UNIVERSITY OF WARSAW

Faculty of Biology

Review of PhD dissertation by Ms. Alisha titled "Phylogenetic and functional studies of *SQUAMOSA PROMOTER BINDING-LIKE* transcription factor gene family members in the liverwort *Marchantia polymorpha*"

SPL (SQUAMOSA promoter binding protein-like) genes encode plant specific transcription factors that play important roles in plant growth and development, and in response to different environmental factors. It is known that *SPL* gene family is widely distributed from unicellular algae to angiosperms and is highly conserved in green plants lineage. Although the literature data indicates large dynamics of the *SPL* genes evolution within angiosperms, however, for other ancient land lineage the data on the role of *SPL* genes is far from complete. Investigation of *SPL* gene family in controlling proper growth and development in the representatives of the oldest living land plants is still needed for better understanding relationships between *SPL* genes of bryophytes and angiosperms, and thus their evolution. The evaluated dissertation is devoted to this subject and focuses especially on liverwort *Marchantia polymorpha*. Thus the scientific significance of the dissertation subject is clear.

The reviewed dissertation was completed at the Department of Gene Expression, Faculty of Biology of Adam Mickiewicz University in Poznan, under the supervision of Professor Zofia Szweykowska-Kulińska, a well-recognized specialist in molecular biology and biotechnology, and of doctor Izabela Sierocka, as an assistant supervisor. The dissertation falls within the field of Natural Sciences in the discipline of Biological Sciences.

The dissertation is prepared according to the traditional rules as a script-book divided into Introduction, Material and Methods, Results, Discussion, and References, preceded by Abstract in English and Polish. The dissertation is 160 pages long including nice illustrations. Part of the results is covered by the original paper placed in BioRxiv (DOI: 10.1101/2023.02.27.530190) with the PhD student as the first and leading author.

The **INTRODUCTION** reviews in a concise way the knowledge of the subject. The author presents characteristics of *Marchantia polymorpha* as a model organism pointing the main advantages of this species, and giving a very nice scheme of its life cycle. Also various tools available for *M. polymorpha* functional studies are described. A separate part of the Introduction concentrates on *SPL* genes, the functions of *SPL* gene family in angiosperms emphasizing *A. thaliana* and *O. sativa*. I appreciate simple but informative Figures 11 and 12. The INTRODUCTION points that the expression levels of many *SPL* genes are controlled by microRNA, and that the changes in the expression levels of each miRNA have their implication in Arabidopsis and rice development. The author also emphasizes the role of *SPL* genes as important factors in plant's response to different stresses. The last section of the Introduction concentrates on the functions of *SPL* transcription factor gene family, described in only a few bryophyte species represented by moss *Physcomitrium patens*, liverwort *Marchantia polymorpha*, hornworts *Anthoceros agrestis* and *Anthoceros angustus*. In my opinion, the INTRODUCTION is of great value; it touches all issues discussed in further parts of the dissertation. In spite of fact that a part of it is already available in BioRxiv, it would be an excellent review paper that deserves separate publication.

There is no **Objectives** in the PhD thesis as a separate chapter which is present in most dissertations. Such part would emphasize the main goals which should be reached.

The next part, **MATERIALS and METHODS** describes in details the broad spectrum of methods including several molecular genetic tools used in the PhD thesis. The methods are properly chosen. I want to stress that applied methods are very advanced and require skills, experience, and patience. In addition to standard methods such as DNA, RNA and protein isolation, PCR and RT-qPCR, the CRISPR/Cas9 system, and artificial miRNA approach are really advanced and could be used by the author due to experience of the home laboratory.

The part **RESULTS** consists of 2 chapters. The first Chapter is devoted to phylogenetic relationships of *SPL* TF genes from representatives of all bryophyte lineages to angiosperms which is one of the main goals of the PhD dissertation. The choice of four bryophytes representatives: *Physcomitrium*

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patens, *Marchantia polymorpha*, *Anthoceros agrestis,* and *Anthoceros punctatus* was interesting in my opinion, also because two of them are known as model plants. The moss *P. patens* is a model organism for division plane positioning and *M. polymorpha*, the main object of this thesis, is known as an attractive evolutionary model plant to study complex biological processes; the two other bryophytes - hornworts *A.agrestis* and *A. punctatus* have never been objects of *SPL* genes identification. I found the phylogenetic analysis of SPL proteins from *A. thaliana*, *P. patens*, *M. polymorpha*, *A. agrestis,* and *A. punctatus*, constructed and presented in Figure 3.2, to be very interesting. Based on the phylogenetic analysis, SPL proteins were classified into four phylogenetic groups. I found the result that the members of the same group have similar gene structure and protein domains as being extremely important because, according to author, it might indicate their similar function. The author found also that similarly to *M. polymorpha*, representatives of hornworts have four members of *SPL* gene family which is the smallest known *SPL* family in comparison to other land plants. In my opinion this Chapter carries a significant contribution to the knowledge of land plants evolution. The results of this Chapter are discussed in research article: "Comparative analysis of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* gene family between bryophytes and seed plants", BioRxiv (DOI: 10.1101/2023.02.27.530190) in which Ms. Alisha is the first and leading author. The second Chapter of the **RESULTS** focuses on functional characterization of Mp*SPL3* and Mp*SPL4* genes from the model liverwort species, *M polymorpha*. Various molecular genetic tools were applied in this part. It was demonstrated by joining promoter activity using GUS reporter gene with RT-qPCR analysis *in planta*, that both genes are expressed during the vegetative and reproductive phase of *M. polymorpha's* life cycle. Another approach, with the help of CRISPR/Cas9 system, showed that the knock-out plants for each gene displayed very strong inhibition of development. Similarly, the knock-down plants for Mp*SPL3* and Mp*SPL4* genes, obtained with an artificial miRNA approach, exhibited delay of their vegetative growth together with completely inhibited formation of gametangiophores or delayed formation of archegoniophores, respectively. Thus, the author proved that proper level of Mp*SPL3* and Mp*SPL4* genes expression is necessary for Marchantia generative organs development, as well as for vegetative development. Moreover, transgenic plants with overexpression of Mp*SPL3* did not show significant changes in vegetative growth, however plants overexpressing Mp*SPL4* protein had narrower thalli and bigger gemma cups as compared to wild-type plants. This chapter, using a variety of approaches, demonstrated that the proper level of

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Mp*SPL3* and Mp*SPL4* genes expression from the model liverwort species, *M. polymorpha* is indispensable for its vegetative and generative development. These significant results on the function of *SPL3* and *SPL4* genes in *M. polymorpha* elucidate to a large extend the regulation of growth and development of this species.

The part **DISCUSSION** refers to obtained results and confronts own results with other authors' publications. It is divided into two sections corresponding to the two chapters of **RESULTS** which contributes to clarity of the whole thesis.

For clarity of the dissertation I would expect the part **Conclusion**, as a separated Chapter, nevertheless the last few sentences in the part **DISCUSSION** fulfill this role.

In my opinion the most significant achievements of this thesis are:

- Providing comparative and phylogenetic analyses which allowed profound understanding of the *SPL* gene family, from bryophytes to land plants. Pointing on archetype of *SPL* genes in liverwort and hornwort as possible ancestors of nowadays land plants.

These results represent a large step towards clarification of *SPL* genes evolution.

- Giving deep understanding of the crucial functions of Mp*SPL3* and Mp*SPL4* genes in control of the development of both vegetative and reproductive organs of *Marchantia polymorpha*.

These results extend the knowledge in the area of plant-specific transcription factors and their involvement in the regulation processes of plant development.

One more remark. The author made a comparison between phenotypes of mutant plants involved in auxin-signaling pathway and Mp*SPL3* and Mp*SPL4* transgenic plants (presented in Figure 4.2) . The author indicated that phenotype changes observed in the obtained knock-out, knock-down and overexpression plant lines of Mp*SPL3* and Mp*SPL4* genes are similar to those observed in mutant plants of several genes engaged in auxin-biosynthesis, suggesting shared pathways between these mutants and auxin signaling network. I would like to hear more about these interactions during the public defense.

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The thesis is clearly written and easy to follow as well as edited quite carefully, with only few typographic mistakes. The remarks and comments summarized above do not influence my very positive opinion about Ms. Alisha' work. The range of applied methods is really impressive. Ms. Alisha has proven to be fully prepared to execute experimental work and has shown that she can pose scientific problems and solve them.

In summary, I am absolutely convinced that the presented thesis meets all requirements for PhD dissertations according to the law (art. 187 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (tekst jednolity: Dz.U. z 2021 r. poz. 478 z późn. zm). Therefore I am applying to the Academic Council of Biological Sciences of Adam Mickiewicz University in Poznań for acceptance of Ms. Alisha PhD thesis and admission Candidate for further steps provided by the PhD procedure. Moreover, taking into account high quality of the results, broad view on the regulation of plant development with strong evolutionary aspects and also comprehensive presentation, I am applying for a distinction of the PhD thesis.

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