative, achieved thanks to the mentioned unique experimental system and the ability to use such large microsporocytes for microscopic analysis. This system allows for detailed analyses, and the interpretation of results is much easier and more reliable. The entire work is written in a language appropriate for this type of study. Only, a very few typographical errors are present; in particular, attention should be paid to the incorrect spelling of species names, as the second part of the Latin name should be written in lowercase. However, these are minor issues.

Evaluation of individual parts of the dissertation

The *Introduction* section is very well written, providing a solid background for the study and an extremely detailed description of post-transcriptional regulation of gene expression in eukaryotes, as well as the formation and the role of P-bodies in cells, including the significance of P-bodies in post-transcriptional mRNA regulation. The chapter includes informative figures that clearly illustrate the various mechanisms of: gene processing in eukaryotic cells, 5'-end capping process of mRNA, pre-mRNA splicing, 3'-end polyadenylation process of mRNA, mRNAs fate in the cytoplasm and the aggregation of P-bodies components, mRNA degradation pathway associated with PBs in plants and the stages of poly(A) RNA cycle. Despite its considerable length, the Introduction goes beyond a simple description of the studied topics and serves as a comprehensive compendium, allowing the reader to understand both the current state of knowledge and the Author's motivation for addressing these issues in the doctoral work. The chapter concludes with a description of the work's aim and hypotheses, which clearly and comprehensively highlight and justify the research topic undertaken.
I have a question: in the exceptionally detailed overview of P-bodies, there is a lack of references to trees. Given that this work is performed on tree species, it is unclear whether P-bodies, as well as SGs, have been noticed in other tree species beyond the larch. Is the function of them well described in trees?

In the *Material and Methods* chapter, the research material, sampling and the developed experimental setup were presented. To achieve the main objectives of this work, fully appropriate and modern techniques and methods from the fields of cell and molecular biology were employed. The research methods are described in accordance with the standards adopted for doctoral theses, clearly presenting both the methodology and the scope of the analyses performed. As such, this chapter may serve as a ready-made protocol for similar studies. The entire chapter also includes a description of the statistical methods used. In this respect, a

sufficient number of analyses and samples were performed to achieve statistical significance of the results.
While reading this chapter, some questions and points of clarification arose, which, as a reviewer, I would like to address. The Author stated that the material was fixed in 4% paraformaldehyde. In fact: ParaFormAldehyde (PFA) is a polymerized, solid form of formaldehyde (FA). When heated in water (often with slight alkalinization), it depolymerizes to monomeric formaldehyde, which is the actual fixing agent. So, during fixation, the real fixative agent is not PFA itself, but formaldehyde released from PFA. So did the Author dissolve PFA and heat it to obtain FA?

The *Results* chapter is presented in nearly 60 pages. The sheer volume of the analyzed results is noteworthy, as is their generally clear presentation, substantive analysis, and insightful observations in each subchapter. The results are original and undoubtedly contain a novel element. The Results section provides the end results of experiments to prove that and how P-bodies assemble and actively mediate decapping-dependent decay during plant development, and how quantitative changes in P-body architecture relate to substrate load. All microscopic results and subsequent events during diplotene in meiosis were additionally presented in quantitative form, based on fluorescence intensity and colocalization measurements. The most valuable results of the doctoral thesis include a detailed presentation of the distribution of individual analyzed proteins, also in the context of high-quality images of microsporocytes in diplotene. Each localized protein was presented through a plate with microscopic analyses with 4 panels: Poly(A), protein analyzed, and DNA separately and then in colocalization, the same scheme for each stage-specific section. The division of P-bodies into size classes/categories facilitated the description of the observed relationships and helped to systematize the results, thereby enabling the formulation of accurate conclusions. Such a visualization allows the readers to follow the fate of each component of the experiment. However, the detailed way of proving the results of the Author's own research included in the work required many time-consuming microscopic analyses, which were performed correctly and this is evidenced by the quality of the documentation presented. Similarly, statistical outcomes of the distribution pattern of each component were presented. The same clear pattern was really helpful in understanding all the interdependencies and interactions. What is more, RIP-seq analysis yielded several mRNA sequences, representing shared and protein-specific groups enriched in mitochondrial, chloroplast, and membrane-associated functions, which constitute potential candidate substrates for subsequent experimental validation. It is worth underlining that the Results chapter was written in a very clear and communicative way, which was certainly not easy, considering the multitude of factors analyzed and the repetitive scheme. Collectively, this multi-level study demonstrates the stage-dependent programmed cytoplasmic mRNA turnover in *Larix decidua* microsporocytes, in which P-bodies serve as principal reaction hubs coordinating decapping-dependent 5′→3′ decay during meiosis. ln my opinion, this is a valuable presentation of reliable results, dealing with original solutions to a scientific problem, which is the

understanding of the mechanisms of the post-transcriptional regulation of mRNA mediated by decapping and degradation proteins in the cytoplasm and processing bodies of larch.

I would like to ask whether, in Author's opinion, the research model applied in this study and especially the results obtained can be considered universal across the plant kingdom, or whether their applicability might be limited to specific taxonomic group(s), developmental stages, or environmental conditions. In other words, do you think that the results from this study and the resulting conclusions can be generalized?

The *Discussion* chapter. Noteworthy is the author's ability to discuss the obtained research results and to relate them to the available literature. This represents a demanding task, given the number of factors examined and the scope of the experimental work, which included multiple experiments employing carefully selected immunocytochemical analyses and specific plant material. The Discussion is divided into subsections, which improves the clarity of the presentation. The discussion in each subsection indicates that the Author can interpret his results appropriately and draw justified conclusions. The comparison with the relevant literature is generally accurate, and the cited works reflect a good knowledge of the subject. The doctoral candidate formulates clear conclusions based on the presented results. The chapter entitled: *Conclusions*, although de facto a summary of the results, is quite helpful and raises the sequence of events described unambiguously.

Chapter 7 *Perspectives* outlines potential directions for future research, which opens up a field for some queries. Does the Author intend to pursue these directions, and what possibilities exist for further work on this topic? Which of the presented perspectives seems to be the most important? In section 7.3 (Development-stress crosstalk), it was mentioned about the application of environmental and physiological stress. It would be helpful to clarify during the doctoral defense which type of stress is referred to (biotic or abiotic) and to provide a more detailed explanation. Is it possible to specify it?

Overall, I am impressed by the amount of experimental work carried out by Arash Matinahmadi and I am convinced that at least a few major publications will result from it. I would therefore like to ask what the status of the manuscripts listed as submitted/revised on page V is? In general, the manner in which the results are presented and discussed clearly demonstrates that the Author is a mature researcher who masters most aspects of scientific work and is a good candidate for a plant biology specialist. For all these reasons, I fully support that He proceeds to the final stages toward the award of the doctoral degree and wish to emphasize that this is an innovative dissertation, conceptually and methodologically advanced.

ADAM MICKIEWICZ UNIVERSITY, POZNAŃ
Faculty of Biology
Institute of Experimental Biology

In conclusion, I state that the dissertation's goals were fully achieved, and the PhD candidate has demonstrated the capability to conduct independent scientific research. Thus, I am absolutely convinced that the reviewed PhD thesis entitled 'Post-Transcriptional regulation of mRNA mediated by decapping and degradation proteins in the cytoplasm and processing bodies of *Larix decidua*' meets the criteria set out in the Article 187 of the Act of 20 July 2018 'Law on Higher Education and Science' (Journal of Laws of 2018, item 1668, as amended). On this basis, I respectfully recommend that the Scientific Council in Biological Sciences of the Nicolaus Copernicus University admits ARASH MATINAHMADI for next stages of the doctoral procedures in the Discipline of Biological Sciences.

Poznań, 08.01.2026

Podpisano przez/ Signed by:
Agnieszka
Bagniewska-Zadworna
Data/ Date: 08.01.2026 08:43
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