

Prof. dr hab. Jakub Sawicki

Department of Botany and Nature Protection

Faculty of Biology and Biotechnology

University of Warmia and Mazury in Olsztyn

Plac Łódzki 1

10-719 Olsztyn

email:jakub.sawicki@uwm.edu.pl

tel: (89) 523 34 94

REVIEW OF

PHD THESIS OF ALISHA ALISHA REALIZED IN DEPARTMENT OF GENE EXPRESSION, FACULTY OF BIOLOGY, ADAM MICKIEWICZ UNIVERSITY IN POZNAŃ UNDER SUPERVISION OF PROF. DR HAB. ZOFIA SZWEYKOWSKA-KULIŃSKA AND DR IZABELA SIEROCKA, ENTITLED “PHYLOGENETIC AND FUNCTIONAL STUDIES OF SQUAMOSA PROMOTER BINDING-LIKE TRANSCRIPTION FACTOR GENE FAMILY MEMBERS IN THE LIVERWORT *MARCHANTIA POLYMORPHA*.”

The review was prepared at the request of the Scientific Council of the Discipline of Biological Sciences at Adam Mickiewicz University in Poznań, in accordance with Article 190(2) of July 20, 2018 (Journal of Laws of 2022, item 574 with amendments).

The doctoral thesis of Mr. Alisha Alisha was prepared in the form of a single-author manuscript consisting of 160 pages. The thesis explores the functional diversity of the SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors in plant growth and development. The study focuses on understanding the evolutionary significance and functional roles of SPL genes in basal lineages of land plants, particularly among bryophytes.

The work begins with an extensive, 27-page introduction that introduces the reader to topics related to the biology and genetics of the model species *Marchantia polymorpha*, available methods of genetic transformation, and the characteristics of SPL genes. Subsection 1.1 is entirely dedicated to *Marchantia polymorpha* and is quite general, providing more of an overview of liverworts in general than focusing on the specific species discussed. I cannot agree with the statement 'The bryophytes lineage, liverworts, consists of simple thalloid and complex thalloid clades,' as at least five major evolutionary lineages have been identified within this group, including early divergent (Haplortriopsida), the mentioned complex thalloids, two simple thalloid lineages (Pellidae and Metzgeridae), and leafy liverworts. Additionally, several important pieces of information about this model species are missing, such as its evolutionary position within Marchantiales, low evolutionary rate, and the lack of an organellar RNA editing mechanism. On the other hand, all genomic resources are very well described, including recent publications and advances. Since the evolutionary relationships of an early land plant lineage are still under debate, are there alternative phylogenetic hypotheses to the one presented in Figure 1 in the context of Bryophyte evolution? This question seems significant, especially in the context of the results presented in Figure 3.2.

Subsection 1.2 precisely characterizes the SPL gene family in both vascular plants and bryophytes, with a particular focus on *Physcomitrium patens*, where these genes have been best studied so far. It also introduces the reader to issues related to their function and expression regulation. The cited literature is current and includes the latest available information on the addressed topic. Summing up this part of the doctoral dissertation, it must be noted that, with few exceptions, it contains all the necessary introductory information and is written in accessible language. In my assessment, this chapter should also include clearly defined research goals and hypotheses, which were lacking in the reviewed manuscript.

Equally extensive, spanning 25 pages, is the chapter 'Material and Methods,' meticulously describing the conducted procedures and research materials. Subsection 2.1 includes a comprehensive list of used reagents, the characterization of Tak-1 lines, and bacterial strains employed for genetic transformation. In subsection 2.2, procedures utilized during the research are detailed, with few exceptions. The variety of methods and procedures applied in the work is extensive, encompassing both basic molecular biology techniques and still innovative approaches like CRISPR/Cas9 genome editing or the application of artificial miRNA. Amidst the detailed descriptions, the method for DNA sequencing (subsection 2.2.14) is broadly characterized and, in my opinion, despite being delegated to the faculty core lab, should be described in more detail. Additionally, there is a lack of information in this chapter about the analysis of obtained sequences, including trimming conditions and sequence assembly. Sanger sequencing typically generates reads with low-quality initial and terminal nucleotides that require trimming before downstream analyses.

The bioinformatics methods are usually well described and standard, well performing tools were used during research. However, there's lack of information about evolutionary models

used during phylogenetic tree reconstruction, which in the case of poorly supported nodes could impact on tree topology.

In summary, aside from the aforementioned issues, the chapter 'Material and Methods' is generally well-written and provides most of the information necessary for the potential replication of the performed experiments. It is primarily text-based, although some procedures, methodological assumptions, and pipelines would be more accessible with corresponding figures.

The chapter 'Results' is the most extensive part of the reviewed work, spanning 51 pages. It consists of two parts: the first one entitled 'Phylogenetic, structural, and functional relationships between SPL transcription factors from bryophytes and angiosperms,' and the second dedicated to the functional characterization of Mp*SPL3* and Mp*SPL4* transcription factors.

In the first part of this chapter (3.1), phylogenetic analysis is conducted on SPL family members from various lineages of bryophytes, including hornworts, liverworts, and mosses, as well as angiosperms. The analysis reveals four distinct phylogenetic groups within the SPL family, with members within the same group sharing similar gene structures and protein domains. Interestingly, no SPL genes were initially identified in hornworts, but the study establishes the presence of a minimal set of SPL genes in the *Anthoceros* lineage, similar to liverworts. It's worth noting that *Pellia endiviifolia* is moved to the separate genus *Apopellia*.

The interpretation of the data described in this subsection is generally correct; my only reservations concern the interpretation of the results presented in Figure 3.2. Based on the phylogenetic analysis, genes AtSPL9 and AtSPL15 cannot be classified into Group 1, as their common clade lacks statistical significance (only 47% bootstrap support).

The second part (3.2) focuses on the molecular genetic characterization of Mp*SPL3* and Mp*SPL4* genes from the model liverwort species *Marchantia polymorpha*. Through in planta promoter activity, RT-qPCR analysis, CRISPR/Cas9 knockout, and artificial miRNA knockdown approaches, the study demonstrates that both Mp*SPL3* and Mp*SPL4* genes are ubiquitously expressed and play crucial roles in the growth and development of *Marchantia*. Loss-of-function mutations result in significant phenotypic changes, including reduced thalli, delayed growth, and altered reproductive structures. Additionally, overexpression of Mp*SPL4* leads to distinct phenotypic changes, such as smaller thalli with larger gemma cups.

Overall, the findings contribute valuable insights into the functional roles of Mp*SPL3* and Mp*SPL4* genes in *Marchantia polymorpha*. These genes are identified as essential regulators controlling the proper growth and development of both vegetative and reproductive organs in this liverwort species.

The main part of the work is crowned by a 15-page discussion referring to the latest discoveries in the addressed topic. It is worth emphasizing that the cited literature is very current and includes many works published in 2023, which indicates a significant interest within the scientific community in the issues addressed in the paper.

While compiling the doctoral dissertation in manuscript form, it's hard not to notice minor editorial and punctuation errors, which are also present in the reviewed work. These errors predominantly involve formatting issues with subsections and the frequent absence of italics for genus and species names. However, they do not affect the reception of the thesis and its substantive value. Presumably, they have been or will be corrected by the doctoral student during the preparation of the dissertation or its parts for publishing.


Final conclusions

The entirety of the doctoral thesis presented to me for evaluation constitutes a valuable contribution to the understanding of the biology of *Marchantia polymorpha*, both in the context of the evolution of MpSPL genes and in the exploration of their roles and functions. Modern molecular biology methods were applied for functional characterization, enabling the publication of the results obtained in this work in reputable journals.

The reviewed doctoral dissertation gives the impression of a thorough scientific thesis and fulfills all the requirements set for doctoral theses according to the law of July 18, 2018 (Article 187) Journal of Laws of 2022, item 574.

In light of the above, I kindly request the High Council of the Discipline of Biological Sciences at Adam Mickiewicz University in Poznań to accept the doctoral thesis of Ms. Alisha Alisha and to allow the doctoral candidate to proceed to the next stages of the doctoral process.

Sincerely,


Jakub Sawicki

Olsztyn, 12.11.2023