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R E V I E W

of the dissertation

by

Syed Muhammad Muntazir Mehdi, MSc

entitled

"Identification of novel and ABA-regulated miRNA and characterisation of its target gene-*AtBro1* upon growth and abiotic stress response in *Arabidopsis thaliana*."

The PhD dissertation of Syed Muhammad Muntazir Mehdi, which I am reviewing, was prepared under the supervision of Prof. UAM dr hab. Agnieszka Ludwików (Adam Mickiewicz University, Poznan). The group of Prof. UAM dr hab. Agnieszka Ludwików is among the best working with ABA signalling in plants. The research was carried out as part of the Sonata Bis project, The European Union: Passport to the future – Interdisciplinary doctoral studies and Dean's grant led by Prof. UAM dr hab. Agnieszka Ludwików, financed by the National Science Centre (Poland), the EU and Adam Mickiewicz University.

The agricultural sector faces the daunting challenge of securing adequate food and other needs for a growing population projected to reach nine billion by 2050. The ability to increase arable land is limited, and a new threat to agriculture is emerging in the form of climate change, with unpredictable weather changes, floods and other catastrophic consequences, as well as other kinds of stress, making it even more challenging to provide the world's population with enough food. Since the agricultural sector is still one of the most critical sectors of the economy in many developing

countries, providing employment and being the primary source of income for people experiencing poverty, it is unsurprising that most of them are interested in technologies for adapting agriculture to different kinds of stress. So, the PhD thesis of Syed Muhammad Muntazir Mehdi is on top of the need to understand the molecular changes which plants face upon growth and abiotic stress response.

The doctoral dissertation is based on two thematically coherent publications that appeared in the years 2021-2023 (according to the chronology of their inclusion in the dissertation):

1. **Mehdi, S.M.M.,** Krishnamoorthy S., Szczesniak, M.W. and Ludwików, A., 2021. "Identification of novel miRNAs and their target genes in the response to abscisic acid in Arabidopsis" International Journal of Molecular Sciences 22, no. 13: 7153. Ministry of Education and Science – 140; Impact factor (2022-2023) – 6.208 (IF_5 = 6.628);

2. **Mehdi, S.M.M.,** Szczesniak, M.W. and Ludwików, A., 2023. The Bro1-like domain containing protein, AtBro1, modulates growth and abiotic stress responses in Arabidopsis. Front. Plant Sci. 14: 1157435. Ministry of Education and Science – 100; Impact factor (2022-2023) – 6.627 (IF_5 = 6.3).

It should be noted that the publications in the dissertation have already been subject to a thorough process of substantive review by independent reviewers who are experts in their subject and appreciate the value of the presented results. In terms of content, I rate the PhD thesis highly. The assumptions of the doctoral project are well thought out. I consider the choice of the research topic to be right - the undertaken topic is significant and future-proof. Therefore, my review focuses on the overall assessment of the PhD student's research and the doctoral dissertation's formal assessment.

The first paper included in the dissertation was published in the International Journal of Molecular Sciences, which according to Clarivate, has an impact factor for the year of publication (IF2022-2023) of 6.628 and the number of Ministry of Education and Science points equal to 140. Subsequent papers were published in Frontiers in Plant Science (IF2022-2023=6.627 and 140 Ministry of Education and Science points). In all the works, the PhD student is the first Author. All

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presented publications are experimental works. Scoring achievements and a high impact factor testify to an excellent scientific workshop of the PhD student. Syed Muhammad Muntazir Mehdi's contribution in preparing publications that make up the doctoral dissertation was specified in the statements of the co-authors. I consider the contribution of the PhD student to the creation of these works as excellent, taking into account his position among the authors and the number of authors.

In the first publication contribution of the PhD student was 75 %, which is higher for this type of work. In this publication, authors aimed to identify novel ABA-responsive miRNAs and their putative target mRNAs and to investigate whether and how the ABA response in Arabidopsis involves miRNA regulation of the ABA signalling pathway. Authors identified ten novel miRNAs in wild type after ABA treatment, while in *abi1td*, *mkkk17*, and *mkkk18* mutants, three, seven, and nine known miRNAs, respectively, were differentially expressed after ABA treatment. PhD student used in this work such methodologies as sequencing, differential expression analysis, validation of miRNA expression with stem-loop qRT-PCR, target validation by 5′ RLM-RACE-PCR and miRNA target gene expression by qRT-PCR and target gene identification, gene ontology, and KEGG pathway analysis. At the end of the work, the authors represent a hypothetical model of the ABA-responsive miRNA regulatory network and their target genes in Arabidopsis. The authors also provided a database for expanding our knowledge and understanding of regulatory networks in the ABA core signalling pathway.

In the second publication contribution of the PhD student was 80 %, which is higher for this type of research work. In this publication, the authors studied the mechanism involving the *AtBro1* gene. This gene encodes one of the small family of poorly characterised Bro1-like domain-containing proteins. Authors subjected Arabidopsis to different stress, including salt, mannitol and drought. PhD student used such methodologies as constructing and generating the transgenic lines, subcellular localisation of the AtBro1 protein, analysis of the overexpression lines and promoter activity, RNA sequencing with subsequent data analysis etc. The main conclusion of this work was that AtBro1 plays a significant role in regulating the plant transcriptional response to ABA and the induction of resistance responses to abiotic stress. All this witnessed the excellent preparation of the PhD student.

In the end, I want to ask PhD student a few questions about the thesis, asking for an answer during the public part of the doctoral defence:

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1. In the publication #1 and #2 you proposed some models for 'A hypothetical model of regulatory networks of ABA-responsive miRNAs and their target genes in *Arabidopsis*.' and represent the schematic model of the AtBro1 in plant response to abiotic stress. Do you think these models can be universal for monocot and dicot plants?

2. In publication #2, you mentioned that the *AtBro1* gene is a promising candidate for interventions that improve plant stress resistance. Can you provide some examples of what we can do to improve, for example, such crops as wheat, barley, or any other, to improve stress resistance? Can we use CRISPR/Cas9 technique in this case? Or do we have any limitations in the EU?

3. Why did you work on Arabidopsis and not directly on essential crops? Do we have any limitations with research on Arabidopsis?

I, hereby, declare that the reviewed PhD thesis entitled "Identification of novel and ABA-regulated miRNA and characterisation of its target gene-*AtBro1* upon growth and abiotic stress response in *Arabidopsis thaliana*" by Syed Muhammad Muntazir Mehdi meets the criteria under art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), and request that the Research Discipline Council of Biological Sciences of the Adam Mickiewicz University accepts Syed Muhammad Muntazir Mehdi for further stages of doctoral proceedings in the field of biological sciences.

Concluding my review, I want to emphasise that this is a conceptually and methodologically advanced PhD project. This is an outstanding achievement of the Author and his supervisor, Prof. UAM dr hab. Agnieszka Ludwików. I hereby request that the thesis be accepted with distinctions due to the valuable, innovative and published results. The two publications published in good journals prove that the PhD candidate fulfilled his research project perfectly.

Signed by /
Podpisano przez:

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