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## REVIEW

of the doctoral dissertation of Ms. Marta Saldat, MSc. entitled:  
“Biotechnological potential of diacylglycerol acyltransferases from *Nannochloropsis oceanica*  
for boosting lipid production in diverse hosts”

**Supervisor:** dr habil. Krzysztof Zienkiewicz, prof. NCU

**Co-Supervisor:** prof. Zhi-Yan (Rock) Du

This review has been prepared following my appointment by the Scientific Discipline Council of Biological Sciences at Nicolaus Copernicus University in Toruń, 20.03.2026, as a reviewer in the doctoral proceedings of MSc. Marta Saldat. The candidate submitted a doctoral dissertation entitled “*Biotechnological potential of diacylglycerol acyltransferases from Nannochloropsis oceanica for boosting lipid production in diverse hosts*” in the form of a monograph.

### General characteristics of the doctoral dissertation

The dissertation was prepared under the supervision of dr. habil. Krzysztof Zienkiewicz, Prof. NCU, with Prof. Zhi-Yan (Rock) Du serving as co-supervisor. The monograph consists of an abstract, introduction, materials and methods, results, discussion, conclusions, and references. It also includes a declaration of the candidate’s contribution in the work and originality of the results. The dissertation comprises 149 pages, including 49 figures and 27 tables.

The work is written in a concise and clear scientific language, using appropriate terminology. The introduction, an integral part of which is a review publication co-authored by the PhD Candidate, provides a comprehensive and detailed introduction to the topic of the doctoral dissertation. This section ends with an explanation of the dissertation's objectives and a presentation of its basic assumptions, formulated clearly and precisely. The methodological section presents a detailed description of biological material and applied techniques, enabling evaluation of the methodological correctness and appropriateness of the chosen methods. The research results are presented in the form of photographs, diagrams, and numerical data, accompanied by textual commentary. The results are described with exceptional care, yet concisely and clearly. I consider the graphical presentation a

valuable part of this chapter, allowing for an in-depth analysis of the doctoral Candidate's achievements.

The discussion, that follows the description of the results, is divided into thematic subchapters focusing on the functional specialization of the analyzed enzymes, the biosynthesis of triacylglycerols, the importance of these substances for the functioning of photoautotrophic cells, and the significance of the research for the broader biotechnology of plants and algae. The discussion, culminating in clearly formulated conclusions, is based on well-selected, current literature, a list of which complements the work. Editorially, the work is well prepared, with clear figures and tables, and properly structured text.

An undeniable strength of the doctoral dissertation is the clearly visible consistency in the implementation of subsequent stages of research, constructed based on previously obtained results in a systematic, thoughtful manner, leading to increasingly detailed analysis of the phenomena under investigation. In my opinion, this demonstrates the doctoral Candidate's development as a **scientist capable of conducting independent research**. It is also important to note the multithreaded nature of the research and the wide range of research techniques and methods employed, the application of which, and above all, the interpretation of the results obtained, require **general knowledge in the field of biological sciences**. The doctoral Candidate's knowledge is also demonstrated by her choice of current, well-founded research topics in the field of plants biotechnology and the very accurate selection of organisms on which the research was conducted. Such research has not only cognitive value but also application, contributing to the development of methods for sustainable development, which, in an era of growing demand for food and industrial raw materials, is undoubtedly one of the priorities of contemporary science. For this reason, the research conducted by the PhD Candidate is not only an element of her personal scientific development, but also constitutes a significant contribution to the development of the discipline of biological sciences and creates a **coherent, original solution to a scientific problem**.

### Detailed evaluation of the doctoral dissertation

During her doctoral project, Marta Saldat's primary goal was to functionally characterize poorly researched genes (NoDGTT7 - NoDGTT12) encoding diacylglycerol acyltransferase type 2 (DGTT; often referred to in the literature as DGAT2) and determine their importance for triacylglycerol (TAG) biosynthesis. She also determined whether the functional hierarchy within the DGTT family was limited solely to isoforms induced by nitrogen deficiency. In my opinion, this goal was achieved through a comprehensive, multifaceted research approach, including bioinformatics analyses, heterologous expression in a model yeast system, studies in the parent organism (a unicellular alga

*Nannochloropsis oceanica*), and a model organism belonging to higher plants (*Arabidopsis thaliana*). The obtained results clearly demonstrated the existence of distinct functional differences between the studied enzyme isoforms and allowed for the establishment of a hierarchy of their activity. In my opinion, the particularly important achievement of the PhD Candidate is the identification of the NoDGTT12 isoform as the most effective TAG synthesis enzyme, which suggests that the high catalytic efficiency of this type of enzymes does not have to be limited only to isoforms induced by nitrogen stress.

Ms. Marta Saldat was able to achieve the overarching goal of her doctoral thesis by successfully implementing the following specific research goals: (1) conducting a comparative analysis of the sequence and structure of the NoDGTT7 - NoDGTT12 genes to identify conserved and differential features that may underlie functional specialization within the nitrogen-insensitive DGTT group; (2) functional characterization of these genes through heterologous expression in the H1246 yeast strain with impaired TAG synthesis, which enabled quantitative analysis of their ability to synthesize TAG; (3) identifying high-efficiency DGTT isoforms and characterizing them biochemically and lipidomically, as well as assessing their substrate preferences and impact on TAG composition; (4) examining the effect of the most efficient isoform in the parent organism through stable overexpression and phenotypic analysis of algal cells; (5) evaluation of the most efficient isoform from an evolutionary perspective by heterologous expression in a higher plant to verify its performance in an evolutionarily distant organism.

Regarding the first specific objective, it should be noted that the sequence and structural analyses performed demonstrated significant diversification of the proteins encoded by individual genes, including differences in the number of transmembrane domains and structural organization, which clearly suggests their functional specialization. This objective was therefore fully achieved and provides a solid basis for the interpretation of subsequent results.

The second specific objective, concerning the functional characterization of the studied genes in a heterologous system, was also achieved. Marta Saldat, MSc, demonstrated that individual DGTT isoforms differ significantly in their ability to induce TAG synthesis in a yeast strain lacking this function, enabling a direct comparison of their enzymatic activity.

The implementation of the next (3rd) objective was confirmed by the identification of the isoforms with the highest activity, followed by their detailed characterization. First, the substrate preferences of NoDGTT12 and its significant impact on the fatty acid composition of TAG were observed, demonstrating the high level of sophistication of the analyses performed.

Another successfully completed goal involved analyzing the function of the NoDGTT12 isoform in the parent organism. This goal was achieved by generating transgenic *Nannochloropsis oceanica*

lines overexpressing NoDGTT12. The PhD Candidate demonstrated increased TAG accumulation in algal cells and significant changes in lipid profile, confirming the importance of this enzyme isoform in regulating lipid metabolism in *N. oceanica* cells.

The final goal of this work, closing the research (if it is possible to close any scientific topic), was to investigate how NoDGTT12 functions in an evolutionarily distant plant model, *Arabidopsis thaliana*. Expression of the gene in a mutant lacking DGAT activity led to the restoration of TAG synthesis and the reconstruction of lipid droplets, which strongly indicates the high functional versatility of the enzyme and its biotechnological potential.

In summary, the presented dissertation consistently and convincingly achieves all of the research objectives. Particularly noteworthy is not only the comprehensiveness of the experimental approach, but also the fact that new, cognitively and appliedly significant results have been obtained, which expand our current knowledge of the functional organization of the DGTT family and the mechanisms of TAG biosynthesis in microalgae. I am convinced of its high scientific standard. Nevertheless, while reading it, certain observations and questions arose, which I would like to express, hoping that there will be an opportunity to discuss them during Marta Saldat's doctoral defense.

First, the PhD Candidate posed a clear hypothesis regarding the functional hierarchy within the DGTT enzyme family, suggesting the possibility of highly efficient enzymes among the isoforms not induced by nitrogen deficiency. However, although the results clearly indicate the dominant role of the NoDGTT12 isoform, I lacked a clearly formulated reference to the main research goal, which, for me, was formulated like „distinguishing between "nitrogen-responsive" and "non-nitrogen-responsive" isoforms". I found it difficult to find a clear summary in the discussion that addresses this hypothesis. I hope the PhD Candidate will be willing to address this issue during her dissertation presentation at the defense.

The second issue concerns the achievement of the first specific objectives of the dissertation, which refers to the *in silico* sequence and structural analysis. These analyses provide an excellent interpretive framework, but I would have hoped that their direct connection to the functional results would have been more clearly highlighted in the discussion section. If the connection between the observed structural diversity and enzymatic activity could have been clearly presented during the defense, it would have been a great highlight, elevating the value of the research.

My next comment concerns objective 5 (evolutionary). Marta Soldat, MSc., conducted a very ambitious functional comparison of the studied enzymes and the expression of the genes encoding them between organisms with varying degrees of organization and evolutionarily distant, which is undoubtedly valuable. However, the conclusions regarding functional conservation could be formulated more carefully or more broadly contextualized in the literature. My comment stems from

the differences I know about lipid metabolism in higher plants and microalgae, especially since, to my knowledge, *Nannochloropsis oceanica*, like the more familiar to me *Chlamydomonas reinhardtii*, possesses a hybrid model of lipid metabolism pathways, combining features of both eukaryotic and prokaryotic organisms.

I would like to emphasize that the above comments are merely supplementary and do not undermine the high scientific value of the work.

In summary, in my opinion, the most important achievements of Marta Soldat, MSc. include, above all, the comprehensive functional characterization of the previously unstudied NoDGTT7-NoDGTT12 genes, which significantly expands our understanding of the diversity of enzymes encoded by these genes. Of particular note is the identification of the NoDGTT12 isoform as the enzyme with the highest efficiency of TAG synthesis, which challenges the belief often expressed in the literature that isoforms induced by nitrogen deficiency are dominant. Another significant achievement is the demonstration of the existence of a functional hierarchy within the studied enzyme family, based on direct comparative analyses. The use of a multi-model experimental approach, encompassing both heterologous systems and the parent organism and plant model, is of great value. The in-depth characterization of selected isoforms is also noteworthy. The results obtained have both cognitive significance and potential applications in the context of lipid biotechnology.

### Final conclusion

The doctoral dissertation by Marta Soldat, MSc., entitled "Biotechnological potential of diacylglycerol acyltransferases from *Nannochloropsis oceanica* for boosting lipid production in diverse hosts," written under the supervision of dr. habil. Krzysztof Zienkiewicz, prof. NCU, and prof. Zhi-Yan (Rock) Du, meets all the requirements for doctoral dissertations specified in Art. 187 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws 2024, item 1571, as amended), i.e.:

- it constitutes an original solution to the scientific problem of functional characterization of previously little-known genes (NoDGTT7 - NoDGTT12) encoding diacylglycerol acyltransferase type 2 (DGTT; often referred to in the literature as DGAT2) and determining their significance for triacylglycerol (TAG) biosynthesis in a cognitive and applied context,

- it demonstrates the PhD Candidate's general theoretical knowledge in the biological sciences and specialized knowledge in molecular biology, plant physiology, and biotechnology;

- it demonstrates the ability to independently conduct scientific work at all stages, from formulating hypotheses and research planning, to interpreting results and drawing conclusions.

Gdańsk, 06.06.2026

**In light of the above, I request that the Disciplinary Council for Biological Sciences at Nicolaus Copernicus University in Toruń accept the doctoral dissertation of Ms. Marta Saldat and allow her to proceed to the next stages of the proceedings for the award of a doctoral degree in the field of natural sciences in the discipline of biological sciences.** Furthermore, given the high scientific value of the doctoral dissertation submitted to me for evaluation, **I request that it be distinguished**, and the justification for this request is attached to this review.



**JUSTIFICATION FOR APPLICATION FOR A DISTINCTION**

for the doctoral dissertation of Marta Saldat, MSc., entitled "Biotechnological potential of diacylglycerol acyltransferases from *Nannochloropsis oceanica* for boosting lipid production in diverse hosts," which is an appendix to my review of this work.

**Supervisor:** dr habil. Krzysztof Zienkiewicz, prof. NCU

**Co-Supervisor:** prof. Zhi-Yan (Rock) Du

In my opinion, Marta Saldat's doctoral dissertation is characterized by an **exceptional level of merit**. The research problem itself is important and interesting, as it concerns research in the field of biotechnology of photoautotrophic organisms and their potential applications, which can contribute to the development of methods for sustainable social and economic development in many countries. The scope of the research encompasses an exceptionally broad range of molecular, biochemical, cytological, and physiological analyses, demonstrating a comprehensive approach to the topic. The spectrum of **research methods and techniques employed** is exceptionally broad. Moreover, the dissertation under consideration is distinguished by the research methods and tools employed. The set of these methods should be considered modern and adequate to the stated goals. A particular value of the work lies in the skillful integration of bioinformatic analyses with experimental studies conducted in three model systems, which significantly enhances the credibility of the obtained results and allows for a broad functional interpretation.

In summary, Marta Saldat's doctoral dissertation is distinguished by its comprehensive approach to research, combining various fields of science, such as molecular biology, biochemistry, and cytology. Furthermore, the work makes a significant contribution to the development of knowledge about the functioning of lipid metabolic pathways in various non-animal organisms and their biotechnological potential. With the above in mind, I request that the Disciplinary Council for Biological Sciences of Nicolaus Copernicus University in Toruń consider the possibility of distinguishing the work under consideration and honoring the PhD candidate in accordance with the procedures adopted by Nicolaus Copernicus University.

