Evaluation of PhD Thesis

PhD candidate: **Michał Szopiński**

Supervisors: **dr hab. Eugeniusz Małkowski**[1]**, prof. UŚ**; **prof. Nathalie Verbruggen**[2]

Faculty/Research group: joint PhD prepared in: 1) Ecophysiology Team, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Poland, and 2) Laboratory of Physiology and Molecular Genetics, Faculty of Science, Université Libre de Bruxelles, Belgium

Title of PhD thesis: **Characterization of Arabidopsis species from metalliferous and non-metalliferous sites in Southern Poland**

Reviewer: **dr hab. Małgorzata Wójcik, prof. UMCS**, Department of Plant Physiology and Biophysics, Institute of Biological Sciences, Faculty of Biology and Biotechnology, University of Maria Curie-Skłodowska in Lublin, Poland

1. Scientific value of the PhD thesis

a) Originality

Although metal toxicity and tolerance in plants have been extensively studied for many years, still the mechanisms underlying are not well understood, especially at the molecular level. These studies were mainly focused on plants exposed to acute short-term metal stress in hydroponic cultures, like part of the experiments presented in the thesis. Such investigations are useful for fast screening of metal resistance between the species or populations within a species and provide valuable information on the physiological and molecular basis of metal tolerance mechanisms. However, they may not accurately predict the plant response to sublethal, chronic metal stress, to which plants are exposed in heavily metal-polluted environments and where (micro)evolutionary processes lead to species divergence and formation of new local forms better adapted to their adverse environmental conditions. The great value of the presented dissertation is combining the hydroponic experiments with *in situ* examination of metallicolous and nonmetallicolous populations of *Arabidospis helleri* and *Arabidopsis arenosa* in order to reveal the inter- and intra-specific variability in metal uptake and tolerance at the physiological and molecular level. The thesis as a whole is an original in its concept, multifaceted and comprehensive approach to the research problem and provided very interesting results which give new insight into mechanisms of metal tolerance and hyperaccumulation evolved in the two related species inhabiting the same metalliferous habitat.

b) Scientific quality

Obtained with up-to-date techniques and appropriate statistical analyses, the results are reliable and of high scientific relevance, which was already confirmed by the publication of some of them in international peer-reviewed journals characterised by very high as for this discipline Impact Factor (Plant Cell Environment – IF = 7.228; Frontiers in Plant Science – IF = 5.753). I am

convinced that the other results (chapter III prepared for submission) will also be published in a very good journal as it presents the most up-to-date molecular studies.

The PhD candidate provided strong evidence for at least partially convergent evolution of the well known Cd/Zn hyperaccumulator *A. halleri* and the related species *A. arenosa* originating from the same calamine site. This was evidenced by some physiological parameters as well as molecular features, including triplication of *HMA4* gene, responsible for Cd/Zn hyperaccumulation in both species (xylem loading) and increased expression of *HMA3* gene, responsible for these metals tolerance (vacuolar sequestration). It was found that *A. arenosa* has a tetraploid genome (the new discovery) and that autotetraploidisation of is not connected with its hyperaccumulation traits. Especially valuable and filling the gap in our knowledge are the studies of the photosynthetic apparatus in these two species *in situ* – such research has not been carried out to date. Pigment content and chlorophyl *a* fluorescence parameters under metal stress showed higher tolerance of *A. helleri* than *A. arenosa*, resulting most probably from more efficient mechanisms of Cd and Zn detoxification by their sequestration in leaf cell vacuoles. Other contrasting features, such as flavonol and anthocyanin content and expression of genes involved in their biosynthesis as well as metal accumulation pattern and genes involved in Cd and Zn uptake, remobilisation from the roots and cellular detoxification suggest different mechanisms of metal uptake and homeostasis the these two species in spite of co-occuring at the same polluted site. In view of the presented results *A. arenosa* emerges as a new interesting model object for studying metal tolerance and hyperaccumulation in plants.

2. Presentation of the text

The structure and format of the PhD thesis are correct and typical for PhD books in the countries of western Europe. The thesis is divided into 12 chapters. The first chapter presents summary in Polish, all the other chapters are provided in English. The Introduction gives a comprehensive and detailed background for future studies, followed by the chapters devoted to clarification of the PhD objectives and a scientific strategy. The objectives of the study are clearly formulated in the form of three questions and the specific research tasks leading to the answers to these questions are detailed. The description of the methods is briefly listed in the chapter “Methods used” with more attention focused on chlorophyll *a* fluorescence analysis, illustrated by an excellent figure combining the scheme of electron transport chain and the fluorescence curve. The next three chapters present the results of three independent studies, which form a clear entity each. Two first ones are copies of the published articles, the last one is a manuscript under preparation for submission. Although the published papers are available in the Internet, it would be good to include the supplementary figures and tables in the doctoral dissertation along with the copies of the articles. It would greatly facilitate analysing and interpretation of the results. The thesis is closed by overview of main achievements and conclusions, followed by the list of references used elsewhere besides the experimental chapters. The thesis is written with mature and correct language, is prepared very thoroughly and aesthetically. The figures and the tables are adequate to the content of each chapter, they are of good quality, clear and informative, supported by the statistical analysis data where necessary.

3. General evaluation

The presented three original studies are sufficiently extensive for a PhD thesis. The number of techniques used and the results obtained are of high quality and international level. The way of preparing the thesis, and especially the presentation and interpretation of the results, as well as rising new research questions and opportunities proves that the PhD candidate has not only

extensive knowledge concerning the subject of research but also analytical mind and ability to draw brilliant synthesis from his work.

4. Conclusion

In my opinion, the doctoral dissertation submitted for review meets all the requirements set out in Article 13 of the Act of 14 March 2003 on Scientific Degrees and Academic Title and Degrees and Title in Art (Journal of Laws of 2017, item 1789) and in Article 179(1) of the Act of 3 July 2018 Introductory provisions of the Act - Law on higher education and science (Journal of Laws of 2018, item 1669). In view of the above, **I hereby apply to the High Council of the Institute of Biology, Biotechnology and Environmental Protection of the Faculty of Natural Sciences of the University of Silesia in Katowice to admit Mr. Michał Szopiński, M.Sc. to further stages of the doctoral procedure**.

At the same time, **in recognition of a wide range of research and valuable, original results, I recommend awarding the thesis with an appropriate prize.**

Uważam, że przedstawiona do recenzji rozprawa doktorska spełnia wszelkie wymagania określone w art. 13 Ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. z 2017 roku, poz. 1789) oraz w art. 179 ust. 1 Ustawy z dnia 3 lipca 2018 r. Przepisy wprowadzające ustawę – Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2018 roku, poz. 1669). W związku z powyższym, ***przedkładam Wysokiej Radzie Instytutu Biologii, Biotechnologii i Ochrony Środowiska Wydziału Nauk Przyrodniczych Uniwersytetu Śląskiego w Katowicach wniosek o dopuszczenie mgr Michała Szopińskiego do dalszych etapów przewodu doktorskiego***.

Jednocześnie, w uznaniu szerokiego zakresu badań i wartościowych, oryginalnych wyników, rekomenduję wyróżnienie rozprawy stosowną nagrodą.

Date: 05/09/2021

Signature: 

Małgorzata Wójcik