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RE: Ms. Halina Pietrykowska dissertat ion

December 26, 2019

Faculty of Biology, Adam Mickiewicz University, Poznan,  
Uniwersytetu Poznanskiiego 6 str.  
61-614 Poznan, Poland

Dear respected faculty members and defense committee members:

I have read the doctoral dissertation prepared by Ms. Halina Pietrykowska entitled: "Identification and characterization of microRNAs involved in sexual reproduction of *Marchantia polymorpha*".

As a whole, I agreed and was satisfied with the novel findings on expression and function of *Marchantia* microRNAs discovered by her study. Especially the analysis of one miRNA named Mpo-miR11889, that is thought to be shared among liverwort and *Chlamydomonas*, provided her with a new stage to study how sporophytes (basal plant specific organ) become determinate organs from the point of gene expression. She gave an original solution to a scientific problem.

She made a good introduction and covered nicely background of the research field in the thesis, also showed general theoretical knowledge in biology at a high level.

I respect great supervision by Prof. Zofia Szweykowska-Kulinska and furthermore admit that Halina Pietrykowska can carry out independent scientific research skill and talent. Otherwise, she could not accomplish such a novel finding.

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Best regards,

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P.S. I noticed several typos, texts, ambiguities to be amended to make a better dissertation in a proper way, but such points do not deny her efforts, establishment in and contribution to the basic science. I definitely agree with that Ms. Halina Pietrykowska is worth Ph.D. status.



Again, I am satisfied with the thesis of Ms. Halina Pietrykowska entitled, "Identification and characterization of microRNAs involved in sexual reproduction of *Marchantia polymorpha*" and well-qualified to reach a sufficient level as an independent natural scientist. Before finalizing the thesis, I would like to suggest the following points.

Major point:

A Typical example is in Fig.24, P.90. Complementary alignments are provided, but they allow G..A pairs. Why or Is it intended?

The ultimate final part relating to miR11889:

Pietrykowska found that deltaMpo-miR11889ge male plants showed abnormal antheridiophore development and deltaMpo-miR11889ge female plants showed altered sporophyte development. Can she explain something common between the molecular mechanisms what occurs in male and female liverwort reproductive process?

P.126, Table 64: I could not understand the detailed content of this table. She conducted 46 crossings but could not find their details of the male and female parents.

P.127, Fig.63 result: If my understanding is correct, archegoniophores keep some vegetative tissues in stalks. If so, how can she exclude the green parts shown in Fig.63 are originated from such vegetative tissues ?

Minor points:

Description of CRISPR knockout lines; at first, Mpo-miR11889<sup>ge#1 or 2</sup> like in Page 116 is OK. But after Page 119 or later, we could see <sup>#1ge</sup> or <sup>#2ge</sup> in a reversed order. The order of ge#"number" is recommended.

P.87. L.5: four different tissue types..

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P.93, Fig 27A and C: I wondered whether or not she could detect RNA-seq reads more closely to genomic sequence provided in C. So different from each other.

P.94, Fig28. C and D: Data are consistent with each other.

P.98-99: Better to describe what 190, 2877, 1042 mean in Figure 32.

P.100, Table 62: Better to include Pellia information in the same columns as well.

P.102-3, Fig.34: There are some differences between the results of NGS and Northern blot. Do you have any comments on that? In miR156/529 blot, I could see a band shift of Ma and Fa lane samples. Do you have any ideas?

P.114, Fig.47: Quantification of the decrease of splice variant in Ma is possible to make contrast with other samples.

P.129, Fig. 67: Has she tried crossing between male and female MpDUSP12 overexpressing plants?

Typos:

P.23, L.2 from the bottom: endophytic fungi

P.36, column list: Length

P.37, L.2: plastocyanin or something.

P.41, L.1: discussion

P.109, last: dissertation

P.115, L10-11. Fig.49 not 48.

FIN

Best regards,

Y. Watanabe